PREVALENCE AND MOLECULAR PATHOGENIC MARKERS OF MYCOPLASMA GALLISEPTICUM INFECTION IN COMMERCIAL CHICKENS AND PROGENIES

ZAHRAA FAISAL AHMED

FPV 2011 37
PREVALENCE AND MOLECULAR PATHOGENIC MARKERS OF MYCOPLASMA GALLISEPTICUM INFECTION IN COMMERCIAL CHICKENS AND PROGENIES

BY

ZAHRAA FAISAL AHMED

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

September 2011
This thesis is dedicated to my father, mother, and sisters for their patience, support and encouragements in completion of this study
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

PREVALENCE AND MOLECULAR PATHOGENIC MARKERS OF MYCOPLASMA GALLISEPTICUM INFECTION IN COMMERCIAL CHICKENS AND PROGENIES

BY

ZAHRAA FAISAL AHMED

September 2011

Chairman: Professor Datin Paduka Aini Ideris, PhD
Faculty: Faculty of Veterinary Medicine

Mycoplasma gallisepticum (MG) causes chronic respiratory disease and the infection is very costly to the poultry industry. There are few published data on avian mycoplasmosis and there is no report on molecular pathogenicity of MG infection in Malaysia. Therefore, this study was carried out to determine the prevalence of MG, and the molecular pathogenic markers of MG infection in the commercial chickens and their progenies (pipped embryos, normal chicks and poor quality chicks), in order to understand the molecular level of pathogenicity. The prevalence of MG infection in chickens was determined in selected commercial farms (breeder, broiler and layer) and the progenies [pipped embryos (PE), day old poor quality chicks (PQC) and normal chicks (NC)]. All samples were obtained from farms in Peninsular Malaysia. A total of 3056 swab samples were collected of which 1243 are from pipped embryos, 248 from day-old poor quality chicks, 340 from day-old normal chicks and 1225 from adult commercial chickens. Conventional polymerase chain
reaction (PCR) test was performed using specific gene target sequence and encoding the surface protein for detection of MG directly from the clinical samples without prior isolation of the target MG. The primer used was designed to bind to the Adherence protein A gene (gapA) and amplify a 505 bp DNA fragment.

In this study, 571 positive samples of MG out of 3056 samples with overall prevalence of 18.68% were detected from different progenies and adult commercial chickens. The total prevalence rates were 13.7 % in the pipped embryos, 16.9 % in the poor quality chicks, 12.6% in the normal chicks, and 25.8% in the adult commercial chickens. This study shows the high prevalence of MG infection through vertical and horizontal transmission from many geographically distinct areas of the country, although these farms have vaccination and treatment history. The present study demonstrated that the control of MG was not successful, despite the use of live and/or killed MG vaccines, an extensive medication program and strict biosecurity.

These positive MG samples were used for molecular characterization by amplification of selected gene target specific sequences to MG, hemagglutinin protein A gene (pMGA) and Phase-variable putative adhesin protein A gene (pvpA), using conventional PCR of published sequence specific primers. These two genes, pMGA and pvpA genes have gene size polymorphism on specific target sequence. The PCR results demonstrated, a total of 281 MG positive field samples out of 571 MG samples were detected with the primer targeted pMGA gene and a total of 188 MG positive field samples out of 571 MG samples were detected with the primer targeted pvpA gene. Similar and identical banding patterns were observed among MG positive samples obtained from progenies, however there was a variable on the
banding pattern among MG positive samples obtained from adult commercial chickens using the agarose gel electrophoresis. The sequencing and phylogenetic analysis results of MG based on selected genes targeted specific sequences were obtained using Bioinformatics software (Bioedit and MEGA 4. software).

