UNIVERSITI PUTRA MALAYSIA

ELUCIDATION OF HOST CELL RESPONSE TO THE IMMUNOGEN
MYCOLIC ACID EXTRACT OF Corynebacterium pseudotuberculosis IN
GOATS

MOHAMMED NAJI AHMED ODHAH

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By

MOHAMMED NAJI AHMED ODHAH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To the spirit my parents: I will do all that I can do for your satisfaction in your graves, thank you of unconditional support with my life, I am honored to have you as my parents, thank you for giving me a chance to prove and improve myself through all my walk of life.

To my beloved wife, whose unconditional encouragement and support made it possible for me to commence PhD. I wish to express my heartfelt love to my children (Haya, Tasneem, Basel, Naji and Kareem) for coping with the undue paternal deprivation during the journey of my study. Most of all I pledge allegiance to the Lord Almighty for the strength and encouragement He has given me.

To my brothers and sisters: Hoping that with this research I have proven to you that there is no mountain higher as long as God is on our side. Hoping that, you will walk again and be able to fulfill your dreams.

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I would like to conclude by again expressing my deepest gratitude and love to all.
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MOHAMMED NAJI AHMED ODHAH

March 2018

Chairman : Associate Professor Faez Firdaus Jesse Abdullah, PhD
Faculty : Veterinary Medicine

Corynebacterium pseudotuberculosis is the causative agent of caseous lymphadenitis (CLA), a chronic disease of sheep and goats, characterized by the formation of suppurative abscesses in superficial and visceral lymph nodes and in the internal organs of small ruminants. The occurrence of CLA has been documented in the United States, Canada, Australia, New Zealand, South Africa, Brazil and Malaysia with the small ruminants being predominant. The prevalence of CLA among small ruminants in Malaysia is currently estimated at 30% and thus is a noteworthy disease in Malaysia. However, the paucity of information in the literature relating to the extraction of mycolic acids (MAs) from C. pseudotuberculosis and its host cell response and the need to investigate the mechanism by which C. pseudotuberculosis and its immunogen MAs adversely affects goats is an attempt to undertake this study. This study was also designed to test the hypothesis that acute phase protein, antibodies, mainly IgM and IgG, estrogen and progesterone hormones, cytokine, especially, IL-1β and IL-6 can be used as a diagnostic aid for CLA.

Twelve clinically healthy crossbred female Boer goats aged between 16-20 months, weighing 25-35 kg were used in this study. The experimental animals were non-pregnant, non-lactating and had no history of vaccination against CLA. The goats were divided into three groups (A, B and C) consisting of 4 animals each. The control group A was administered with 2 ml of sterile phosphate buffered saline (PBS) intradermally, whereas group B was inoculated with 2 ml of mycolic acid (1 gm/ml) intradermally and group C was inoculation with 2 ml of 1×10⁹ CFU of C. pseudotuberculosis intradermally. Animals were monitored for the entire period (3 months) of the experiment for signs of the disease and blood samples were collected intermittently. The blood sample was collected in heparinized, EDTA plain tubes for
haematological, acute phase protein (APP), IgM and IgG antibodies, sex hormones (estrogen and progesterone) and cytokine (IL-1β and IL-6) analysis. At the end of the study, post-mortem examination of gross lesions was conducted mainly on lung, heart, liver, kidneys, spleen, ovaries, fallopian tubes, uterus, cervix, vagina as well as external and internal lymph nodes were examined to morphologic diagnosis tissues samples also collected for histopathology for analysis of cellular changes.

The inoculated goats in both C. pseudotuberculosis and MAs groups showed significant increase (p<0.05) in body temperature, heart rate, respiratory rate and sharp decrease in rumen motility was observed. Moreover, body condition score was significantly decreased (p<0.05) and enlargement with rupture of injection sites in both treated groups. Superficial lymph nodes showed abscess formation in C. pseudotuberculosis inoculated group only. Varying levels of effect on hematological profile in both treatment compared to the control groups was revealed. The C. pseudotuberculosis inoculated goats showed significant decrease (p<0.05) in red blood cell count and significant increase (p<0.05) in packed cell volume. On the other hand, significant decreases (p<0.05) in the haemoglobin and mean corpuscular hemoglobin concentration in both C. pseudotuberculosis and MAs groups. There was also a notable increase in the levels of white blood cells, neutrophils and lymphocytes in both C. pseudotuberculosis and MAs groups. Meanwhile, haptoglobin was increased from weeks 1-3 and reduced to within the normal range at week 4 in C. pseudotuberculosis group while the MAs group showed significant increase (p<0.05) in the these only in weeks 1 and 2. Similar treats were observed in serum amyloid A except that the increase observed in the C. pseudotuberculosis group was only in weeks 1 to 4. In addition, a significant increase (p<0.05) in IgM in C. pseudotuberculosis group was observed from weeks 2 to 5, while MAs group showed significant increase (p<0.05) from weeks 1 to 3. The level of the IgG was response in C. pseudotuberculosis group was significantly (p<0.05) higher throughout the study period for both groups. The concentration of IgG was steadily increased in the C. pseudotuberculosis and MAs treated groups peaking at week 9 with 32.82±8.56 ng/ml in C. pseudotuberculosis group and 28.41±1.27 ng/ml in MAs group. In addition estrogen hormone showed significant increase (p<0.05) in both treated groups from week 1 to 5 in C. pseudotuberculosis group and weeks 1 to 3 in MAs group. On the other hand progesterone hormone showed significant increase (p<0.05) from weeks 1 to 6 and 1 to 3 in C. pseudotuberculosis and MAs groups respectively. Furthermore, the cytokine (IL-1β) and was increased significantly (p<0.05) in weeks 2, 3, 5, 6, 9 and 10 for the C. pseudotuberculosis group, and week 5 to 10 in MAs group. In a similar vein, IL-6 showed significant increased (p<0.05) in week 5, 6, 7 and 8 for the C. pseudotuberculosis group, while MAs group showed significant increased (p<0.05) in week 2, 3, 4, 5, 6 and 8 compared to the untreated control group.

Post mortem examination of vital and reproductive organs and also internal and external lymph nodes showed mild to severe pathology in the C. pseudotuberculosis group, while the MAs group showed mild to moderate lesions. On the other hand, high rate of gross pathological changes were recorded in both groups in the vital organs and lymph nodes. There were, no significant gross pathological changes were observed in
the reproductive organs in both groups. In vital organs such as lung, heart, liver, kidneys, spleen, the main gross pathological lesions observed were congestion, haemorrhage, fatty atrophy and abscessation in *C. pseudotuberculosis* group. Moreover, the lymph nodes, revealed high percentage of abscess formation in different sizes in all goats in group C. While MAIs group did not show any abscess in the visceral organs or the lymph nodes and reproductive organs despite enlargement of the lymph nodes.

The histopathological changes observed were significant in *C. pseudotuberculosis* inoculated group and the pathologies include congestion, oedema, and infiltration of inflammatory cells, degeneration and necrosis in the vital, reproductive organs and lymph nodes. The MAIs inoculated group showed significant congestion, oedema, degeneration and necrosis.

