UNIVERSITI PUTRA MALAYSIA

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 phylogenic 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 *pMGA* and *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 *pMGA* gene and 49 field positive samples of MG using the *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 *pMGA* and *pvpA* genes, which encode putative cytadhesion proteins. The gene size variation patterns of the *pMGA* and *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 *pMGA* gene primer set and a deletion bp fragments by using the *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 *pMGA* gene might be considered as a vertical genetic marker, and the gene size polymorphism patterns by both of selected primer sets of the *pMGA* and *pvpA* genes might be considered as potential pathogenic molecular markers. The present study proved the ability of both selected primer sets of the *pMGA* and *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 berumur 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 progeni, namun ada pembolehubah 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.).

Ciri-ciri sampel lapangan yang positif MG telah ditentukan. Kepelbagaian genetik gen *pMGA* dan *pvpA* lapangan MG sampel positif yang berasal dari ayam dewasa komersial dan progeni dikaji. Dalam kajian ini, kami menilai kepelbagaian genetik 77 sampel lapangan positif MG menggunakan gen *pMGA* dan 49 sampel lapangan positif MG menggunakan gen *pvpA*, dikesan dari progeni dan ayam dewasa komersial, dibandingkan dengan strain rujukan serta strain vaksin MG yang diperolehi dalam kajian ini. Pola variasi genetik dinilai oleh urutan nukleotida separa dari gen *pMGA* dan *pvpA*, yang mengekod protein *cytadhesion* putatif. Pola gen variasi saiz *pMGA* dan gen *pvpA* antara MG sampel positif bersama pola saiz gen variasi identikal dengan strain rujukan patogenik dan strain vaksin, iaitu, “insertion”sebuah fragmen pb dengan menggunakan gen asas set *pMGA* dan “deletion” fragmen bp dengan menggunakan set primer gen *pvpA*. Oleh kerana itu, ianya menunjukkan bahawa ada pola variasi gen saiz identical bagi sampel positif MG dengan strain rujukan patogenik dan strain vaksin yang patogenik semula jadi dan boleh disebarkan secara menegak. Namun, pola variasi gen saiz sangat berbeza dari pola variasi strain vaksin kurang patogenik yang tidak dapat menular secara menegak.

Kajian ini mengesahkan pengenalan berdasarkan amplifikasi dua penanda genetik yang sangat berkaitan dengan kajian patogenisiti sedia ada jangkitan MG. Ianya juga membuktikan pentingnya dua set primer ini dan menunjukkan bahawa asas gen *pMGA* mungkin dianggap sebagai penanda genetik menegak, dan pola polimorfisme gen saiz kedua-dua set pilihan primer bagi gen *pMGA* dan *pvpA* mungkin dianggap sebagai potensi penanda molekul patogen. Kajian ini membuktikan kemampuan kedua-dua set primer pilihan daripada gen *pMGA* dan gen *pvpA* dalam membezakan strain tidak virulen ts11 dan strain rujukan virulen. Oleh itu, kedua-dua set primer pilihan ini adalah penanda yang baik bagi MG patogenik dan boleh digunakan untuk membezakan sama ada strain MG lapangan bersifat patogenik atau kurang patogenik.

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

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
AFLP	Amplified fragment length polymorphism
bp	Base pair
^{o}C	Degree in Celsius
CO_2	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 meleagridis*
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 (Yogev *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.*, 1999 a, 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 *pvpA* and *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

Abdelmoumen, B. B., Mohamed, R. B., Gueriri, I., Boughattas, S., and Mlik, B. (2005). Duplex PCR to differentiate between *Mycoplasma synoviae* and *Mycoplasma gallisepticum* on the basis of conserved species-specific sequences of their hemagglutinin genes. *Journal of Clinical Microbiology*, 43 (2): 948-958.

Abd-el-Motelib, T. Y., and Kleven, S. H. (1993). A comparative study of *Mycoplasma gallisepticum* vaccines in young chickens. *Avian Diseases,* 37(4): 981-987.

Abdu, P. A., Bishu, G., Adysiyun, A. A., and Adegboye, D. S. (1983). Survey for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies in chickens in Zaria, Nigeria. *Journal of Animal Production Research,* 3: 63-69.

Adler, H. E., Bryant, B. J., Cordy, D. R., Shifrine, M., and DaMassa, A. J. (1973). Immunity and mortality in chickens infected with *Mycoplasma gallisepticum*: Influence of the bursa of Fabricius. *The Journal of Infectious Diseases,* 127: 61-66.

Alls, A. A., Benton, W. J., Krauss, W. C., and Cover, M. S. (1963). The mechanics of treating hatching eggs for disease prevention. *Avian Diseases,* 7(1): 89-97.

Amin, M. M., Siddique, M.A.B., and Rahman, M.M. (1992). Investigation on chronic respiratory disease in chickens: Part-II. *BAU Research Progress* 6: 262-266.

Anderson, D. P., Wolfe, R. R., Cherms, F. L., and Roper, W. E. (1968). Influence of dust and ammonia on the development of air sac lesions in turkeys. *American Journal of Veterinary Research,* 29: 1049-1058.

Anonymous, A. (1996). National Poultry Improvement Plan and Auxiliary Provisions. *Publication APHIS 91—55—031.Animal and Plant Health Inspection Service, U.S. Department of Agriculture: Hyattsville, MD,* 59-72.

Avakian, A. P., Kleven, S. H., and Glisson, J. R. (1988). Evaluation of the specificity and sensitivity of two commercial enzyme-linked immunosorbent assay kits, the serum plate agglutination test and the hemaagglutination-inhibition test for antibodies formed in response to *Mycoplasma gallisepticum*. *Avian Diseases*, 32: 262-272.

Avakian, A. P., and Ley, D. H. (1993). Inhibition of *Mycoplasma gallisepticum* growth and attachment to chick tracheal rings by antibodies to a 64-kilodalton membrane protein of *Mycoplasma gallisepticum*. *Avian Diseases*, 37(3): 706-714.

Barbour, E. K., Newman, J. A., Sasipreeyajan, J., Caputa, A. C., and Muneer, M. A. (1989). Identification of the antigenic components of the virulent *Mycoplasma gallisepticum* (R) in chickens: their role in differentiation from the vaccine strain (F). *Veterinary Immunology and Immunopathology,* 21(2): 197-206.

Baseggio, N., Glew, M. D., Markham, P. F., Whithear, K. G., and Browning, G. F. (1996). Size and genomic location of the *pMGA* multigene family of *Mycoplasma gallisepticum. Microbiology*, 142: 1429-1435.

Bencina, D. (2002). Haemagglutinins of pathogenic avian Mycoplasma. *Avian Pathology*, 31(6): 535-547.

Bencina, D., Kleven, S. H., Elfaki, M. G., Snoj, A., Dovc, P., Dorrer, D. and Russ, I. (1994). Variable expression of epitopes on the surface of *Mycoplasma gallisepticum* demonstrated with monoclonal antibodies. *Avian Pathology,* 23(1): 19-36.

Bencina, D., and Bradbury, J. M. (1992). Combination of immunofluorescence and immunoperoxidase techniques for serotyping mixtures of Mycoplasma species. *Journal of Clinical Microbiology,* 30: 407-410.

Bencina, D., and Dorrer, D. (1984). Demonstration of *Mycoplasma gallisepticum* in tracheas of healthy carrier chickens by fluorescent-antibody procedure and the significance of certain serologic tests in estimating antibody response. *Avian Diseases,* 28(3): 574-578.

Bencina, D., Tadina, T., and Dorrer, D. (1988a). Natural infection of ducks with *Mycoplasma synoviae* and *Mycoplasma gallisepticum* and mycoplasma egg transmission. *Avian Pathology,*17(2): 441-449.