The characteristics of the positive MG field positive samples were determined. The genetic diversity of the \textit{pMGA} and \textit{pvpA} genes of MG positive samples originated from adult commercial chickens and progenies were investigated. In the present study, we evaluated the genetic variability of 77 field positive samples of MG using the \textit{pMGA} gene and 49 field positive samples of MG using the \textit{pvpA} gene, detected in progenies and adult commercial chickens and compared them to the reference and vaccine strains of MG obtained in this study. Genetic variation patterns were evaluated by partial nucleotide sequencing of the \textit{pMGA} and \textit{pvpA} genes, which encode putative cytadhesion proteins. The gene size variation patterns of the \textit{pMGA} and \textit{pvpA} genes among MG field positive samples shared identical gene size variation patterns with the pathogenic reference and vaccine strains, that is, an insertion bp fragments by using the \textit{pMGA} gene primer set and a deletion bp fragments by using the \textit{pvpA} gene primer set. Therefore, it showed that there was identical genes size variation patterns of the MG positive samples with the pathogenic reference and vaccine strains which are pathogenic by nature and can be transmitted vertically. However, the gene size variation patterns are quite different from the variation pattern of the less pathogenic vaccine strain that cannot be transmitted vertically.
This study concluded the identification of two amplification based genetic markers that highly correlate with the existing pathogenicity studies of MG infection. It also proved the importance of these two primer sets and showed that the primer of \textit{pMGA} gene might be considered as a vertical genetic marker, and the gene size polymorphism patterns by both of selected primer sets of the \textit{pMGA} and \textit{pvpA} genes might be considered as potential pathogenic molecular markers. The present study proved the ability of both selected primer sets of the \textit{pMGA} and \textit{pvpA} genes in differentiating avirulent ts11 strain and virulent reference strains. Therefore both these selected primer sets are good pathogenic markers of MG that can be used to differentiate whether the MG field strains are pathogenic or less pathogenic.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PREVALENS DAN PETANDA MOLEKUL PATOGEN BAGI INFEKSI MYCOPLASMA GALLISEPTICUM PADA AYAM KOMERSIAL DAN PROGENI

Oleh

ZAHRAA FAISAL AHMED

September 2011

Pengerusi:   Profesor Datin Paduka Aini Ideris, Phd
Fakulti:  Fakulti Perubatan Veterinar

Mycoplasma gallisepticum (MG) menyebabkan penyakit pernafasan kronik dan jangkitan ini melibatkan kos yang tinggi kepada industri poltri. Hanya ada beberapa data yang diterbitkan pagi “mycoplasmosis” unggas dan tidak ada laporan mengenai patogenisiti molekul jangkitan MG di Malaysia. Oleh kerana itu, kajian ini dilakukan untuk menentukan prevalens MG, dan petanda molekul patogen bagi jangkitan MG pada ayam komersial dan progeni mereka (embrio “pipped”, anak ayam normal dan anak ayam yang rendah kualitinya), untuk memahami patogenisiti pada tahap molekul. Prevalens jangkitan MG pada ayam ditentukan, di ladang komersial yang dipilih (ayam pembiakbaka, ayam pedaging dan petelur) dan progeni [embrio “pipped” (PE), anak ayam berumor satu hari yang rendah kualitinya (PQC) dan anak ayam normal (NC)]. Semua sampel diperolehi dari ladang di Semenanjung Malaysia. Sejumlah 3056 swab sampel telah dikumpulkan dimana 1243 daripada nya ialah embrio “pipped”, 248 daripada anak-anak ayam berumor satu hari dan mempunyai
kualiti rendah, 340 dari anak-anak ayam normal dan 1225 daripada ayam dewasa komersial. Ujian rantaian reaksi polimerase konvensional (PCR) dilakukan dengan menggunakan gen urutan target khusus dan mengekod protein permukaan untuk mengesan MG secara langsung dari sampel klinikal, tanpa mengisolat sasaran MG terlebih dahulu. Primer yang digunakan direka bentuk untuk mengikat protein “Adherence” A (gapA) dan mengembangkan 505 bp fragmen DNA.

Dalam kajian ini, 571 sampel positif MG daripada 3056 sampel dengan prevalens keseluruhan 18.68% dikesan dari progeni berbeza dan ayam dewasa komersial. Kadar prevalens adalah 13.7% dalam embrio “pipped”, 16.9% pada anak ayam berkualiti rendah, 12.6% pada anak ayam normal, dan 25.8% dalam ayam dewasa komersial. Kajian ini menunjukkan prevalens jangkitan MG yang tinggi melalui sebaran menegak dan mendatar, dari banyak kawasan geografi yang berbeza di negara ini, walaupun ladang-ladang ini mempunyai sejarah vaksinasi dan rawatan. Kajian ini menunjukkan bahawa kawalan MG tidak berjaya, walaupun penggunaan vaksin MG hidup dan / atau mati, program ubatan yang ekstensif dan biosekuriti yang ketat.