Detection of the bacteria using conventional PCR revealed 100% detection of the bacteria in the *C. pseudotuberculosis* inoculated group.

Conclusively, this study was able to demonstrate varying clinical manifestations from both *C. pseudotuberculosis* and MAIs inoculated groups. Classical signs of CLA were observed in the *C. pseudotuberculosis* group specifically abscess formation in superficial lymph nodes, while MAIs group showed no abscessation. On the other hand, *C. pseudotuberculosis* and MAIs showed significant changes in all haematological parameters, acute phase protein, IgM and IgG antibodies, estrogen and progesterone concentrations. Significant changes were also observed in the cytokines (IL-1β and IL-6). Different response pattern in the group compared to *C. pseudotuberculosis* for the haematological parameters, acute phase protein, IgM and IgG antibodies, reproductive hormones as well as cytokines indicate different mechanisms. The gross and cellular changes were typical of CLA lesions in *C. pseudotuberculosis* inoculated group whilst MAIs inoculated group showed less gross changes however, the cellular changes were severe, indicating the effect of MAIs on tissues.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

MENGENAL PASTI TINDAK BALAS SEL BADAN KAMBING TERHADAP Corynebacterium pseudotuberculosis DAN EKSTRAK IMMUNOGEN ASID MYCOLIC

Oleh

MOHAMMED NAJI AHMED ODHAH

Mac 2018

Pengerusi : Profesor Madya Faez Firdaus Jesse Abdullah, PhD
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Corynebacterium pseudotuberculosis adalah merupakan agen penyakit “caseous lymphadenitis” ataupun CLA, penyakit kronik kepada kambing dan biri-biri yang boleh menyebabkan bisul yang berelah pada nodus limfa luaran dan dalam haiwan ruminan kecil tersebut. Penyakit CLA ini telah dilaporkan berlaku di Amerika Syarikat, Kanada, Australia, New Zealand, Afrika Selatan, Brazil dan Malaysia, terutamanya terhadap haiwan ruminan kecil. Dianggarkan bahawa kelaziman penyakit CLA di Malaysia adalah sebanyak 30%, menjadikan CLA satu penyakit yang perlu diberi perhatian di Malaysia. Tujuan kajian ini adalah untuk mengisi kekurangan maklumat mengenai pengekstrakkan asid mycolic daripada C. pseudotuberculosis, tindak balas sel perumah dan mekanisma C. pseudotuberculosis dan imunogen asid mycolic boleh menjajaskan kambing yang terlibat dalam kajian ini. Kajian ini juga direka untuk menguji hipotesis sama ada protein fasa akut, antibodi seperti IgM dan IgG, hormon progesteron dan estrogen dan sitokin seperti IL-1β dan IL-6 boleh digunakan untuk tujuan diagnostik terhadap penyakit CLA.

Sebanyak 12 ekor kambing betina Boer kaucuan yang sihat secara klinikal berumur antara 16-20 bulan, berat badan antara 25-35 kg telah digunakan untuk kajian ini. Semua haiwan untuk eksperimen ini adalah tidak bunting, tidak menyusu dan tiada sejarah vaksinasi terhadap penyakit CLA. Kambing-kambing tersebut telah dibahagi kepada tiga kumpulan (A, B dan C) dengan 4 ekor kambing di dalam setiap kumpulan. Kumpulan kawalan, iaitu kumpulan A telah diinokulasi dengan 2 ml saline buffer fosfat steril (PBS) melalui intradermal, manakala kumpulan B telah diinokulasi dengan 2 ml asid mycolic melalui intradermal dan kumpulan C telah diinokulasi dengan 2 ml 1×10⁹ CFU C. pseudotuberculosis juga melalui intradermal. Semua haiwan telah dipantau sepanjang tempoh eksperimen (3 bulan) untuk tanda-
tanda klinikal penyakit dan sampel darah telah diambil secara berselang-seli. Sampel darah telah dikumpul di dalam tiub yang mengandungi heparin, EDTA dan juga tiub kosong untuk analisis hematologi, protein fasa akut (APP), antibodi IgM dan IgG, hormon pembiakan (estrogen dan progestron) dan sitokin (IL-1β dan IL-6). Pada penghujung kajian, pemeriksaan bedah siasat secara kasar telah dijalankan pada paru-paru, jantung, hati, buah pinggang, limpa, ovari, tiub fallopian, uterus, serviks, faraj serta nodus limfa dalaman dan luaran dan kesemua tisu dikumpul untuk pemeriksaan histopatologi dan perubahan sel.

Kambing-kambing dalam kumpulan yang diinokulasi dengan *C. pseudotuberculosis* dan asid mycolic menunjukkan peningkatan ketara (p<0.05) terhadap suhu badan, kadar degupan jantung dan kadar pernafasan manakala penurunan ketara dalam pergerakan rumen. Skor keadaan badan turut menunjukkan penurunan ketara (p<0.05), disamping pembesaran dan kerosakan tisu di kawasan suntikan di dalam kedua-dua kumpulan rawatan. Hanya kumpulan yang diinokulasi dengan *C. pseudotuberculosis* menunjukkan pembentukan abses pada nodus limfa luaran. Hasil kajian ini turut menunjukkan pelbagai kesan yang berbeza pada profil hematologi pada kedua-dua kumpulan rawatan berbanding dengan kumpulan kawalan. Kumpulan kambing yang diinokulasi dengan *C. pseudotuberculosis* menunjukkan penurunan ketara (p<0.05) dalam kiraan sel darah merah dan peningkatan ketara (p<0.05) dalam jumlah sel berpadat. Manakala penurunan ketara (p<0.05) telah diperhatikan ke atas sel hemoglobin dan purata kepekatan hemoglobin korpuskular dalam kedua-dua kumpulan yang diinokulasi dengan *C. pseudotuberculosis* dan asid mycolic. Peningkatan ketara tersebut diperhatikan dalam tahap sel darah putih, neutrofil dan limfosit dalam kedua-dua kumpulan *C. pseudotuberculosis* dan asid mycolic. Sementara itu, protein fasa akut (haptoglobin) meningkat daripada minggu 1 hingga minggu 3 sebelum turun kepada paras normal pada minggu ke-4 untuk kumpulan *C. pseudotuberculosis* manakala kumpulan asid mycolic menunjukkan peningkatan ketara (p<0.05) hanya pada minggu 1 dan minggu 2, perubahan yang sama diperhatikan dalam tahap serum amyloid A. kecuali peningkatan dalam serum amyloid A diperhatikan dalam kumpulan *C. pseudotuberculosis* pada minggu 1 hingga minggu 4. Peningkatan ketara (p<0.05) turut diperhatikan dalam tahap IgG dalam kumpulan *C. pseudotuberculosis* diperhatikan daripada minggu 2 hingga minggu 5, manakala kumpulan asid mycolic menunjukkan peningkatan ketara (p<0.05) daripada minggu 1 hingga minggu 3. Tahap tindak balas IgG dalam kumpulan *C. pseudotuberculosis* adalah lebih tinggi secara ketara (p<0.05) untuk kedua-dua kumpulan sepanjang tempoh kajian. Tahap IgG meningkat secara berterusan dalam kedua-dua kumpulan *C. pseudotuberculosis* dan asid mycolic dan memuncak pada minggu 9 dengan 32.82±8.56 ng/ml dalam kumpulan *C. pseudotuberculosis* dan 28.41±1.27 ng/ml dalam kumpulan asid mycolic. Pada masa yang sama, hormon estrogen menunjukkan peningkatan ketara (p<0.05) dalam kedua-dua kumpulan rawatan daripada minggu 1 hingga minggu 5 dalam kumpulan *C. pseudotuberculosis* dan daripada minggu 1 hingga minggu 3 dalam kumpulan asid mycolic. Manakala hormon progesteron menunjukkan peningkatan ketara (p<0.05) daripada minggu 1 hingga minggu 6 untuk kumpulan *C. pseudotuberculosis* dan minggu 1 hingga minggu 3 untuk kumpulan asid mycolic. Tambahan pula, tahap sitokin (IL-1β) meningkat secara ketara (p<0.05) pada minggu 2, 3, 5, 6, 9 dan 10 untuk kumpulan *C. pseudotuberculosis* dan pada minggu 5 hingga
minggu 10 untuk kumpulan asid mycolic. Pada masa yang sama, IL-6 menunjukkan peningkatan ketara (p<0.05) pada minggu 5, 6, 7 dan 8 untuk kumpulan C. pseudotuberculosis, manakala kumpulan asid mycolic menunjukkan peningkatan ketara (p<0.05) pada minggu 2, 3, 4, 5, 6 dan 8 berbanding dengan kumpulan kawalan.