Bencina, D., Tadina, T., and Dorrer, D. (1988b). Natural infection of geese with *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and egg transmission of the Mycoplasmas. *Avian Pathology,* 17(4): 925-928.

Boguslavsky, S., Menaker, D., Lysnyansky, I., Liu, T., Levisohn, S., Rosengarten, R., García, M. and Yogev, D. (2000). Molecular characterization of the *Mycoplasma gallisepticum pvpA* gene which encodes a putative variable cytadhesin protein. *Infection and Immunity*, 68: 3956-3964.

Bozeman, L. H., Kleven, S. H., and Davis, R. B. (1984). Mycoplasma challenge studies in budgerigars (*Melopsittacus undulatus*) and chickens. *Avian Diseases*, 28: 426-434.

Bradbury, J. M. (1984). Avian mycoplasma infections: prototype of mixed infections with Mycoplasmas, bacteria and viruses. *Annales de l'* Institut Pasteur *Microbiology,* 135(A): 83-89.

Bradbury, J.M. (2001). Avian Mycoplasmosis. In: Frank Jordan et al, eds. *Poultry Diseases*. 5th ed. W.B.Saunders, 178-193.

Bradbury, J.M. (2005). Poultry Mycoplasmas: Sophisticated pathogens in simple guise. *British Poultry Science,* 46: 125 -136.

Bradley, L. D., Snyder, D. B., and Van Deusen, R. A. (1988). Identification of species-specific and interspecies-specific polypeptides of *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. *American Journal of Veterinary Research,* 49: 511-515.

Branton, S. L., and Deaton, J. W. (1985). Egg production, egg weight, eggshell strength, and mortality in three strains of commercial layers vaccinated with F strain *Mycoplasma gallisepticum*. *Avian Diseases,* 29(3): 832-837.

Branton, S. L., Lott, B. D., Deaton, J. W., Hardin, J. M., and Maslin, W. R. (1988). F strain *Mycoplasma gallisepticum* vaccination of post-production-peak commercial Leghorns and its effect on egg and eggshell quality. *Avian Diseases,* 32: 304-307.

Buim, M.R., Mettifogo, E., Timenetsky, J., Kleven, S., and Ferreira, A. J. P. (2009) Epidemiological survey on *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by multiplex PCR in commercial poultry. *Pesquisa Veterinária Brasileira*, 29(7):552-556.

Buntz, B., Bradbury, J. M., Vuillaume, A., and Rousselot-Paillet, D. (1986). Isolation of *Mycoplasma gallisepticum* from geese. *Avian Pathology,* 15(3): 615-617.

Carlile, F. S. (1984). Ammonia in poultry houses: A literature review. *World's Poultry Science Journal,* 40: 99-113.

Carpenter, T. E., Miller, K. F., Gentry, R. F., Schwartz, L. D., and Mallinson, E. T. (1979). Control of *Mycoplasma gallisepticum* in commercial laying chickens using artificial exposure to connecticut F strain *Mycoplasma gallisepticum*. *Proceedings of the United States Animal Health Association,* 83: 364-370.

Chakraborty, D., Sadhukahan, T., Guha, D., and Chatterjee, A. (2001). Seroprevalence of *Mycoplasma gallisepticum* in West Bengal. *Indian Veterinary Journal,* 78(9):855-856.

Chandiramani, N. K., Van Roekel, H., and Olesiuk, O. M. (1966). Viability studies with *Mycoplasma gallisepticum* under different environmental conditions. *Poultry Science,* 45:1029-1044.

Charlton, B. R., Bickford, A. A., Chin, R. P., and Walker, R. L. (1999a). Randomly amplified polymorphic DNA (RAPD) analysis of *Mycoplasma gallisepticum*

isolates from turkeys from the central valley of California. *Journal of Veterinary Diagnostic Investigation,* 11: 408-415.

Charlton, B. R., Bickford, A. A., Walker, R. L., and Yamamoto, R. (1999b). Complementary randomly amplified polymorphic DNA (RAPD) analysis patterns and primer sets to differentiate *Mycoplasma gallisepticum* strains. *Journal of Veterinary Diagnostic Investigation,* 11: 158-161.

Cherry, J.J., ley, D.H., and Altizer, S. (2006). Genotypic analyses of *Mycoplasma gallisepticum* isolates from songbirds by random amplification of polymorphic DNA and amplified- fragment length polymorphism. *Journal of Wildlife diseases,* 42(2): 421-428.

Chin, R. P., Daft, B. M., Meteyer, C. U., and Yamamoto, R. (1991). Meningoencephalitis in commercial meat turkeys associated with *Mycoplasma gallisepticum*. *Avian Diseases,* 35: 986-993.

Chrysostome, C., Bell, J. G., Demey, F., and Verhulst, A. (1995). Sero prevalences to three diseases in village chickens in Benin. *Preventive Veterinary Medicine,* 22(4): 257-261.

Cobb, D. T., Ley, D. H., and Doerr, P. D. (1992). Isolation of *Mycoplasma gallopavonis* from free-ranging wild turkeys in coastal North Carolina seropositive and culture-negative for *Mycoplasma gallisepticum*. *Journal of Wildlife Diseases,* 28: 105-109.

Cole, B. C., & Cassell, G. H. (1979). Mycoplasma infections as models of chronic joint inflammation. *Arthritis and Rheumatism,* 22: 1375-1381.

Collier, S. D., Pharr, G. T., Branton, S. L., Evans, J. D., Leigh, S. A., and Felfoldi, B. (2006). Initial proteomics analysis of differentially expressed proteins from *Mycoplasma gallisepticum* vaccine strains ts-11 and F detected by Western Blotting. *International Journal of Poultry Science,* 5(4): 330-336.

Cummings, T. S., & Kleven, S. H. (1986). Evaluation of protection against *Mycoplasma gallisepticum* infection in chickens vaccinated with the F strain of *Mycoplasma gallisepticum*. *Avian Diseases,* 30(1): 169-171.

Czifra, G., Kleven, S. H., Engstrom, B., and Stipkovits, L. (1995). Detection of specific antibodies directed against a consistently expressed surface antigen of *Mycoplasma gallisepticum* using a monoclonal blocking enzyme-linked immunosorbent assay. *Avian Diseases*. 39: 28-31.

Dallo, S. F., and Baseman, J. B. (1990). Cross-hybridization between the cytadhesin genes of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* and genomic DNA of *Mycoplasma gallisepticum*. *Microbial Pathogenesis,* 8(5): 371-375.

Davidson, W. R., Nettles, V. F., Couvillion, C. E., and Yoder Jr, H. W. (1982). Infectious sinusitis in wild turkeys. *Avian Diseases,* 26(2): 402-405.

Dierks, R.E., Newman, J. A., and Pomeroy, B.S. (1967). Characterization of Avian Mycoplasma. *Annals of the New York Academy of Sciences*, 143(1):170-189.

Domanska-Blicharz, K., Tomczyk, G., and Minta, Z. (2008). Comparision of different molecular methods for detection of *Mycoplasma gallisepticum. Bulletin of the Veterinary Institute in Pulawy*, 52: 529-532.

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

Dunlop, W. R., Parke, G., Strout, R. G., and Smith, S. C. (1964). The effect of sequence of infection on complex respiratory disease. *Avian Diseases,* 8(3): 321-327.

East I.J. (2008). Addressing the problems of using the polymerase chain reaction technique as a stand-alone test for detecting pathogens in aquatic animals. *Review Science and Technology. International Office of Epizootics*, 27 (3): 829-837.