Sampel MG yang positif digunakan bagi pencirian molekul dengan cara amplifikasi sekuens khusus target gen yang dipilih untuk MG, gen protein A hemaglutinin (pMGA) dan “phase variable putative adhesion gen protein A” (pvpA), melalui PCR konvensional dengan menggunakan primer urutan spesifik yang telah diterbitkan. Kedua-dua gen, pMGA dan pvpA, mempunyai saiz polimorfisme pada urutan target khusus. Keputusan PCR menunjukkan, jumlah keseluruhan 281 MG sampel lapangan positif dari 571 sampel MG yang dikesan dengan menggunakan target gen
primer $pMGA$ dan jumlah 188 MG sampel lapangan positif dari 571 sampel MG yang dikesan dengan target gen primer $pvpA$. Paten band yang serupa dan identikal dilihat antara MG sampel positif yang diperolehi daripada proeni, namun ada pembolehduah pada paten band antara MG sampel positif yang diperolehi dari ayam dewasa komersial dengan menggunakan elektroforesis gel agaros. Urutan dan hasil analisis filogenik MG berdasarkan gen pilihan sasaran urutan khusus telah diperolehi dengan menggunakan perisian bioinformatik (Perisian Bioedit dan MEGA 4.).

ACKNOWLEDGEMENTS

First, I would like to thank our Almighty Allah for his generosity, kindness and mercy and for supporting me throughout my study.

Next, I would like to appreciate my kind supervisor, Professor Datin Paduka Dr. Aini Ideris, for her patience and understanding during my study. Definitely, her support and limitless assistance were of great help to me.

Many thanks and appreciations are also accorded to my supervisory committee members, Prof Dr. Abdul Rahman Omar and Prof Dr. Mohd Hair Bejo, for their precious suggestions and assistance during this study. My thanks also go to Dr. Goh Yong Meng, Dr. Jalila Abu and Prof Dr. Abdul Kareem Al Jashamy for their precious assistance and useful advices.

I am very grateful to my senior, Dr. Tan Ching Giap, for his support, advice, encouragement, time and guidance throughout this project. My sincere thanks to him. The study would not have been possible without his generosity and cooperation. My thanks also go to everyone who has helped me during this study.

My appreciation is also extended to the entire postgraduate students in Biologics Laboratory, Faculty of Veterinary Medicine, UPM for sharing their technical knowledge and advices, towards the completion of my study, as well as their patience and tolerance. Last but not least, I would like to thank all individuals who were directly or indirectly involved in this project.
Lastly, my heartfelt appreciation goes to my wonderful parents, sisters, friends for their ethical encouragement and understanding during my study.

This work was conducted under the financial support of Universiti Putra Malaysia and Ministry of Science, Technology and Innovation (MOSTI), Malaysia, project number 02- 01- 04- SF0370.
I certify that a Thesis Examination Committee has met on 26 September 2011 to conduct the final examination of Zahraa F. A. AL-Barghash on her thesis entitled "Prevalence and Molecular Pathogenic Markers of *Mycoplasma gallisepticum* Infection in Commercial Chickens and Progenies" in accordance with the Universities and University College 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 Master of Science.

Members of the Thesis Examination Committee were as follows:

**Saleha binti Abdul Aziz, PhD**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Jalila binti Abu, PhD**  
Senior Lecturer  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Internal Examiner)

**Abdul Rahim bin Abdul Mutalib, PhD**  
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Internal Examiner)

**Zaini Mohd Zain, PhD**  
Associate Professor  
Faculty of Medicine  
Universiti Teknologi MARA  
(External Examiner)

**SEOW HENG FONG, PhD**  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 22 November 2011
This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

**Aini Ideris, PhD**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Abdul Rahman bin Omar, PhD**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Mohd. Hair Bejo, PhD**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

---

**BUJANG BIN KIM HUAT, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:
DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

ZAHRAA FAISAL AHMED

Date: 26 September 2011
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>xi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xiii</td>
</tr>
<tr>
<td>DECLARATION FORM</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xviii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xix</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xx</td>
</tr>
</tbody>
</table>