Perubahan histopatologi yang ketara telah diperhatikan di dalam kumpulan yang diinokulasi dengan C. pseudotuberculosis dalam bentuk konjesi, edema, penyusupan sel keradangan, degenerasi dan nekrosis di dalam organ-organ penting, organ pembiakan dan nodus limfa. Kumpulan yang diinokulasi dengan asid mycolic pula menunjukkan konjesi, edema, degenerasi dan nekrosis yang ketara.

Pengesanan bakteria menggunakan PCR konvensional telah mengesankan 100% bakteria tersebut di dalam kumpulan yang telah diinokulasi dengan C. pseudotuberculosis, manakala tiada bakteria yang dikesan di dalam kumpulan yang diinokulasi dengan asid mycolic seperti yang dijangka.

Secara keseluruhan, kajian ini dapat menunjukkan manifestasi tanda klinikal yang berbeza-beza di dalam kedua-dua kumpulan C. pseudotuberculosis dan asid mycolic. Tanda-tanda klasik penyakit CLA telah diperhatikan di dalam kumpulan C. pseudotuberculosis terutamanya dalam pembentukan abses di dalam nodus limfa luaran manakala kumpulan asid mycolic pula tidak menunjukkan pembentukan abses. Pada masa yang sama, kedua-dua kumpulan C. pseudotuberculosis dan asid mycolic menunjukkan perubahan ketara dalam semua parameter hematologi, protein fasa akut, antibodi-antibodi IgM dan IgG serta kepekatan estrogen dan progesteron. Perubahan ketara turut diperhatikan di dalam profil sitokin (IL-1β dan IL-6) manakala kumpulan asid mycolic menunjukkan corak tindak balas yang berbeza termasuk di dalam parameter hematologi, protein fasa akut, antibodi-antibodi IgM dan IgG, hormon pembiakan serta profil sitokin, menunjukkan mekanisme tindakan yang
berbeza. Perubahan kasar dan selular yang diperhatikan adalah tipikal bagi penyakit CLA di dalam kumpulan yang diinokulasi dengan *C. pseudotuberculosis* sementara kumpulan yang telah diinokulasi dengan asid mycolic pula menunjukkan perubahan kasar yang lebih sederhana manakala perubahan selular yang diperhatikan adalah lebih teruk, menunjukkan kesan asid mycolic pada tisu organ.
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I certify that a Thesis Examination Committee has met on 6 March 2018 to conduct the final examination of Mohammed Naji Ahmed Odhah on his thesis entitled "Elucidation of Host Cell Response to the Immunogen Mycolic Acid Extract of Corynebacterium pseudotuberculosis in Goats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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xii
TABLE OF CONTENTS

ABSTRACT i
ABSTRAK iv
ACKNOWLEDGEMENTS viii
APPROVAL ix
DECLARATION xi
LIST OF TABLES xviii
LIST OF FIGURES xx
LIST OF APPENDICES xxvi
LIST OF ABBREVIATIONS xxvii

CHAPTER

1 INTRODUCTION 1
1.1 Background 1

2 LITERATURE REVIEW 5
2.1 Background information on Corynebacterium pseudotuberculosis 5
2.1.1 The phylogenic classification of C. pseudotuberculosis 5
2.1.2 Biochemical characteristic of C. pseudotuberculosis 6
2.2 Virulence factors of C. pseudotuberculosis 7
2.2.1 Mycolic acid 7
2.2.1.1 Mycolic acid in Corynebacterium pseudotuberculosis 8
2.2.2 Phospholipase D (PLD) 9
2.3 Infection Source of CLA 12
2.4 Zoonosis of CLA 12
2.5 Epidemiology and economic effect of CLA 13
2.6 Pathogenesis of C. pseudotuberculosis 13
2.7 Haematology of Caseous Lymphadenitis 15
2.8 Clinical signs of CLA 16
2.9 CLA diagnosis 17
2.10 Treatment of CLA 18
2.11 Immuno-pathogenesis of CLA 18
2.12 Vaccine model for CLA infection 20
2.13 The resistance of host cell to Corynebacterium pseudotuberculosis 21
2.14 Acute phase protein 21
2.14.1 The Acute Phase Response (APR) 22
2.14.2 Function of acute phase proteins 22
2.14.3 Positive Acute Phase Proteins 23
2.14.3.1 Haptoglobin (Hp) 23
2.14.3.2 Serum Amyloid A 23
2.14.4 Negative Acute Phase Proteins 23
  2.14.4.1 Albumin 23
  2.14.4.2 Transferrin 23
2.14.5 Acute Phase Proteins in Small Ruminants 24
2.15 Responses of Cytokine (IL-1β and IL-6) 24
2.16 Histopathology of Caseous Lymphadenitis 27
2.17 Reproductive efficiency 30
  2.17.1 Reproductive endocrinology and physiology of goats 30
  2.17.2 Female reproductive anatomy 31
  2.17.3 Female hormones 32
  2.17.4 Folliculogenesis 32
2.18 Caseous lymphadenitis and reproduction 33
2.19 Identification of \textit{Corynebacterium pseudotuberculosis} through Polymerase Chain Reaction (PCR) 34