Elfaki, M. G., Ware, G. O., Kleven, S. H., and Ragland, W. L. (1992). An enzyme-linked immunosorbent assay for the detection of specific IgG antibody to *Mycoplasma gallisepticum* in sera and tracheobronchial washes. *Journal of Immunoassay and Immunochemistry,* 13(1): 97-126.

Enright, M. C., and Spratt, B. G. (1999). Multilocus sequence typing. *Trends in Microbiology, 7*(12): 482-487.

Evans, J.D., Leigh, S. A., Branton, S.L., Collier, S.D., Pharr, G.T., and Bearson, S.M.D. (2005). *Mycoplasma gallisepticum*: current and developing means to control the avian pathogen. *The Journal of Applied Poultry Research*, 14(4):757-763.

Evans, J.D., and Leigh, S. A. (2008). Differentiation of *Mycoplasma gallisepticum* vaccine strains ts-11 and 6/85 from commonly used *Mycoplasma gallisepticum* challenge strains by PCR. *Avian Diseases*, 52(3): 491-497.

Evans, R. D., and Hafez, Y. S. (1992). Evaluation of a *Mycoplasma gallisepticum* strain exhibiting reduced virulence for prevention and control of poultry Mycoplasmosis. *Avian Diseases*, 36: 197-201.

Evans, R. D., Hafez, Y. S., and Schreurs, C. S. (1992). Demonstration of the genetic stability of a *Mycoplasma gallisepticum* strain following in vivo passage. *Avian Diseases,* 36(3): 554-560.

Ewing, M.L., Lauerman, L.H., Kleven, S.H. and Brown, M.B. (1996). Evaluation of diagnostic procedures to detect *Mycoplasma synoviae* in commercial multiplier-breeder farms and commerical hatcheries in Florida. *Avian Diseases*, 40: 798 - 806.

Fan, H. H., Kleven, S. H., and Jackwood, M. W. (1995a). Application of polymerase chain reaction with arbitrary primers to strain identification of *Mycoplasma gallisepticum*. *Avian Diseases,* 39(4): 729-735.

Fan, H. H., Kleven, S. H., Jackwood, M. W., Johansson, K. E., Pettersson, B., and Levisohn, S. (1995b). Species identification of avian Mycoplasmas by polymerase chain reaction and restriction fragment length polymorphism analysis. *Avian Diseases,* 39(2): 398-407.

Feberwee, A., Mekkes, D.R., De Wit, J.J., Hartman, E.G. and Pijpers, A. (2005). Comparison of culture, PCR, and different serologic tests for detection of *Mycplasma gallisepticum* and *Mycoplasma synviae* infections. *Avian Diseases*, 49: 260 -268.

Feberwee, A., W. J. M. Landman, T. von Banniseht-Wysmuller, D. Klinkenberg, J. C. M. Vernooij, A. L. J. Gielkens, and J. A. Stegeman (2006). The effect of a live vaccine on the horizontal transmission of *Mycoplasma gallisepticum*. *Avian Pathoogyl*, 35: 359–366.

Ferguson, N. M., Hepp, D., Sun, S., Ikuta, N., Levisohn, S., Kleven, S. H. and Garcia, M (2005). Use of molecular diversity of *Mycoplasma gallisepticum* by gene-targeted sequencing (GTS) and random amplified polymorphic DNA (RAPD) analysis for epidemiological studies. *Microbiology,* 151: 1883-1893.

Ferguson, N. M., Leiting, V. A. and Kleven, S. H. (2004). Safety and efficacy of the a virulent *Mycoplasma gallisepticum* strain K5054 as a live vaccine in poultry. *Avian Diseases,* 48: 91-99.

Ferraz, N. P. and Danelli, M. M. (2003). Phenotypic and antigenic variation of *Mycoplasma gallisepticum* vaccine strains. *Brazilian Journal of Microbiology,* 34: 238-241.

Frey, M. L., Hanson, R. P., and Andrson, D. P. (1968). A medium for the isolation of avian Mycoplasmas. *American Journal of Veterinary Research,* 29(11): 2163-2171.

Fritz, B. A., Thomas, C. B., and Yuill, T. M. (1992). Serological and microbial survey of *Mycoplasma gallisepticum* in wild turkeys (*Meleagris gallopavo*) from six western states. *Journal of Wildlife Diseases,* 28(1): 10-20.

Ganapathy, K., Bradbury, J.M., Tan, C.G., Mutalib, A.R. and Tan, C.T. (2001). Seroprevalence of *Mycoplasma gallisepticum* in commercial broilers and layer chickens in Malaysia. In: 2nd International Congress/13th VAM Congress and

CVA-Australia/Oceania Regional Symposium, Kuala Lumpur, 27-30 August, 2001, pp 108-109.

Garcia, M., Elfaki, M. G., and Kleven, S. H. (1994). Analysis of the variability in expression of *Mycoplasma gallisepticum* surface antigens. *Veterinary Microbiology,* 42(2-3): 147-158.

Garcia, M., Jackwood, M. W., Levisohn, S. and Kleven, S. H. (1995). Detection of *Mycoplasma gallisepticum*, *Mycoplasma synoviae* and *Mycoplasma iowae* by multispecies polymerase chain reaction and restriction fragment length polymorphism. *Avian Diseases,* 39(3): 606-616.

Gardella, R. S., R. A. Del Giudice, and J. G. Tully. (1983). Immunofluorescence. *In S. Razin and J. G. Tully (eds.). Methods in Mycoplasmology, Vol. I. Academic Press: New York,* 431- 439.

Gaunson, J. E., Philip, C. J., Whithear, K. G., and Browning, G. F. (2006). The cellular immune response in the tracheal mucosa to *Mycoplasma gallisepticum* in vaccinated and unvaccinated chickens in the acute and chronic stages of disease. *Vaccine,* 24(14): 2627-2633.

Geary, S. J., Forsyth, M. H., Saoud, S. A., Wang, G., Berg, D. E., and Berg, C. M. (1994). *Mycoplasma gallisepticum* strain differentiation by arbitrary primer PCR (RAPD) fingerprinting. *Molecular and cellular probes,* 8(4): 311-316.

Glew, M. D., Baseggio, N., Markham, P. F., Browning, G. F., and Walker, I. D. (1998). Expression of the *pMGA* genes of *Mycoplasma gallisepticum* is controlled by variation in the GAA trinucleotide repeat lengths within the 5'noncoding regions. *Infection and Immunity*, 66(12): 5833-5841.

Glew, M. D., Browning, G. F., Markham, P. F., and Walker, I. D. (2000). *pMGA* phenotypic variation in *Mycoplasma gallisepticum* occurs in vivo and is mediated by trinucleotide repeat length variation. *Infection and Immunity,* 68(10): 6027-6033.

Glew, M. D., Markham, P. F., Browning, G. F., and Walker, I. D. (1995). Expression studies on four members of the *pMGA* multigene family in *Mycoplasma gallisepticum* S6. *Microbiology,* 141: 3005-3014.

Glisson, J. R., Cheng, I. H. N., Brown, J., and Stewart, R. G. (1989). The effect of oxytetracycline on the severity of airsacculitis in chickens infected with *Mycoplasma gallisepticum*. *Avian Diseases,* 33(4): 750-752.

Glisson, J. R., and Kleven, S. H. (1984). *Mycoplasma gallisepticum* vaccination: effects on egg transmission and egg production. *Avian Diseases,* 28(2): 406- 415.

Glisson, J. R. and Kleven, S. H. (1985). *Mycoplasma gallisepticum* vaccination: further studies on egg transmission and egg production. *Avian Diseases,* 29: 408- 415.

Goh, M. S., Gorton, T. S., Forsyth, M. H., Troy, K. E., and Geary, S. J. (1998). Molecular and biochemical analysis of a 105 kDa *Mycoplasma gallisepticum* cytadhesin (GapA). *Microbiology,* 144(11): 2971-2978.