## CHAPTER

### I INTRODUCTION

### II LITRATURE REVIEW

2.1 Mycoplasmosis in Chickens 6

2.2 The Organism 9

2.3 Biochemical Properties 9

2.4 Intra-species Heterogeneity in MG 10

2.5 Pathogenesis and Epizootiology 10

2.5.1 Natural and Experimental Hosts 10

2.5.2 Transmission and Predisposing Factors 11

2.5.3 Significant Impact 12

2.5.4 Influence of Concurrent Respiratory Infections 13

2.5.5 Clinical Signs 13

2.5.6 Immunity 14

2.5.7 The Pathogenicity of *Mycoplasma gallisepticum* strains 15

2.5.8 *Mycoplasma gallisepticum* pathogenesis 16

2.6 The Genetic Characterization and the Antigenic Variation of MG 16

2.7 Prevalence of *M. gallisepticum* in Chickens 20

2.8 Diagnosis of MG Infection 21

2.8.1 Isolation and Identification of Causative Agent 21

2.8.2 The Molecular Detection Methods 22

2.8.2 Serological Methods 24

2.9 Treatment, Prevention and Control of MG 25

2.10 *Mycoplasma gallisepticum* Vaccines 27

### III MOLECULAR DETECTION AND PREVALENCE OF *

*MYCOPLASMA GALLISEPTICUM* USING POLYMERASE CHAIN REACTION METHOD 29

3.1 Introduction 29

3.2 Materials and methods 32

3.2.1 Sample Size 32
3.2.2 Samples from Progenies [pipped embryo (PE), day old poor quality chicks (PQC) and day old normal chicks (NC)]

3.2.3 Samples from Adult Commercial Chickens (Breeder, Broiler and Layer)

3.2.4 DNA Extraction

3.2.5 Conventional Polymerase Chain Reaction (PCR)

3.2.6 Agarose Gel Electrophoresis

3.3 Results

3.4 Discussion and Conclusions

IV MOLECULAR IDENTIFICATION OF TWO GENETIC MARKERS THAT DISTINGUISH BETWEEN PATHOGENIC AND NON PATHOGENIC STRAINS OF MYCOPLASMA GALLISEPTICUM