3 MATERIAL AND METHOD 36
3.1 Approval of the Study 36
3.2 Experimental location 36
3.3 Experimental design 36
3.4 The Feeding and Management of the experimental animals 38
3.5 Isolation and identification of \textit{Corynebacterium pseudotuberculosis} 38
3.6 Bacterial preparation for the mycolic acid extraction 38
3.7 Mycolic acid extraction 38
3.8 Inoculum challenge dose preparation 39
3.9 Examination of Clinical Signs in Goats 39
  3.9.1 Rumen Motility 39
  3.9.2 Scoring of Body Condition 39
  3.9.3 Hair Coat Condition Score (HCS) 40
3.10 Haematological analysis 40
3.11 Analyses of Immune Response. 40
  3.11.1 Goat Haptoglobin (Hp) 41
  3.11.2 Goat Serum Amyloid A (SAA) ELISA Kit Assay 41
  3.11.3 Goat Immunoglobulin M (IgM) ELISA Kit assay 41
  3.11.4 Goat Immunoglobulin G (IgG) ELISA KIT assay 41
3.12 Hormonal Assay 41
  3.12.1 Serum estradiol analysis 42
  3.12.2 Serum progesterone analysis 42
3.13 Goat Serum cytokine (IL-1β and IL-6) ELISA Kit Assay 42
3.14 Histopathological Examination of Goats 43
  3.14.1 Post mortem examination 43
  3.14.2 Histopathology 43
  3.14.3 Lesion Scoring 43
3.15 Polymerase Chain Reaction (PCR) Technique Procedure 43
  3.15.1 Bacterial Isolation and Identification 43
  3.15.2 Primer Design 44
3.16 Statistical Analysis 44
4 CLINICAL RESPONSES IN GOATS TOWARDS CHALLENGED OF Corynebacterium pseudotuberculosis and ITS IMMUNOGEN MYCOLIC ACID
4.1 Introduction 45
4.2 Material and Methods 46
4.3 Results 46
4.3.1 Body Condition Scoring (BCS) 48
4.3.2 Temperature 49
4.3.3 Heart rate 50
4.3.4 Respiratory rate 50
4.3.5 Rumen Motility 51
4.4 Discussion 52

5 COMPLETE BLOOD COUNT CHANGES DUE TO Corynebacterium pseudotuberculosis AND ITS MYCOLIC ACID CHALLENGED IN GOATS
5.1 Introduction 56
5.2 Materials and Methods 57
5.3 Results 57
5.3.1 Red blood cell (RBCs) 57
5.3.2 Concentration of Haemoglobin (Hb) 58
5.3.3 Packed cell volume (PCV) 59
5.3.4 Mean corpuscular volume (MCV) 60
5.3.5 Mean Corpuscular Haemoglobin Concentration (MCHC) 61
5.3.6 White Blood Cell Count (WBC) 62
5.3.7 Neutrophil Count 63
5.3.8 Lymphocyte Count 64
5.3.9 Monocyte Count 65
5.3.10 Basophil Count 66
5.3.11 Eosinophil Count 67
5.3.12 Plasma protein Concentration 68
5.4 Discussion 70

6 ACUTE PHASE PROTEINS RESPONSE IN CROSSBRED FEMALE GOATS CHALLENGED WITH Corynebacterium pseudotuberculosis AND MYCOLIC ACID
6.1 Introduction 75
6.2 Materials and Methods 76
6.3 Result 76
6.3.1 Haptoglobin concentration (Hp) 76
6.3.2 Serum Amyloid A concentration (SAA) 77
6.4 Discussion 79
7 EVALUATING THE LEVEL OF ANTIBODIES IN GOATS TREATED WITH *Corynebacterium pseudotuberculosis* AND MYCOLIC ACID

7.1 Introduction 82
7.2 Materials and methods 83
7.3 Results 83
   7.3.1 Immunoglobulin M (IgM) 83
   7.3.2 Immunoglobulin G (IgG) 84
7.4 Discussion 86

8 STERODAL SEX HORMONES CONCENTRATION IN DOES INOCULATED WITH *Corynebacterium pseudotuberculosis* AND MYCOLIC ACID

8.1 Introduction 89
8.2 Material and Methods 89
8.3 Results 90
   8.3.1 Estrogen concentration 90
   8.3.2 Progesterone concentration 91
8.4 Discussion 92

9 ASSESSMENT OF THE CONCENTRATION OF PRO-INFLAMMATORY CYTOKINES IL-1β AND IL-6 IN GOATS INOCULATED OF *Corynebacterium pseudotuberculosis* AND IMMUNOGEN MYCOLIC ACID IN GOATS

9.1 Introduction 96
9.2 Material and Method 97
9.3 Result 97
   9.3.1 Cytokine concentration of Interleukin 1β (IL-1β) 97
   9.3.2 Cytokine concentration of Interleukin 16 (IL-6) 98
9.4 Discussion 100

10 HISTOPATHOLOGICAL EFFECTS OF *Corynebacterium pseudotuberculosis* AND ITS IMMUNOGEN MYCOLIC ACID IN LYMPH NODES, VITAL AND REPRODUCTIVE ORGANS IN DOES

10.1 Introduction 102
10.2 Material and method 103
10.3 Results 103
   10.3.1 Gross lesion 103
   10.3.2 Histopathology assessment of lymph nodes 108
   10.3.3 Histopathology of the vital and reproductive organs 118
10.4 Discussion 132
11 ISOLATION AND IDENTIFICATION OF Corynebacterium pseudotuberculosis IN THE VITAL ORGANS, REPRODUCTIVE ORGANS AND LYMPH NODES OF GOATS INOCULATED WITH Corynebacterium pseudotuberculosis AND ITS IMMUNOGEN MYCOLIC ACID

11.1 Introduction 137
11.2 Material and Method 138
11.3 Result 138
11.3.1 Bacterial Isolation and Identification 138
11.3.2 PCR Results 139
11.4 Discussion 141

12 GENERAL DISCUSSION AND CONCLUSION 143
12.1 General Discussion 143
12.2 Conclusion 146

REFERENCES 147
APPENDICES 178
BIODATA OF STUDENT 204
LIST OF PUBLICATIONS 205
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1</td>
<td>Mean of red blood cells count of inoculated group through the study period</td>
</tr>
<tr>
<td>5.2</td>
<td>Mean of hemoglobin concentration of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.3</td>
<td>Mean of packed cell volume (PCV) of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.4</td>
<td>Mean of corpuscular volume (MCV) of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.5</td>
<td>Mean of corpuscular haemoglobin concentration (MCHC) of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.6</td>
<td>Mean of white blood cells (WBC) of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.7</td>
<td>Mean of neutrophil count of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.8</td>
<td>Mean of Lymphocytes Count of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.9</td>
<td>Mean of Monocyte Count of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.10</td>
<td>Mean of Basophil Count of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.11</td>
<td>Mean of Eosinophil Count of inoculated groups through the study period</td>
</tr>
<tr>
<td>5.12</td>
<td>Mean of Plasma protein concentration of inoculated groups through the study period</td>
</tr>
<tr>
<td>6.1</td>
<td>Mean of Haptoglobin concentration (Hp) of inoculated groups through the study period</td>
</tr>
<tr>
<td>6.2</td>
<td>Mean of Serum amyloid A concentration of inoculated groups through the study period</td>
</tr>
<tr>
<td>7.1</td>
<td>Means (S.E) of IgM concentration (ug/ml) of inoculated group throughout the study period</td>
</tr>
</tbody>
</table>
7.2 Means (S.E) of IgG concentration (ug/ml) of inoculated group throughout the study period