Gorton, T. S., and Geary, S. J. (1997). Antibody-mediated selection of a *Mycoplasma gallisepticum* phenotype expressing variable proteins. *FEMS Microbiology Letters,* 155(1): 31-38.

Gross, W. B. (1990). Factors affecting the development of respiratory disease complex in chickens. *Avian Diseases*, 34: 607-610.

Hall, C. F., Flowers, A. I., and Grumbles, L. C. (1963). Dipping of hatching eggs for control of *Mycoplasma gallisepticum*. *Avian Diseases,* 7(2): 178-183.

Hamdy, A. H. (1970). Therapeutic effect of lincospectin on airsacculitis in chickens. *Avian diseases*, 14: 706-714.

Hartup, B. K., Kollias, G. V., and Ley, D. H. (2000). Mycoplasmal conjunctivitis in songbirds from New York. *Journal of Wildlife Diseases,* 36(2): 257-264.

Herrmann, R. (1992). Genome structure and organization in Mycoplasma: Molecular biology and pathogenesis, R.N.M Jack Maniloff, Lloyd R. Finch, and Joel B. Baseman, Editor. American Society for Microbiology: Washington, D.C. 157-168.

Hess, M., Neubauer, C., and Hackl, R. (2007). Interlaboratory comparison of ability to detect nucleic acid of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* by polymerase chain reaction. *Avian Pathology,* 36(2): 127-133.

Hnatow, L. L., Keeler Jr, C. L., Tessmer, L. L., Czymmek, K., and Dohms, J. E. (1998). Characterization of MGC2, a *Mycoplasma gallisepticum* cytadhesin with homology to the *Mycoplasma pneumoniae* 30-kilodalton protein P30 and *Mycoplasma genitalium* P32. *Infection and Immunity,* 66(7): 3436-3442.

Holt, J. G., Kreig, N. R., Sneath, P.H.A., Staley, J.T., and Williams, S.T. (1994). The Mycoplasmas (or mollicutes): Cell wall- Less Bacteria. *In W. R. Hensyl (ed). Bergey' Manual of Determinative Bacteriology, 9th. Williams and Wilkins: Baltimore, MD,* 705-717.

Hong, Y., García, M., Levisohn, S., Savelkoul, P., Leiting, V., Lysnyansky, I., Ley, D., H., and Kleven, S., H. (2005). Differentiation of *Mycoplasma gallisepticum* strains using amplified fragment length polymorphism and other DNA-based typing methods. *Avian Diseases*: 49(1): 43-49.

Hopkins, S. R., and Yoder Jr, H. W. (1982). Influence of infectious bronchitis strains and vaccines on the incidence of *Mycoplasma synoviae* airsacculitis. *Avian Diseases,* 26(4): 741-752.

Hopkins, S. R., and Yoder Jr, H. W. (1984). Increased incidence of airsacculitis in broilers infected with *Mycoplasma synoviae* and chicken-passaged infectious bronchitis vaccine virus. *Avian Diseases,* 848: 386-396.

Jan, G., C. Brenner, and H. Wroblewski. (1996). Purification of *Mycoplasma gallisepticum* membrane proteins p52, p67 (*pMGA*), and p77 by high-performance liquid chromatography. *Protein Expression and Purification*, 7: 160-166.

Jiang, H.X., Chen, J.R., Yan, H.L., Li, X. N., Chen, Z.L., and Zeng, Z.L. (2009). Molecular Variability of DR-1 and DR-2 within the *PVPA* gene in *Mycoplasma gallisepticum* isolates. *Avian Diseases,* 53(1): 124-128.

John, J. J., Ley, D. H. and Altizer, S. (2006). Genotypic analyses of *Mycoplasma gallisepticum* isolates from songbirds by random amplification of polymorphic DNA and amplified-fragment length polymorphism. *Journal of Wildlife Diseases,* 42(2): 421-428.

Jordan, F.T.W. (1979). In "The Mycoplasma, vol. II. " J.G. Tully and R.F.Whitcomb, ends., *Academic Press, New York,* pp. 1-48.

Jordan, F. T., and Amin, M. M. (1980). A survey of Mycoplasma infections in domestic poultry. *Research in Veterinary Science,* 28(1): 96-100.

Jordan, F.T.W. (1985). Gordan Memorial Lecture; People, Poultry and Pathogenic Mycoplasma. *World's Poultry Science Journal,* 41: 226-239.

Karaca, K. and Lam, K. M. (1987). Efficacy of commercial *Mycoplasma gallisepticum* bacterin (MGBac) in preventing air-sac lesions in chickens. *Avian Diseases,* 31: 202-203.

Keeler Jr, C. L., Hnatow, L. L., Whetzel, P. L., and Dohms, J. E. (1996). Cloning and characterization of a putative cytadhesin gene (mgc1) from *Mycoplasma gallisepticum*. *Infection and Immunity,* 64(5): 1541-1547.

Kelly, P. J., Chitauro, D., Rohde, C., Rukwava, J., Majok, A., Davelaar, F., and Mason, P. R. (1994). Diseases and management of backyard chicken flocks in Chitungwiza, Zimbabwe. *Avian Diseases,* 38(3):626-629.

Kempf, I. (1997). DNA amplification methods for diagnosis and epidemiological investigations of avian mycoplasmosis. *Acta Veterinaria Hungaria* , 45: 373 - 386.

Kempf, I., Blanchard, A., Gesbert, F., Guittet, M. and Bennejean, G. (1993). The polymerase chain reaction for *Mycoplasma gallisepticum* detection. *Avian Pathology*, 22: 739 -750.

Khan, M. I., Kirkpatrick, B. C., and Yamamoto, R. (1989). *Mycoplasma gallisepticum* species and strain-specific recombinant DNA probes. *Avian Pathology*, 18(1): 135-146.

Khan, M. I., Lam, K. M., and Yamamoto, R. (1987). *Mycoplasma gallisepticum* strain variations detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Avian Diseases*, 31: 315-320.

Kheyar, A., Reddy, S. K., and Silim, A. (1995). The 64 kDa lipoprotein of *Mycoplasma gallisepticum* has two distinct epitopes responsible for haemagglutination and growth inhibition. *Avian Pathology*, 24(1): 55-68.

Kiss, I., Matiz, K., Kaszanyitzky, E., Chavez, Y. and Johansson, K. E. (1997). Detection and identification of avian mycoplasmosis by polymerase chain reaction and restriction fragment length polymorphism assay. *Veterinary Microbiology Journal*, 58: 23-30.

Kleven, S. H. (1985). Stability of the F strain of *Mycoplasma gallisepticum* in various diluents at 4, 22, and 37°C. *Avian Diseases*, 29(4): 1266-1268.

Kleven, S. H. (1997). Changing expectations in the control of *Mycoplasma gallisepticum*. *Acta Veterinaria Hungarica*, 45(3): 299-305.

Kleven, S.H. (1998). Mycoplasmosis. In D.E. Swayne, J.R. Glisson, M.W. Jackwood, J.E. Pearson, and W.M. Reed (eds.). A laboratory manual for the isolation and identification of avian pathogens, Fourth ed. American Association of Avian Pathologists: Kennett Square, PA, pp 74-80.

Kleven, S. H. (2002). Recent developments in Mycoplasma diagnosis and control. *Proceedings of the Western Poultry Disease Conference*, 51: 109-113.

Kleven, S.H. (2003). Mycoplasmosis. In: Diseases of Poultry. Edited by Y.M. Saif., H.J. Barnes., A.M. Fadly, J.R. Glisson, L.R. McDougald and D.E. Swayne. *Iowa State Press, Ames, Iowa*. pp 719-721.