4.1 Introduction

4.2 Materials and methods

4.2.1 Polymerase Chain Reaction Targeting the pmGA and ppvA genes

4.2.2 Gel Electrophoresis

4.2.3 DNA Purification

4.2.4 Gene Sequencing and Data Analysis

4.3 Results

4.4 Discussion and Conclusions

V SUMMARY, CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

REFERENCES

APPENDICES

BIODATA OF STUDENT

LIST OF PUBLICATIONS
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Characteristics of avian mycoplasmas</td>
</tr>
<tr>
<td>3.1</td>
<td>Pipped embryos samples</td>
</tr>
<tr>
<td>3.2</td>
<td>Day old poor quality chicks samples</td>
</tr>
<tr>
<td>3.3</td>
<td>Day old normal chicks samples</td>
</tr>
<tr>
<td>3.4</td>
<td>Commercial chicken samples</td>
</tr>
<tr>
<td>3.5</td>
<td>The nucleotide sequences of primer used in this study</td>
</tr>
<tr>
<td>3.6</td>
<td>Reagents used in conventional PCR master mixture reaction</td>
</tr>
<tr>
<td>3.7</td>
<td>Numbers and percentages of pipped embryos from various farms with positive MG by PCR</td>
</tr>
<tr>
<td>3.8</td>
<td>Numbers and percentages of day old poor quality chicks from various farms with positive MG by PCR</td>
</tr>
<tr>
<td>3.9</td>
<td>Numbers and percentages of day old normal chicks from various farms with positive MG by PCR</td>
</tr>
<tr>
<td>3.10</td>
<td>Numbers and percentages of commercial chicken samples from various farms with positive MG by PCR</td>
</tr>
<tr>
<td>3.11</td>
<td>Numbers and percentages of pipped embryos, poor quality and normal chicks from various farms with positive MG by PCR</td>
</tr>
<tr>
<td>4.1</td>
<td>Product sizes and sequence positions for primers used for MG characterization</td>
</tr>
<tr>
<td>4.2</td>
<td>Reagents used in the PCR master mixture reaction</td>
</tr>
<tr>
<td>4.3</td>
<td>The differences of the nucleotides on the gene size of pMGA gene for the MG positive samples from the progenies comparing with MGS6 reference and ts11 vaccine strains</td>
</tr>
<tr>
<td>4.4</td>
<td>The differences of the nucleotides on the gene size of pMGA gene for the MG positive samples from the commercial chickens comparing with MGS6 reference and ts11 vaccine strains</td>
</tr>
<tr>
<td>4.5</td>
<td>The differences of the nucleotides on the gene size of pypA gene for the MG positive samples from the commercial chickens comparing with MGS6 reference and ts11 vaccine strains</td>
</tr>
<tr>
<td>4.6</td>
<td>The differences of the nucleotides on the gene size of pypA gene for the MG positive samples from the progenies comparing with MGS6 reference and ts11 vaccine strains</td>
</tr>
<tr>
<td>4.7</td>
<td>The difference in nucleotides sequences of the pMGA and pypA genes among vaccine and reference strains</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>PCR product of 505bp of positive MG from pipped embryo (PE) samples amplified using the GAP A primer set</td>
<td>38</td>
</tr>
<tr>
<td>3.2</td>
<td>PCR product of 505bp of positive MG from poor quality chicks (PQC) samples amplified using the GAP A primer set</td>
<td>39</td>
</tr>
<tr>
<td>3.3</td>
<td>PCR product of 505bp of positive MG from normal chicks (NC) samples amplified using the GAP A primer set</td>
<td>40</td>
</tr>
<tr>
<td>3.4</td>
<td>PCR product of 505bp of positive MG from commercial chicken samples (layer and broiler farms) amplified using the GAP A primer set</td>
<td>41</td>
</tr>
<tr>
<td>4.1</td>
<td>PCR product of ~329bp of MG positive samples from progenies amplified using the AU-AT TS11 F + R primer set</td>
<td>59</td>
</tr>
<tr>
<td>4.2</td>
<td>PCR product of ~329bp of MG positive samples from progenies amplified using the AU-AT TS11 F + R primer set</td>
<td>60</td>
</tr>
<tr>
<td>4.3</td>
<td>PCR product of ~329bp of MG positive sample from adult commercial chickens, reference and vaccine strains amplified using the AU-AT TS11 F + R primer set</td>
<td>61</td>
</tr>
<tr>
<td>4.4</td>
<td>PCR product of ~702bp of MG positive samples from progenies amplified using the pvpA 1F + 2R primer set</td>
<td>62</td>
</tr>
<tr>
<td>4.5</td>
<td>PCR product of ~702bp of MG positive samples from adult commercial chickens, reference and vaccine strains amplified using the pvpA 1F + 2R primer set</td>
<td>63</td>
</tr>
<tr>
<td>4.6</td>
<td>PCR product of ~702bp of MG positive samples from adult commercial chickens, reference and vaccine strains amplified using the pvpA 1F + 2R primer set</td>
<td>64</td>
</tr>
<tr>
<td>4.7</td>
<td>Nucleotide sequences alignment of the pMGA gene from vaccine, reference and MG field isolates</td>
<td>71</td>
</tr>
<tr>
<td>4.8</td>
<td>Nucleotide sequences alignment of the pvpA gene from vaccine, reference and MG field isolates</td>
<td>72</td>
</tr>
<tr>
<td>4.9</td>
<td>The phylogenetic tree for the reference strains, vaccine strains and the positive MG samples from the progenies based on the partial pMGA gene sequence constructed using MEGA version 4 software</td>
<td>73</td>
</tr>
<tr>
<td>4.10</td>
<td>The phylogenetic tree for the reference strains, vaccine strains and the positive MG samples from the adult commercial chickens based on the partial pMGA gene sequence constructed using MEGA version 4 software</td>
<td>74</td>
</tr>
<tr>
<td>4.11</td>
<td>The phylogenetic tree for the reference strains, vaccine strains and the positive MG samples from the progenies based on the partial pvpA gene sequence constructed using MEGA version 4 software</td>
<td>75</td>
</tr>
<tr>
<td>4.12</td>
<td>The phylogenetic tree for the reference strains, vaccine strains and the positive MG samples from the adult commercial chickens based on the partial pvpA gene sequence constructed using MEGA version 4 software</td>
<td>76</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