8.1 Mean of Estrogen concentration (pg/ml) of inoculated groups through the study period

8.2 Mean of progesterone concentration (ng/ml) of inoculated groups through the study period

9.1 Chart showed of IL-16 concentration of the goats after inoculated with mycolic acid and *Corynebacterium pseudotuberculosis* (n=12)

9.2 Mean of Cytokine concentration IL-6 of inoculated groups through the study period

10.1 The gross lesions observed on the harvested organs in both *Corynebacterium pseudotuberculosis* and Mycolic acid

10.2 Histo-pathological findings of lymph nodes after inoculation *C. pseudotuberculosis* and Mas

10.3 Histo-pathological findings of vital and reproductive organs with inoculation *Corynebacterium pseudotuberculosis* and Mycolic acid

11.1 Bacteriology of C. pseudotuberculosis culture results of vital, reproductive organs and lymph nodes from the *Corynebacterium pseudotuberculosis* and Mycolic acid challenged group

11.2 PCR Detection culture results of vital, reproductive organs and lymph nodes isolation from the *Corynebacterium pseudotuberculosis* and Mycolic acid challenged groups
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Chemical Structure of Mycolic Acids (Glickman et al., 2001)</td>
<td>9</td>
</tr>
<tr>
<td>2.2</td>
<td>Showing response to bacterial invasion where interleukin-1 and IL-1β produce expressions of epithelial anti-microbial protein alongside with IL-6 and IL-23 stimulating helper T 17 cells which in turn secretes IL-17A, IL-17F and IL-22. The IL-17A then induces secretion of ligand 20 that performs the antimicrobial activity thereby attracting the dendritic cells and neutrophils. In an acute phase, this is of beneficial to the host.</td>
<td>27</td>
</tr>
<tr>
<td>3.1</td>
<td>Experimental design depicting the preliminary study of the effects of <em>C. pseudotuberculosis</em> and Mycolic acid in goats</td>
<td>37</td>
</tr>
<tr>
<td>4.1</td>
<td>Showing the site of <em>C. pseudotuberculosis</em> injection in treated group with evidence of abscess with purulent discharge after two (A) and four (B) weeks of intradermal inoculation</td>
<td>46</td>
</tr>
<tr>
<td>4.2</td>
<td>Showing the site of injection in Mycolic acid treated group with evidence of enlargement after one (C) and two (D) weeks of intradermal inoculation</td>
<td>47</td>
</tr>
<tr>
<td>4.3</td>
<td>Photo of Mycolic acid group characterized by marked emaciation</td>
<td>47</td>
</tr>
<tr>
<td>4.4</td>
<td>Parotid lymph node showed enlargement of site of inoculated with <em>C. pseudotuberculosis</em> in group C</td>
<td>48</td>
</tr>
<tr>
<td>4.5</td>
<td>Pre-scaphular lymph node shows enlargement and rupture oozing with suppurative material after inoculation with the viable bacterium <em>C. pseudotuberculosis</em> group</td>
<td>48</td>
</tr>
<tr>
<td>4.6</td>
<td>Body condition scoring of the goats post inoculated with <em>C. pseudotuberculosis</em> and Mycolic acid</td>
<td>49</td>
</tr>
<tr>
<td>4.7</td>
<td>Body temperature of the goats post inoculated with <em>C. pseudotuberculosis</em> and mycolic acid</td>
<td>49</td>
</tr>
<tr>
<td>4.8</td>
<td>Heart rate (bpm) of the goats post inoculated with <em>C. pseudotuberculosis</em> and mycolic acid</td>
<td>50</td>
</tr>
<tr>
<td>4.9</td>
<td>Respiratory rate (bpm) of the goats post inoculated with <em>C. pseudotuberculosis</em> and Mycolic acid</td>
<td>51</td>
</tr>
<tr>
<td>4.10</td>
<td>Rumen motility of the goats post inoculated with <em>C. pseudotuberculosis</em> and Mycolic acid</td>
<td>51</td>
</tr>
<tr>
<td>5.1</td>
<td>Chart showed of red blood cell count of the goats after inoculated with mycolic acid and <em>C. pseudotuberculosis</em>. N=12 animals</td>
<td>58</td>
</tr>
<tr>
<td>5.2</td>
<td>Chart showed of haemoglobin concentration of the goats after inoculated with mycolic acid and <em>C. pseudotuberculosis</em>. n=12 animals</td>
<td>59</td>
</tr>
</tbody>
</table>
5.3 Chart showed of packed cell volume of the goats after inoculated with Mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.4 Chart showed of means corpuscular volume of the goats after inoculated with mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.5 Chart showed of means corpuscular hemoglobin concentration of the goats after inoculated with mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.6 Chart showed of white blood cell count of the goats after inoculated with Mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.7 Chart showed of means neutrophil count of the goats after inoculated with mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.8 Chart showed of Lymphocyte count of the goats after inoculated with Mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.9 Chart showed of monocyte count of the goats after inoculated with Mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.10 Chart showed of basophil count of the goats after inoculated with Mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.11 Chart showed of eosinophil count of the goats after inoculated with Mycolic acid and *C. pseudotuberculosis*. n=12 animals.

5.12 Chart showing the plasma protein concentration of goats after inoculated with mycolic acid and *C. pseudotuberculosis*. n=12 animals.

6.1 Chart showed of Haptoglobin concentration of the goats after inoculated with *C. pseudotuberculosis*. n=12.

6.2 Chart showed of Serum amyloid A concentration in goats after inoculated with *C. pseudotuberculosis*. n=12.

7.1 Chart showed of IgM concentration of the goats after inoculated with Mycolic acid and *C. pseudotuberculosis* (n=12).

7.2 Chart showed of IgG concentration of the goats after inoculated with mycolic acid and *C. pseudotuberculosis* (n=12).

8.1 Chart showed of estrogen concentration of the goats after inoculated with mycolic acid and *C. pseudotuberculosis*. (n=12).

8.2 Chart showed of progesterone concentration of the goats after inoculated with mycolic acid and *C. pseudotuberculosis* (n=12).

9.1 Chart showed of IL-1β concentration of the goats after inoculated with mycolic acid and *C. pseudotuberculosis* (n=12).

9.2 Chart showed of IL-16 concentration of the goats after inoculated with mycolic acid and *C. pseudotuberculosis* (n=12).