Kleven S.H. (2008). Control of avian Mycoplasma infections in commercial poultry. *Avian Disease*, 52(3): 367-374.

Kleven, S. H., Browning, G. F., Bulach, D. M., Ghiocas, E., Morrow, C. J., and Whithear, K. G. (1988a). Examination of *Mycoplasma gallisepticum* strains using restriction endonuclease DNA analysis and DNA-DNA hybridisation. *Avian Pathology*, 17(3): 559-570.

Kleven, S. H., Fan, H. H., and Turner, K. S. (1998). Pen trial studies on the use of live vaccines to displace virulent *Mycoplasma gallisepticum* in chickens. *Avian Diseases*, 42: 300-306.

Kleven, S. H., Fulton, R. M., Garcia, M., Ikuta, V. N., Leiting, V. A., Liu, T., Ley , D. H., Opegart, K. N., Rowland, G. N. and Wallner-Pendleton E. (2004). Molecular characterization of *Mycoplasma gallisepticum* isolates from turkeys. *Avian Disease*, 48(3): 562-569.

Kleven, S. H. and Levisohn, S. (1996). Mycoplasma infections of poultry. In J. G. Tully (ed.). Molecular and diagnostic procedures in mycoplasmology. Volume II—Diagnostic Procedures, Vol. II. *Academic Press, Inc.: New York*, pp 283-292.

Kleven, S. H., Morrow, C. J., and Whithear, K. G. (1988b). Comparison of *Mycoplasma gallisepticum* strains by hemagglutination-inhibition and restriction endonuclease analysis. *Avian Diseases*, 32(4): 731-741.

Labarere, J., and Barroso, G. (1984). Ultraviolet irradiation mutagenesis and recombination in Spiroplasma citri. *Isreal Journal Medicine Scienc*e. 20: 826-829.

Lauerman, L. H., Chilina, A. R., Closser, J. A., and Johansen, D. (1995). Avian Mycoplasma identification using polymerase chain reaction amplicon and restriction fragment length polymorphism analysis. *Avian Diseases*, 39(4): 804-811.

Levisohn, S. (1981). Antibiotic sensitivity patterns in field isolates of *Mycoplasma gallisepticum* as a guide to chemotherapy. *Israel Journal of Medical Sciences*, 17(7): 661-666.

Levisohn, S., and Dykstra, M. J. (1987). A quantitative study of single and mixed infection of the chicken trachea by *Mycoplasma gallisepticum*. *Avian Diseases*, 31(1): 1-12.

Levisohn, S., Dykstra, M. J., Lin, M. Y., and Kleven, S. H. (1986). Comparison of in vivo and in vitro methods for pathogenicity evaluation for *Mycoplasma gallisepticum* in respiratory infection. *Avian Pathology*, 15(2): 233-246.

Levisohn, S., Glisson, J. R., and Kleven, S. H. (1985). In ovo pathogenicity of *Mycoplasma gallisepticum* strains in the presence and absence of maternal antibody. *Avian Diseases*, 29(1): 188-197.

Levisohn, S., and Kleven, S. H. (2000). Avian mycoplasmosis (*Mycoplasma gallisepticum*). *Review Science and Technology*, 19: 425-442.

Levisohn, S., Rosengarten, R., and Yogev, D. (1995). In vivo variation of *Mycoplasma gallisepticum* antigen expression in experimentally infected chickens. *Veterinary Microbiology, 45*: 219-231.

Ley, D.H. (2003). *Mycoplasma gallisepticum* infection. In: Diseases of Poultry. Edited by Y.M. Saif., H.J. Barnes., A.M. Fadly, J.R. Glisson, L.R. McDougald and D.E. Swayne. Iowa State Press, Ames, Iowa. P, pp 722-744.

Ley D.H. (2008). *Mycoplasma gallisepticum* infection. In:*Diseases of Poultry.* Edited by Saif Y.M., Fadly A.M., Gllison J.R., McDougald L.R., Nolan L.K., and Swayne D.E., Blackwell Publishing, Ames, USA, pp. 807-834.

Ley, D. H., Avakian, A. P., and Berkhoff, J. E. (1993). Clinical *Mycoplasma gallisepticum* infection in multiplier breeder and meat turkeys caused by F strain: identification by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, restriction endonuclease analysis, and the polymerase chain reaction. *Avian Diseases*, 37: 854-862.

Ley, D. H., Berkhoff, J. E., and Levisohn, S. (1997a). Molecular epidemiologic investigations of *Mycoplasma gallisepticum* conjunctivitis in songbirds by random amplified polymorphic DNA analyses. *Emerging Infectious Diseases*, 3: 375-380.

Ley, D. H., McLaren, J. M., Miles, A. M., Barnes, H. J., Miller, S. H., and Franz, G. (1997b). Transmissibility of live *Mycoplasma gallisepticum* vaccine strains ts-11 and 6/85 from vaccinated layer pullets to sentinel poultry. *Avian Diseases*, 41: 187-194.

Ley, D.H. and Yoder, H.W., Jr. 1997. Mycoplasmosis/*Mycoplasma gallisepticum* infection. In Calnek B.W. et al., (Eds.) Diseases of Poultry. 10th edn. Ames:Iowa State University Press, pp 194-207.

Lin, M. Y., and Kleven, S. H. (1982a). Egg transmission of two strains of *Mycoplasma gallisepticum* in chickens. *Avian Diseases*, 26(3): 487-495.

Lin, M. Y., and Kleven, S. H. (1982b). Pathogenicity of two strains of *Mycoplasma gallisepticum* in turkeys. *Avian Diseases*, 26(2): 360-364.

Lin, M. Y., Wu, Y. H., Cheng, J. H., Lin, G. J., Tung, M. C., Lan, Y. C., Sung, H. T. and Cheng, C. P. (1996). Isolation of avian mycoplasmas and Salmonella spp. and serological survey of Newcastle disease, egg drop syndrome, pullorum disease

and two avian mycoplasmas in sparrows flying around chicken farms. *Taiwan Journal of Veterinary Medicine and Animal Husbandry*, 66 (2): 125-131.

Liu, L., Dybvig, K., Panangala, V. S., Van Santen, V. L., and French, C. T. (2000). GAA trinucleotide repeat region regulates M9/*pMGA* gene expression in *Mycoplasma gallisepticum. Infection and Immunity,* 68: 871-876.

Liu, L., Payne, D. M., Van Santen, V. L., Dybvig, K., and Panangala, V. S. (1998). A protein (M9) associated with monoclonal antibody-mediated agglutination of *Mycoplasma gallisepticum* is a member of the *pMGA* family. *Infection and Immunity*, 66: 5570–5575.

Liu, T., Garcia, M., Levisohn, S., Yogev, D., and Kleven, S. H. (2001). Molecular variability of the adhesin-encoding gene *pvpA* among *Mycoplasma gallisepticum* strains and its application in diagnosis. *Journal Clinical Microbiology*, 39: 1882-1888.

Luttrell, M. P., Stallknecht, D. E., Kleven, S. H., Kavanaugh, D. M., Corn, J. L., and Fischer, J. R. (2001). *Mycoplasma gallisepticum* in house finches (*Carpodacus mexicanus*) and other wild birds associated with poultry production facilities. *Avian Diseases,* 45(2): 321-329.

Lysnyansky, I., Garcia, M., and Levisohn, S. (2005). Use of mgc2-polymerase chain reaction–restriction fragment length polymorphism for rapid differentiation between field isolates and vaccine strains of *Mycoplasma gallisepticum* in Israel. *Avian diseases,* 49(2): 238-245.