%  Percentage
AFLP  Amplified fragment length polymorphism
bp  Base pair
℃  Degree in Celsius
CO₂  Carbon dioxide
CRD  Chronic respiratory disease
CCRD  Complicated chronic respiratory disease
DNA  Deoxyribonucleic acid
dNTPs  Deoxynucleotide triphosphate
E. coli  *Escherichia coli*
EDTA  Ethylene diamine tetra acetic acid
ELISA  Enzyme linked immunosorbent assay
gapA  Adherence protein A
GTS  Gene-targeted sequencing
HI  Hemagglutination inhibition
IBV  Infectious bronchitis virus
IFA  Immunofluorescence antibody
IGSR  16S-23S rRNA intergenic spacer region sequencing
Kbp  kilobase pairs
KDa  kilo Daltons
LP  Surface lipoprotein
MI  *Mycoplasma iowae*
MG  *Mycoplasma gallisepticum*
mg  Milligram
mgc2  Cytadhesion membrane protein
ml  milliliter
MM  *Mycoplasma meleagrisidis*
mm  Millimeter
mM  Milli molar
MS  *Mycoplasma synoviae*
NC  Normal chick
NDV  Newcastle disease virus
ng  Nanogram
nm  Nanometer
PBS  Phosphate buffered saline
PCR  Polymerase chain reaction
PCR-RFLP  PCR based restriction fragment length polymorphism
PE  Pipped embryo
pMGA  Hemagglutinin protein
pmole  Picomole
PPLO  Pleuropneumonia like organism
PQC  Poor quality chick
pvpA  Phase-variable putative adhesin protein
RAPD  Random amplified polymorphic DNA
REA  Restriction endonuclease analysis
RFLP  Restriction fragment length polymorphism
RNA  Ribonucleic acid
rpm  Radius per minute
rRNA  Ribosomal ribonucleic acid
SDS  Sodium dodecyl sulphate
SPA  Serum plate agglutination
TAE  Tris-acetate EDTA
USA  United States of America
UV  Ultraviolet
μg  Microgram
μl  Microlitre
μm  Micro-meter
VTP  Vertical transmission progeny
CHAPTER I
INTRODUCTION

*Mycoplasma gallisepticum* (MG) is one of the important pathogens and the infection has a high prevalence causing major economic losses to the poultry industry. Therefore MG is considered as one of the costly diseases for the poultry industry worldwide. *Mycoplasma gallisepticum* caused complicated chronic respiratory disease (CCRD) of chickens when there are multiple infections with *E. coli* and respiratory viral infections such as Newcastle disease virus and infectious bronchitis virus (Ley and Yoder, 1997; Ley, 2003; 2008).

The horizontal transmission of MG infection occurs in poultry flocks and consequently the breeder progeny flocks become infected by the vertical transmission (Bradbury, 2005). The vaccination program was practiced in some countries for controlling the spread of the infection but it has proven to be ineffective at clearing MG infection from the breeder flocks (Ley, 2003). MG control program should be based on the elimination of the organism from the primary breeder flocks and on the maintenance of Mycoplasma free conditions in the breeders and breeder progeny flocks using premises biosecurity (Kleven, 2008).

The detection by culture for isolation and identification are complicated because MG is identified as a fastidious bacteria (Ley, 2003). Usually the serological methods are used for the MG infection diagnosis, however the non specific reactions limit these methods effectiveness (Avakian et al., 1988; Levisohn and Kleven, 2000; Hess et al., 2007). Additionally the antigenic variation (Bencina et al., 1988a, b; Garcia et al.,
1994; Ferraz and Danelli, 2003) and the interspecies cross reactivity (Yogevev et al., 1989) may cause the delay in MG diagnosis.