10.1 Photograph of goat necropsy from *C. pseudotuberculosis* inoculated group shows abscess oozing from the surface of cut of the pre-scapular lymph node (A).
10.2 Photograph of goat necropsy from *C. pseudotuberculosis* inoculated group shows blood tinged fluids inside the chest cavity (A), opaque pericardium (B) and focal abscess on the surface of the liver (C)

10.3 Photograph of goat necropsy of liver from *C. pseudotuberculosis* inoculated group shows pale coloured and flabby structure of the liver (A) and engrement of GB with bile fluid filled gall bladder and discoloration (B)

10.4 Photograph of goat necropsy of lung from *C. pseudotuberculosis* inoculated group showing an open abscess on the surface of the lung (A), congestion (B) and flabby pale lungs (C)

10.5 Photograph of lung necropsy from MA inoculated group shows different stages of pneumonia. Congestion (A), red hepatization (B) and gray hepatization (C)

10.6 Photograph of goat necropsy of lung from MA inoculated group shows congested trachea (A), congested lung (B) with marble appearance of the lung surface (C)

10.7 Photograph of goat necropsy from *C. pseudotuberculosis* inoculated group shows single abscess on the surface of the spleen (A)

10.8 Photograph of goat necropsy of heart from MA inoculated group shows thick opaque pericardium (A), pericardium effusion (B) and generally, flabby heart

10.9 Section of the parotid lymph node in group C (*C. pseudotuberculosis*) showing extensive depletion of follicles with presence of microabscesses (black arrows), H&E x 100

10.10 Section of the parotid lymph node in group B (Mycolic acid) showing extensive areas of follicular edema and lymphocyte depletion (black arrows), H&E x 100

10.11 Section of the sub-mandibular lymph node in group C (*C. pseudotuberculosis*) showing areas of medullary cord rich in cellularity including macrophage, plasma cells and lymphocyte (black arrows), and slight hemorrhage (yellow arrow) H&E x 100

10.12 Section of the sub-mandibular lymph node in group B (Mycolic acid) showing areas of follicular depletion (black arrows), H&E x 100

10.13 Section of the pre-scapular lymph node in group C (*C. pseudotuberculosis*) showing vascular congestion (yellow arrows), with areas of follicular lymphocyte depletion (black arrows), H&E x 100

10.14 Section of the pre-scapular lymph node in group B (Mycolic acid) showing an extensive area of follicular depletion characterized by lymphocyte depopulation (Black arrows), H&E x 100

10.15 Section of the pre-femoral lymph node in group C (*C. pseudotuberculosis*) showing extensive areas of follicular necrosis and
lymphocyte depletion, resulting in thin walled abscesses (black arrows), H&E x 100

10.16 Section of the pre-femoral lymph node in group B (Mycolic acid) showing an area of lymphoid depletion around a medullary cord (black arrow), H&E x 200

10.17 Section of the popliteal lymph node in group C (C. pseudotuberculosis) showing vascular congestion (yellow arrows), with extensive areas of follicular necrosis and depletion (black arrows), H&E x 100

10.18 Section of the popliteal lymph node in group B (Mycolic acid) showing vascular congestion (black arrows), with extensive areas of follicular necrosis and depletion (yellow arrows), H&E x 100

10.19 Section of the supra mammary lymph node in group C (C. pseudotuberculosis) showing dilated sinusoid (due to edema) in medulla with some macrophages and neutrophils, H&E x 100

10.20 Section of the supra mammary lymph node in group B (Mycolic acid) showing medulla area with lymphadenitis, dilated and high cellularity sinusoid and vascular congestion (black arrows), H&E x 100

10.21 Section of the mesenteric lymph node in group C (C. pseudotuberculosis) showing vascular congestion (yellow arrows), with extensive areas of follicular necrosis and depletion (black arrows), H&E x 100

10.22 Section of the mesenteric lymph node in group B (Mycolic acid) showing extensive areas of edema (yellow arrows) with reduced lymphocyte population in the lymphoid follicles (black arrows), H&E x 100

10.23 Section of the lung in group C (C. pseudotuberculosis) showing extensive areas of pulmonary hemorrhage (red arrows) and vascular congestion (yellow arrow) with thickening of the interstitium due to leucocytic infiltration (black arrows), Note also the desquamation of bronchiolar epithelium (green arrows), H&E x 100

10.24 Section of the lung in group C (C. pseudotuberculosis) showing extensive areas of pulmonary edema (green arrows) and vascular congestion (yellow arrows) with fibrous and inflammatory cell infiltration (blue arrow), also diffuse leucocytic infiltration (red arrows) in the interstitium, H&E x 100

10.25 Section of the lung in group B (Mycolic acid) showing vascular congestion (yellow arrows) with multifocal peri-bronchiolar lymphocytic infiltration (black arrows), H&E x 100

10.26 Section of the heart in group C (C. pseudotuberculosis) showing vascular congestion (yellow arrow), H&E x 100

10.27 Section of the heart in group B (Mycolic acid) showing areas of vascular congestion (black arrows), H&E x 100
10.28 Section of the liver in group C (*C. pseudotuberculosis*) showing degenerating hepatocytes (yellow arrow), and hepatocyte necrosis (black arrows), H&E x 100

10.29 Section of the liver in group B (Mycolic acid) showing an area of increased sinusoidal spaces with vascular congestion (yellow arrows) and pyknotic hepatocytes, adjacent areas have normal hepatocyte lined sinusoids (black arrow), H&E x 200

10.30 Section of the renal cortex in group C (*C. pseudotuberculosis*) showing a locally extensive area of hemorrhage (red arrows) with diffuse areas of tubular necrosis (yellow arrow), multifocal areas of lamphocytes infiltration were also observed in the renal intestitium (black arrow), H&E x 100

10.31 Section of the kidney medulla in group B (mycolic acid) showing a locally extensive area of tubular degeneration and necrosis (red arrows) with areas of vascular congestion (yellow arrow), H&E x 100

10.32 Section of the spleen in group C (*C. pseudotuberculosis*) showing lymphocyte depletion in the white pulp (black arrow) with extensive vascular congestion in the red pulp (yellow arrow), H&E x 100

10.33 Section of the spleen in group B (Mycolic acid) showing lymphocyte depletion in the marginal zone of the white pulp (green arrow) with vascular congestion in the red pulp (red arrow), H&E x 100

10.34 Section of the ovary in group C (*C. pseudotuberculosis*) showing vascular congestion (black arrow) H&E x 100

10.35 Section of the ovary in group B (Mycolic acid) showing vascular congestion (black arrow) and leucocytic infiltration dominated by neutrophils around primordial in the stromal tissue (red arrows), H&E x 100

10.36 Section of the fallopian tube in group C (*C. pseudotuberculosis*) showing vascular congestion (black arrows), H&E x 100

10.37 Section of the fallopian tube in group B (Mycolic acid) showing vascular congestion (black arrows) and leucocytic infiltration dominated by neutrophils in the stromal tissues (red arrow), H&E x 100

10.38 Section of the uterine myometrium in group C (*C. pseudotuberculosis*) showing a congested blood vessel (black arrow) and myocyte degeneration and necrosis with few leucocyte infiltration (yellow arrow), H&E x 100

10.39 Section of the uterine endometrium in group C (*C. pseudotuberculosis*) showing extensive necrosis of endometrial glands with infiltration of neutrophils (yellow arrows), H&E x 100

10.40 Section of the uterine endometrium in group B (Mycolic acid) showing congested blood vessels (black arrows) and degenerating uterine glands
characterized by vacolation of glandular epithelia (yellow arrows) H&E x 100