Mahadevan Jaganathan. (2007). Prevalence of *Mycoplasma gallisepticum* in domestic chickens and free flying birds and molecular characterisation of the isolates. Master Dissertation. Universiti Putra Malaysia. pp 150.

Maiden, M. C. J., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D.A., Feavers, I.M., Achtman, M., and Spratt, B. G. (1998). Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proceedings of the National Academy of Sciences of the United States of America,* 95(6): 3140–3145.

Maniloff, J. and Quinlan. (1973). Biosynthesis and subcellular organization of nucleic acids in *Mycoplasma gallisepticum* strain A5969. *Annals of New York Academy of Science*, 225: 181-189.

Markham, J. F., Morrow, C. J., Scott, P. C., and Whithear, K. G. (1998a). Safety of a temperature-sensitive clone of *Mycoplasma synoviae* as a live vaccine. *Avian Diseases, 42*(4): 677-681.

Markham, P. F., Glew, M. D., Brandon, M. R., Walker, I. D., and Whithear, K. G. (1992). Characterization of a major hemagglutinin protein from *Mycoplasma gallisepticum*. *Infection and Immunity,* 60: 3885-3891.

Markham, P. F., Glew, M. D., Browning, G. F., Whithear, K. G., and Walker, I. D. (1998b). Expression of two members of the *pMGA* gene family of *Mycoplasma gallisepticum* oscillates and is influenced by *pMGA*-specific antibodies. *Infection and Immunity,* 66: 2845-2853.

Markham, P. F., Glew, M. D., Sykes, J. E., Bowden, T. R., Pollocks, T. D., Browning, G. F., Whithear, K.G., and Walker, I. D. (1994). The organisation of the multigene family which encodes the major cell surface protein, *pMGA*, of *Mycoplasma gallisepticum*. *FEBS Letters,* 352(3): 347-352.

Markham, P. F., Glew, M. D., Whithear, K. G., and Walker, I. D. (1993). Molecular cloning of a member of the gene family that encodes *pMGA*, a hemagglutinin of *Mycoplasma gallisepticum*. *Infection and Immunity,* 61(3): 903-909.

Marois, C., Dufour-Gesbert, F., and Kempf, I. (2000). Detection of *Mycoplasma synoviae* in poultry environment samples by culture and polymerase chain reaction. *Veterinary Microbiology,* 73(4): 311-318.

McBride, M. D., Hird, D. W., Carpenter, T. E., Snipes, K. P., Danaye-Elmi, C., and Utterback, W. W. (1991). Health survey of backyard poultry and other avian species located within one mile of commercial California meat-turkey flocks. *Avian Diseases,* 35(2): 403-407.

McLaren, J. M., Ley, D. H., Berkhoff, J. E., and Avakian, A. P. (1996). Antibody responses of chickens to inoculation with *Mycoplasma gallisepticum* membrane proteins in immunostimulating complexes. *Avian Diseases,* 40(4): 813-822.

Mekkes, D. R., and Feberwee, A. (2005). Real-time polymerase chain reaction for the qualitative and quantitative detection of *Mycoplasma gallisepticum*. *Avian Pathology,* 34(4): 348-354.

Minion, F. C. (2002). Molecular pathogenesis of Mycoplasma animal respiratory pathogens. *Frontiers in Bioscience*, 7:1410 -1422.

Mohammed, H. O., Carpenter, T. E., and Yamamoto, R. (1987a). Economic impact of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial layer flocks. *Avian Diseases,* 31(3): 477-482.

Mohammed, H. O., Carpenter, T. E., and Yamamoto, R. (1987b). Evaluation of factors associated with infection of commercial layers with *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. *Avian Diseases*, 31: 470-476.

Mohammed, H. O., Yamamoto, R., Carpenter, T. E., and Ortmayer, H. B. (1986). Comparison of egg yolk and serum for the detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies by enzyme-linked immunosorbent assay. *Avian Diseases*, 30: 398-408.

Much, P., Winner, F., Stipkovits, L., Rosengarten, R., and Citti, C. (2002). *Mycoplasma gallisepticum*: influence of cell invasiveness on the outcome of experimental infection in chickens. *FEMS Immunology and Medical Microbiology Journal*, 34:181–186.

Mudahi-Orenstein, S., Levisohn, S., Geary, S. J., and Yogev, D. (2003). Cytadherence-deficient mutants of *Mycoplasma gallisepticum* generated by transposon mutagenesis. *Infection and Immunity,* 71(7): 3812-3820.

Mushi, E. Z., Binta, M. G., Chabo, R. G., Mathaio, M., and Ndebele, R. T. (1999). Detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* antibodies in the sera of indigenous chickens by Rapid Plate agglutination test at Mnopane, Gabornone, Botswana, Onderstepeort. *Journal of Veterinary Research*, 66: 333-334.

Mutalib, A.R., Yardi, A., Pargini, N., Ganapathy, K. and Zakaria, Z. (2001). Polymerase chain reaction as an alternative method for diagnosis of *Mycoplasma gallisepticum* in chickens. 2nd International Congress/ 13th VAM Congress and CVA-Australia/Oceania Regional Symposium, Kuala Lumpur, 27-30 August, 2001, pp 75-76.

Muto, A. (1987). The genome structure of *Mycoplasma capricolum*. *Israel Journal of Medical Sciences*, 23: 334-341.

Nakamura, K., Ueda, H., Tanimura, T., and Noguchi, K. (1994). Effect of mixed live vaccine (Newcastle disease and infectious bronchitis) and *Mycoplasma gallisepticum* on the chicken respiratory tract and on *Escherichia coli* infection. *Journal of Comparative Pathology,* 111(1): 33-42.

Nascimento, E. R., Pereira, V. L. A., Nascimento, M. G. F. and Barreto, M. L. (2005). Avian mycoplasmosis update. *Brazilian Journal of Poultry Science,* 7(1): 01-09.

Nascimento, E. R., Yamamoto, R., Herrick, K. R., and Tait, R. C. (1991). Polymerase chain reaction for detection of *Mycoplasma gallisepticum*. *Avian diseases*, 35: 62-69.

Nascimento, E. R., Yamamoto, R., and Khan, M. I. (1993). *Mycoplasma gallisepticum* F-vaccine strain-specific polymerase chain reaction. *Avian Diseases,* 37(1): 203-211.

Nunoya, T., Yagihashi, T., Tajima, M., and Nagasawa, Y. (1995). Occurrence of keratoconjunctivitis apparently caused by *Mycoplasma gallisepticum* in layer chickens. *Veterinary Pathology*, 32(1): 11-18.

OIE, (2004). Avian mycoplasmosis. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th edition.

Ortiz, A., Froyman, R., and Kleven, S. H. (1995). Evaluation of enrofloxacin against egg transmission of *Mycoplasma gallisepticum*. *Avian Diseases*, 39(4): 830-836.

Pakpinyo, S., Pitayachamrat, P., Saccavadit, S., Santaswang, T., Tawatsin, T., and Sasipreeyajan, J. (2006). Laboratory diagnosis of *Mycoplasma gallisepticum* (MG) infection in experimental layer chicken receiving MG vaccines and MG organisms. *Thai Journal of Veterinary Medicine,* 36: 29-37.

Pandey, G. S., and Hasegawa, M. (1998). Serological survey of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* infection in chickens in Zambia. *Bulletin of Animal. Health Production in Africa*, 46: 113-117.

Papazisi, L., Gorton, T. S., Kutish, G., Markham, P. F., Browning, G. F., Nguyen, D. K., Swartzell, S., Madan, A., Mahairas, G., and Geary, S. J. (2003). The complete genome sequence of the avian pathogen *Mycoplasma gallisepticum* strain R_{low}. *Microbiology*, 149: 2307-2316.