The rapid and appropriate differentiation of MG strains are essential for the epidemiological study of the MG occurrence, to find the origin sources of the MG infection, and to design effective control measures (Ley et al., 1997a). Many techniques have been used for MG strain differentiation, which include profile analysis (Khan et al., 1987; John et al., 2006), restriction fragment length polymorphism (RFLP) (Kleven et al., 1988a; Hong et al., 2005), PCR with strain specific primers (Nascimento et al., 1993; Feberwee et al., 2006, Fan et al., 1995b), gene-targeted sequencing (GTS) (Ferguson et al., 2005) and random amplification of polymorphic DNA (RAPD) (Geary et al., 1994; Fan et al., 1995a; Rawadi, 1998; Charlton et al., 1999a, b). Also the RAPD method has been used effectively for the identification of MG vaccine strains in field and experimental conditions (Ley et al., 1997a; Kleven et al., 1998; Turner and Kleven, 1998; Kleven et al., 2004), as well as for tracking epidemiologically related isolates in the field (Charlton et al., 1999a, b; Levisohn and Kleven, 2000; Ferguson et al., 2005). However, there was difficulty in standardizing protocols among the laboratories. Therefore, the RAPD test was not permitted for long term epidemiological investigations or inter laboratory comparisons.

Most of the investigations have switched to the molecular techniques as the basis of MG identification. Sequencing methods have been developed as an approach for molecular biology of MG and the complete genome sequence availability has driven the idea to estimate the gene target sequencing as a typing tool for differentiating
MG strains (Raviv et al., 2007). With reference to Papazisi et al. (2003), the authors completed sequencing of the genome of MG. It was also mentioned that some of the genes are conserved while others are not. Such information provides an added advantage during differentiation, particularly when the PCR assay is being investigated.

Significant attempt has been made to recognize MG antigens, especially the cytadhesion properties antigens, which may play key roles in the pathogenesis and immune response to infection. Two of MG gene families, pMGA and pvpA genes, have been described and these genes encode major surface proteins with pathogenic, antigenic and immune evasion properties (Boguslavsky et al., 2000; Evans et al., 2005). The expression of pMGA and pvpA genes and the antigenic variation, major immunogenic surface proteins, were correlated with the response of antibody in vivo studies, suggesting that modulation of the immune system may have important role in producing the surface diversity (Levisohn et al., 1995; Bencina, 2002; Papazisi et al., 2003; Razin, 2006).

Avian mycoplasma serotypes were found to differ in their potential for producing embryo mortality, with most strains of MG being pathogenic for chicken embryos (Levisohn et al., 1985). Pathogenic MG strains cause high embryo mortality, but it may be possible that the in ova virulence was enhanced by egg adaptation (Levisohn et al., 1986). Studies showed that MG strains varied in their in ovo pathogenicity and there was no correlation between in ovo pathogenicity and in vivo or in vitro methods for pathogenicity evaluation.
The concern in many poultry farms in Malaysia is that most of the farms practice protective measures including vaccination and treatment, to control the epidemic MG infection. The investigation on the diagnosis of MG infection is inadequate, thus reliable tests for MG detection and strain differentiation might help in understanding the occurrence and spread of the infection since they produce the essential information to recognize and identify new MG outbreaks. Due to lack of studies and understanding of the molecular pathogenicity of MG infection, it is therefore crucial to determine their molecular level of pathogenicity using commercial birds and progenies. Therefore, the correlation of the molecular finding towards existing pathogenicity study of the MG strains may be carried out to detect and identify the potential pathogenic molecular marker. The combination of the gene size polymorphism in \textit{pvpA} and \textit{pMGA} genes act as potential pathogenic molecular marker of MG infection.

The hypothesis of this study was that MG positive samples from commercial chickens under different conditions in different farms in Malaysia, have high degree of gene size polymorphism of certain genes, suggesting that some of the MG positive samples are highly pathogenic and others are less pathogenic. Therefore, the objectives of this study were to determine:

1) the prevalence of MG infection, in selected commercial farms (breeder, broiler and layer), progeny-pipped embryos (PE), day old poor quality chicks (PQC) and normal chicks (NC) obtained from Peninsular Malaysia.
2) the molecular characteristics of the local MG positive samples based on selected target genes specific sequences encode for a putative surface cytadhesion protein.

3) the phylogenetic tree of MG positive samples based on targeted specific sequences of specific selected genes.

4) the pathogenic marker that can facilitate the specific molecular detection, characterization and differentiation between the highly and the less pathogenic MG strains.
REFERENCES


Dulali, R.S. (2003). Seroprevalence and patholology of mycoplasmosis in sonali chickens. MS Thesis. Submitted to the Department of Pathology. Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.


99