10.41 Section of the uterine myometrium in group B (Mycolic acid) showing a congested blood vessel (black arrow) and multifocal perivascular infiltration of neutrophils around the blood vessels (yellow arrows), H&E x 100

10.42 Section of the cervix in group C (C. pseudotuberculosis) showing a mild disarrangement of cervical musculature and oedema (yellow arrow) and inflammatory cell infiltration (black circle) H&E x 100

10.43 Section of the cervix in group B (Mycolic acid) showing vascular congestion (yellow arrow) and leucocytic infiltration in the lamina propria (black arrow), H&E x 100

11.1 Agrose gel electrophoresis showing amplification of 816 bp bands specific for C. pseudotuberculosis isolated from vital, reproductive organs and lymph nodes of positive group goats (+ve)
**LIST OF APPENDICES**

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>The approval letter of the experimental procedures by the “Institutional Animal Care and Use Committee” Universiti Putra Malaysia</td>
</tr>
<tr>
<td>B</td>
<td>General observation sheet</td>
</tr>
<tr>
<td>C</td>
<td>Goat Haptoglobin (Hp) ELISA Kit Assay (Cat. No. QY-E140048)</td>
</tr>
<tr>
<td>D</td>
<td>Goat Serum Amyloid A (SAA) ELISA Kit Assay (Cat. No. QY-E140047)</td>
</tr>
<tr>
<td>E</td>
<td>Goat Immunoglobulin M (IgM) ELISA Kit Assay (Cat No. QY-E140013)</td>
</tr>
<tr>
<td>F</td>
<td>Goat Immunoglobulin G (IgG) ELISA Kit Assay (Cat No. Qy-E140013)</td>
</tr>
<tr>
<td>G</td>
<td>Goat Radioimmunoassay Estradiol (Lot 150622C/ Ref. 21854)</td>
</tr>
<tr>
<td>H</td>
<td>Goat Radioimmunoassay Progesterone (Lot 150622C/ Ref. 1188)</td>
</tr>
<tr>
<td>I</td>
<td>Goat Interleukin-1beta (IL-1β) ELISA Kit Assay (Cat No. QY-E140037)</td>
</tr>
<tr>
<td>J</td>
<td>Goat Interleukin-6 (IL-6) ELISA Kit Assay (Cat No. QY-E140039)</td>
</tr>
<tr>
<td>K</td>
<td>Histopathology procedure</td>
</tr>
<tr>
<td>L</td>
<td>Primer Design</td>
</tr>
<tr>
<td>M</td>
<td>White blood cell differential count</td>
</tr>
<tr>
<td>N</td>
<td>Reference Range</td>
</tr>
</tbody>
</table>
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>AGID</td>
<td>Agar gel immunodiffusion</td>
</tr>
<tr>
<td>APP</td>
<td>Acute phase proteins</td>
</tr>
<tr>
<td>CBC</td>
<td>Complete Blood Count</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CLA</td>
<td>Caseous lymphadenitis</td>
</tr>
<tr>
<td>DVS</td>
<td>Department of Veterinary Service</td>
</tr>
<tr>
<td>E2</td>
<td>Estrogen Hormone</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>H&amp;E</td>
<td>Hematoxylin and Eosin</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Hp</td>
<td>Haptoglobin</td>
</tr>
<tr>
<td>I.V</td>
<td>Intravenous</td>
</tr>
<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Interleukin 1β</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>IM</td>
<td>Intradermally</td>
</tr>
<tr>
<td>MAs</td>
<td>Mycolic Acid</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean Corpuscular Hemoglobin Concentration</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>°C</td>
<td>Degree celsius</td>
</tr>
<tr>
<td>OD</td>
<td>Optical Density</td>
</tr>
<tr>
<td>OIE</td>
<td>Office of International Epizootic</td>
</tr>
<tr>
<td>P4</td>
<td>Progesterone Hormone</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PCV</td>
<td>Packed Cell Volume</td>
</tr>
<tr>
<td>PLD</td>
<td>Phospholipase D</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>SAA</td>
<td>Serum Amyloid A</td>
</tr>
<tr>
<td>TPU</td>
<td>Taman Pertanian Universiti</td>
</tr>
<tr>
<td>UPM</td>
<td>Universiti Putra Malaysia</td>
</tr>
<tr>
<td>VLSU</td>
<td>Veterinary Laboratories Service Unit</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background

*Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*) is the etiological agent that causes caseous lymphadenitis (CLA), a well-known disease of sheep and goats. The CLA is described by formation of one or many abscesses in lymph nodes (Paton, 2010).

The *C. pseudotuberculosis* is an intracellular, gram positive, facultative anaerobe, small curved rod. It is non-spore forming bacterium that is responsible for caseous lymphadenitis (CLA), a chronic contagious disease of sheep and goats worldwide (Dorella *et al.*, 2006a; Fontaine & Baird, 2008; Jesse *et al.*, 2011). Ruptured abscesses are the main source of *C. pseudotuberculosis* contaminating the environment. Animal exposure to the bacteria can be either by direct physical contact with the infected animal or indirectly via contaminated fomite (Stoops *et al.*, 1984; Collet *et al.*, 1994; Jesse *et al.*, 2008a).

Corynebacterineae is a suborder of pathogenic bacteria species that have cell membrane covered with waxy substances known as mycolic acids (MAs) such as *Corynebacterium diphtheria* and *Corynebacterium pseudotuberculosis*. Mycolic acids are the main and specific constituents of the Bacteria cell envelope. It is a long chain of fatty acids with a complex, structural design that ensures the impermeability of these bacteria cell membrane (Damien *et al.*, 2004; Jesse *et al.*, 2013a). Mycolic acids, a 2-alkyl, 3-hydroxy long-chain fatty acids, are part of the cell envelope of *Mycobacterium tuberculosis* (Daffé & Draper, 1997). MAs are found extractable using organic solvents or terminal esterification of the penta-arabinofuranosyl units of arabinogalactan (McNeil *et al.*, 1991).

*Corynebacterium* strains have been reported to be viable in the absence of MAs (Portevin *et al.*, 2004; Portevin *et al.*, 2005). It has been proven that the outer membrane was no longer observed in other mutant strains devoid of MAs (Zuber *et al.*, 2008). However there is still lack of information on the mechanism of action and host cell responses towards MAs (Carne, 1939; Onon, 1979). Therefore, this study was conducted to fill in the gap of the research of MAs in *C. pseudotuberculosis*.

It was clinically observed in *C. pseudotuberculosis* that a typical of CLA disease appearance is seen with a short incubation period of about two weeks. But the phospholipase D (PLD) inoculation showed little or no clinical signs which did not lead to any form of abscesses formation. PLD plays an important role in CLA development but it has a weaker triggering ability (Mahmood *et al.*, 2015).
The effects of *C. pseudotuberculosis* and PLD on the reproductive performances of non-pregnant does is very paramount. PLD and *C. pseudotuberculosis* causes infertility by its pathologic effects on the reproductive organs, coupled with the imbalances observed in hormonal levels which decreases and hence impede reproductive efficiency (Khuder et al., 2012; Othman et al., 2016). The changes in hormonal activities involves a sharp rise in the oestrogen and progesterone levels which is the main reason why it sequels to infertility as it impairs ovulation and subsequent implantation (Othman et al., 2016). A lot of attempt were made to study the effects of *C. pseudotuberculosis*, PLD and MAs on the reproductive performances in doe, but information at hand only gives insights on how *C. pseudotuberculosis* and PLD affects reproduction. Therefore, there is a paucity of information on the effects of mycolic acid on the reproductive performances in does.