Papazisi, L., Silbart, L. K., Frasca,jr., S., Rood, D., Liao, X., Gladd, M., Javed, M. A. and Geary, S.J. (2002). A modified live *Mycoplasma gallisepticum* vaccine to protect chickens from respiratory disease. *Vaccine,* 20(31-32): 3709-3719.

Parker, T.A., Branton, S.L., Jones, M.S., Peebles, E.D., Gerard, P.D., Willeford, K.O., Pharr, G.T. and Maslin, W. R. (2003). Effect of S6 strain of *Mycoplasma gallisepticum* challenges at onset of lay on digestive and reproductive tract characteristics in commercial layers. *Avian Diseases,* 47(1): 96-100.

Peterson, M. J., Aguirre, R., Ferro, P. J., Jones, D. A., Lawyer, T. A., Peterson, M. N. and Silvy, N. J. (2002). Infectious disease survey of Rio Grande wild turkeys in the Edwards Plateau of Texas. *Journal of Wildlife Diseases*, 38: 826-833.

Pharr, G. T., Branton, S. L., Cooksey, A. M., Hanson, L. A., Burgess, S. C., Bottoms, C. C., McCarthy, F. M., and Collier, S. D. (2006). The recognition of a vlhA protein from the F-Strain of *Mycoplasma gallisepticum* with monoclonal antibody 6F10. *International Journal of Poultry Science,* 5(8): 789-795.

Pharr, G. T., Branton, S. L., Hanson, L. A., Minion, F. C., Hughlett, M. B., and Wan, X. (2002). Characterization of *pMGA* genes from the F-strain (vaccine strain) of *Mycoplasma gallisepticum*. *International Journal of Poultry Science,* 1: 63-73.

Pillai, S. R., Mays, H. L., Ley, D. H., Luttrell, P., Panangala, V. S., Framers, K. L. and Roberts, S. R. (2003). Molecular variability of house finch *Mycoplasma gallisepticum* isolates as revealed by sequencing and restriction fragment length polymorphism analysis of the *pvpA* gene. *Avian Diseases*, 47: 640-648.

Power, J. and Jordan, F.T.W. (1976). A comparison of the virulence of three strains of *Mycoplasma gallisepticum* and one strain of *Mycoplasma gallinarum* in chicks, turkey poults, tracheal organ cultures and embryonated fowl eggs. *Research Veterinary Science*, 21: 41-46.

Pradhan, M.A.M.(2002). Studies on Avian mycoplasmosis: Prevalence, Isolation, Characterization and Antigenic properties. PhD Thesis Submitted to the Dept. of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Raviv, Z., Callison, S., Ferguson-Noel, N., Laibinis, V., Wooten, R., and Kleven, S. H. (2007). The *Mycoplasma gallisepticum* 16S–23S rRNA intergenic spacer region sequence as a novel tool for epizootiological studies. *Avian Diseases*, 51(2): 555-560.

Raviv, Z., Callison, S. A., Ferguson-Noel, N., and Kleven, S.H. (2008). Strain differentiating real-time PCR for *Mycoplasma gallisepticum* live vaccine evaluation studies. *Veterinary Microbiology*, 129(1-2): 179-187.

Rawadi, G. A., (1998). Characterization of Mycoplasmas by RAPD fingerprinting. *Method Molecular Biology*, 104:179-187.

Razin, S. (1985). Molecular biology and genetics of Mycoplasmas (Mollicutes). *Microbiological Reviews*, 49(4), 419-455.

Razin, S. (1988). Molecular approach to Mycoplasma phylogeny. In: The Mycoplasma, R.F. Whitcomb and J.G. Tully, Editors. *Academic Press: Orlando*, pp 33-69.

Razin, S. (1995). Molecular properties of mollicutes. In: Molecular and diagnostic procedures in Mycoplasmology, S. Razin and J.G. Tully, Editors. *Academic Press: New York*, pp 1-25.

Razin, S. (2006). The genus Mycoplasma and related genera (class Mollicutes). *Prokaryotes*, 4: 836-904.

Razin, S., and Freundt, E. A. (1984). The Mycoplasmas. In Kreig, N.R. and J.G. Holt (ed). Bergey's Manual of Systematic Bacteriology. 9th ed., vol. 1. Williams & Wilkins, Baltimore, pp. 740-793.

Razin, S., Yogev, D., and Naot, Y. (1998). Molecular biology and pathogenicity of Mycoplasmas. *Microbiology and Molecular Biology Reviews*, 62(4): 1094-1156.

Reece, R. L., Ireland, L., and Barr, D. A. (1986). Infectious sinusitis associated with *Mycoplasma gallisepticum* in game-birds. *Australian Veterinary Journal,* 63(5): 167-168.

Roberts, D. H. (1970). Non-specific agglutination reactions with *Mycoplasma gallisepticum* antigens. *The Veterinary Record,* 87(5): 125-126.

Roberts, D. H. and J. W. McDanial. (1967). Mechanism of egg transmission of *Mycoplasma gallispeticum. Journal of Comparative Pathology*, 77: 439-442.

Rodriguez, R., and Kleven, S. H. (1980a). Evaluation of a vaccine against *Mycoplasma gallisepticum* in commercial broilers. *Avian Diseases,* 24(4): 879-889.

Rodriguez, R., and Kleven, S. H. (1980b). Pathogenicity of two strains of *Mycoplasma gallisepticum* in broilers. *Avian Diseases,* 24(4): 800-807.

Rogers, M. J., Simmons, J., Walker, R. T., Weisburg, W. G., Woese, C. R., Tanner, R. S., Robinson, I. M., Stahl, D. A., Olsen, G., Leach, R. H., and Maniloff, J.(1985). Construction of the Mycoplasma evolutionary tree from 5S rRNA sequence data. *Proceedings of the National Academy of Sciences of the United States of America,* 82(4): 1160-1164.

Rosengarten, R., Levisohn, S., and Yogev, D. (1995). A 41-kDa variable surface protein of *Mycoplasma gallisepticum* has a counterpart in *Mycoplasma imitans* and *Mycoplasma iowae*. *FEMS Microbiology Letters,* 132(1-2): 115-123.

Rosengarten, R., and Yogev, D. (1996). Variant colony surface antigenic phenotypes within Mycoplasma strain populations: implications for species identification and strain standardization. *Journal of Clinical Microbiology,* 34(1), 149-158.

Saif-Edin, M. (1997). Situation of Mycoplasma infection among chickens in Upper Egypt with evaluation of different diagnostic techniques. *Assiut Veterinary Medical Journal,* 37: 54-67.

Salisch, H., Hinz, K.H., Graack, H.D. and Ryll, M. (1998). A comparison of a commercial PCR-based test to culture methods for detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in concurrently infected chickens. *Avian Pathology*, 27: 142-147.

Sasipreeyajan, J., Halvorson, D. A., and Newman, J. A. (1987). Effect of *Mycoplasma gallisepticum* bacterin on egg-transmission and egg production. *Avian Diseases,* 31(4): 776-781.

Shah-Majid, M. (1996). Detection of *Mycoplasma gallisepticum* antibodies in the sera of village chickens by the enzyme-linked immunosorbent assay. *Tropical Animal Health and Production,* 28(2): 181-182.

Soeripto, K. E., Whithear, K. G., Cottew, G. S. and Harrigan, K. E. (1989). Virulence and transmissibility of *Mycoplasma gallisepticum*. *Australian Veterinary Journal*, 66: 65-72.

Sprygin, A.V., Andreychuk, D.B., Elatkin, N.P., Zinyakov, N.G., Kolosov, S.N., Mudrak, N.S., Irza, V. N., Drygin, V.V., Borisov, A.V. and . Perevozchikova, N. A. (2010). Genetic diversity of *Mycoplasma gallisepticum* field isolates using partial sequencing of the *pvpA* gene fragment in Russia. *Avian diseases*, 54(2): 899-904.

Stanbridge, E. J. (1985). The molecular biology of Mycoplasma. In: The Mycoplasma, M. F. Barile and S. Razin, Editors. *Academic Press: New York*, pp 157-158.

Stipkovits, L., Czifra, G., and Sundquist, B. (1993). Indirect ELISA for the detection of a specific antibody response against *Mycoplasma gallisepticum*. *Avian Pathology*, 22(3): 481-494.

Tajima, M., Yagihashi, T., and Nunoya, T. (1985). Ultrastructure of Mycoplasmal capsules as revealed by stabilization with antiserum and staining with ruthenium red. *Japanese Journal of Veterinaty Science*, 47(2): 217-223.

Talha, A. F. S., Bisgaard, M. and Das, P. M. (Thesis, 2003). Investigation of the prevalence and significance of *Mycoplasma gallisepticum* in layer chickens in Bangladesh. The royal Veterinary and Agricultural University: Department of veterinary Microbiology, 17 Bulowsvej, DK-1870, Mymensingh, Bangladesh.

Talkington, F. D., and Kleven, S. H. (1985). Evaluation of protection against colonization of the chicken trachea following administration of *Mycoplasma gallisepticum* bacterin. *Avian Diseases*, 29(4): 998-1003.

Tan Ching Giap. (2004). Prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in commercial and village chickens in Penang. DVM Dissertation. Universiti Putra Malaysia. pp 68.

Tan, C. G. (2008). Gene size polymorphism and pathogenicity in embryonated eggs of *Mycoplasma gallisepticum* isolated from commercial chickens, Chapter 4 and 6. Master Thesis, Universiti Putra Malaysia.pp 83, 84, 91,164.

Timms, L. M., Marshall, R. N., and Breslin, M. F. (1989). Evaluation of the efficacy of chlortetracycline for the control of chronic respiratory disease caused by Escherichia coli and *Mycoplasma gallisepticum*. *Research in Veterinary Science*, 47(3): 377-382.

Troy, K. E. (1998). M.Sc. Genetic analysis of a MGP1-mutant high passage *Mycoplasma gallisepticum*. The University of Connecticut, Storrs, Conn.

Turner, K. S., and Kleven, S. H. (1998). Eradication of live F strain *Mycoplasma gallisepticum* vaccine using live ts-11 on a multiage commercial layer farm. *Avian Diseases,* 42(2): 404-407.

Tyrrell, P., and Andersen, P. (1994). Efficacy of sample pooling for the detection of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* utilizing PCR. Proceedings of the Western Poultry Disease Conference, 43: 62.

Villegas, P., Kleven, S. H., and Anderson, D. P. (1976). Effect of rout of Newcastle disease vaccination on the incidence of airsacculitis in chickens infected with *Mycoplasma synoviae*. *Avian Diseases,* 20: 395-400.

Walker, R. L. (2004). Mollicutes. Pages 240-9 in D. C. Hirsh, N. J. MacLachlan, and R. L. Walker, ed. Veterinary Microbiology. Blackwell Publishing Professional, Ames, IA, USA.

Wang, C., Ewing, M., and A'Arabi, S. Y. (2001). In vitro susceptibility of avian Mycoplasmas to enrofloxacin, sarafloxacin, tylosin, and oxytetracycline. *Avian Diseases,* 45(2): 456-460.

Whithear, K .G. (1996). Control of avian mycoplasmoses by vaccination. In J. Nicolet (ed.). Animal Mycoplasmosis and Control, Vol. 15. *Scientific and Technical Review - International Office of Epizootics,* pp 1527-1553.

Whithear, K. G., Soeripto, K. E. H., and Ghiocas, E. (1990). Safety of temperature sensitive mutant *Mycoplasma gallisepticum* vaccine. *Australian veterinary Journal,* 67(5): 159-165.

Wieslander, A., M. J. Boyer, and H. Wroblewski. (1992). Membrane Protein Structure. *In J. Maniloff, R. N. McElhaney, L.R. Finch, and J. Baseman (eds.). Mycoplasmas: Molecular Biology and Pathogenesis. American Society for Microbiology: Washington, DC,* 93-112.

Winner, F., Rosengarten, R., and Citti, C. (2000). In vitro cell invasion of *Mycoplasma gallisepticum. Infection and Immunity,* 68(7), 4238-4244.

Woese, C. R., Maniloff, J., and Zablen, L. B. (1980). Phylogenetic analysis of the Mycoplasmas. *Proceedings of the National Academy of Sciences of the United States of America,* 77(1): 494-498.

Yap Mee Ling. (2005). Prevalence of *Mycoplasma gallisepticum* and *Mycoplasma synoviae* in pipped embryos. DVM Dissertation. Universiti Putra Malaysia. pp 68.

Yagihashi, T., and Tajima, M. (1986). Antibody responses in sera and respiratory secretions from chickens infected with *Mycoplasma gallisepticum*. *Avian Diseases,* 30(3): 543-550.

Yoder Jr, H. W. (1986). A historical account of the diagnosis and characterization of strains of *Mycoplasma gallisepticum* of low virulence. *Avian Diseases,* 30(3): 510-518.

Yoder, H. W., Jr. (1994). Characterization of avian Mycoplasma. *Avian Diseases,* 8: 481-512.

Yoder Jr, H. W., and Hofstad, M. S. (1964). Characterization of avian Mycoplasma. *Avian Diseases,* 8(4): 481-512.

Yoder Jr, H. W., and Hofstad, M. S. (1965). Evaluation of tylosin in preventing egg transmission of *Mycoplasma gallisepticum* in chickens. *Avian Diseases,* 9(2): 291-301.

Yoder Jr, H. W., and Hopkins, S. R. (1985). Efficacy of experimental inactivated *Mycoplasma gallisepticum* oil-emulsion bacterin in egg-layer chickens. *Avian Diseases,* 29(2): 322-334.

Yoder Jr, H. W., Hopkins, S. R., and Mitchell, B. W. (1984). Evaluation of inactivated *Mycoplasma gallisepticum* oil-emulsion bacterins for protection against airsacculitis in broilers. *Avian Diseases,* 28(1): 224-234.

Yogev, D., Levisohn, S., Kleven, S. H., Halachmi, D., and Razin, S. (1988). Ribosomal RNA gene probes to detect intraspecies heterogeneity in *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. *Avian Diseases,* 32(2): 220-231.

Yogev, D., Levisohn, S., and Razin, S. (1989). Genetic and antigenic relatedness between *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. *Veterinary Microbiology,* 19(1): 75-84.

Yogev, D., Menaker, D., Strutzberg, K., Levisohn, S., Kirchhoff, H., Hinz, K. H., and Rosengarten, R. (1994). A surface epitope undergoing high-frequency phase variation is shared by *Mycoplasma gallisepticum* and *Mycoplasma bovis*. *Infection and Immunity,* 62(11): 4962-4968.

Zander, D. V. (1961). Origin of S6 strain Mycoplasma. *Avian Diseases,* 5: 154-156.

Zelenika, T. A., Savic, V., and Balenovic, M. (1999). Mycoplasmosis in heavy hybrid hens in Croatia from 1993 to 1998. Stocarstvo. 5: 411-418.

Zhang, J. H., D. R. Bi, M. H. Wang, B. Han, and A. X. Gao. (2001). Prevalence and pathogenicity of *Mycoplasma gallisepticum* in broilers in Inner Mongolia. *Chinese Journal of Veterinary Science and Technology,* 31: 12-13.