The Acute-phase proteins (APPs) are synthetic proteins from the liver which either rise or decreases in plasma concentration during infections (Ceciliani et al., 2002; Murata et al., 2004). The APPs are basically summoned during tissue injury, trauma, infections and inflammatory conditions (Cray et al., 2009; Abdullah et al., 2013b). Haptoglobin and serum amyloid A are basic positive APPs of interest in this infection, whereby Zaid et al., (2016) reported that haptoglobin (Hp) increases in PLD and *C. pseudotuberculosis* infections which is an indication that *C. pseudotuberculosis* and PLD affects the titre of concentration of APPs in these infections. The researchers further indicated that the level of SAA is less elevated as compared to Hp, therefore, SAA is suggested to be less influenced by *C. pseudotuberculosis* and PLD. As stated above, a lot of attempts were made to study the effects of PLD, *C. pseudotuberculosis* and Mycolic acid on the concentrations of APPs in doe, but information at hand just as obtained for the reproductive functions only gives insights on how *C. pseudotuberculosis* and PLD affects serum Hp and SAA. Currently, there is no information available on the effects of mycolic acid on the concentrations of APPs in does.

The immunity to *C. pseudotuberculosis* has been described to be complex involving both cellular and humoral responses (Ellis et al., 1991) with the cellular phase response, of mainly Th1 type, having more response than the humoral response in sheep and goats (Pepin et al., 1997; Lan et al., 1999). Cytokines involved in CLA infections are mainly associated with interleukin-1β and interleukin-6, and tumor necrosis factor-α (TNF-α) (Pepin et al., 1997; Jesse et al., 2016).

In *C. pseudotuberculosis*, there is a significant stimulatory changes in the levels of cytokines mainly IL 1 β and IL 6. The cytokine IL 1 β concentration rises in *C. pseudotuberculosis* indicating that it infection actually occurs. Also, IL 6 was reported to have increased in its concentration in an in vivo study thereby indicating a chronic form of the infection. This information of the effects of this infection on cytokine concentration is only in relation to *C. pseudotuberculosis*. Hence, there is, inadequate or rather lack of viable information on the effects of PLD and mycolic acid on cytokine in CLA.
In *C. pseudotuberculosis*, abscess was formed in kidneys, liver, spleen, tongue, eyes, diaphragm, heart, mammary glands, testes, joints and other nervous tissues (de Sá Guimarães *et al.*, 2011). In ewes, neonatal infection, still-birth and in a worst case scenario abortion are a common occurrence in this infection (Alonso *et al.*, 1992). In PLD, congestion, oedema, thrombosis, necrosis are seen as a result of the generalized toxemia. Also, degenerative changes, neutrophilic infiltration, macrophagic inclusions and necrosis are seen in the ovaries and uterus (Khuder *et al.*, 2012). The histologic trend indicates that the foremost inflammatory alterations in lymph nodes of sheep and goats contain numerous neutrophilic and eosinophilic transmigration and micro-abscesses, the myeloperoxidase in the primary granules of the granulated leukocytes gives the pyogenic greenish pus colouration (Valli, 1993). Abscesses augment and coalesce constantly during inflammatory responses related to innate immune phase (Pepin *et al.*, 1994a).

The histopathological effects of MAs in this infection had not been widely studied and understood. However, a singular study in North Korea in 2014 indicated that MAs has immunogenic effects on a mice model (Kim *et al.*, 2014), which suppressed bronchoalveolar inflammation and pulmonary eosinophilic inflammation. An anti-CD25mAb treatment with MAs in this mouse model depleted CD4⁺CD25⁺Foxp3⁺ T cells in the spleen (Kim *et al.*, 2014). However, on the other hand, there is no record showing any evidence of MAs effect on histopathological findings as well as clinical signs in this disease condition. Therefore there is a gap of information on clinical signs as it relates to MAs.

**Problem statement**

Caseous lymphadenitis (CLA) is currently considered untreatable disease because of its encapsulated nature. It also has insidious effects on productivity of the small ruminant. Additionally, little is known about the host cell response of mycolic acid extract from *Corynebacterium pseudotuberculosis* and its role in pathogenicity and disease pathogenesis.

**Hypothesis**

- *Corynebacterium pseudotuberculosis* Mycolic acid’s has immunogenic properties and a key role in host cell response that eventually contributes to the pathogenicity of *Corynebacterium pseudotuberculosis*.
- The host cell response induced by Mycolic acid has direct and/or indirect effects on female goat fertility.
- Reproductive hormones concentration altered with Mycolic acid immunogenic properties.
- Pathological changes has to appear as lesion in key reproductive, vital organs and lymph nodes.
Thus, the objectives of this study are:

1. To determine changes in clinical response and complete blood count (CBC) in goats after inoculation of *C. pseudotuberculosis* and its immunogen mycolic acid extract.
2. To estimate the acute phase proteins responses and the hormones concentration estrogen and progesterone between infection by *C. pseudotuberculosis* and Mycolic acid extract as immunogen.
3. To estimate the concentrations of antibodies (IgM, IgG) and pro-inflammatory cytokines between the infection by *C. pseudotuberculosis* and its immunogen Mycolic acid extract.
4. To identify *C. pseudotuberculosis* using PCR and histopathological changes of vital organs, reproductive organs, and lymph nodes between the infected goats with *C. pseudotuberculosis* and its immunogen Mycolic acid extract.
REFERENCES


of caseous lymphadenitis in sheep. *BMC veterinary research, 9*(1), 254.


Microbiol, 45(9), 2761-2764.


susceptibility to 39 antimicrobial agents. *Veterinary microbiology*, 27(2), 145-150.


Klein, M. B., Miller, J. S., Nelson, C. M., & Goodman, J. L. (1997). Primary bone marrow progenitors of both granulocytic and monocytic lineages are susceptible to infection with the agent of human granulocytic ehrlichiosis. Journal of Infectious Diseases, 176(5), 1405-1409.


pseudotuberculosis infection in mice. Microbiology and immunology, 43(12), 1103-1106.


McFarland, J. (1907). The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. Journal of the American Medical Association, 49(14), 1176-1178.

McKean, S. C., Davies, J. K., & Moore, R. J. (2007). Expression of phospholipase D, the major virulence factor of Corynebacterium pseudotuberculosis, is regulated by multiple environmental factors and plays a role in macrophage death. Microbiology, 153(7), 2203-2211.


126(1), 131-141.


strain isolated from a cow in Israel with bovine mastitis. *Journal of bacteriology, 193*(1), 323-324.


dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. *Blood, 115*(8), 1519-1529.


