UNIVERSITI PUTRA MALAYSIA

PREVALENCE OF MYCOPLASMA GALLISEPTICUM IN DOMESTIC CHICKENS AND FREE FLYING BIRDS AND MOLECULAR CHARACTERISATION OF THE ISOLATES

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By

MAHADEVAN JAGANATHAN

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

May 2006

This project paper is especially dedicated to

my beloved parents, my wife and

my son SUDEESSVAN,

for their patience, support, encouragement and understanding

of my interest in Veterinary Medicine.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

PREVALENCE OF *MYCOPLASMA GALLISEPTICUM* IN DOMESTIC CHICKENS AND FREE FLYING BIRDS AND MOLECULAR CHARACTERISATION OF THE ISOLATES

By

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May 2006

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Chronic respiratory disease (CRD) and complicated chronic respiratory disease (CCRD) are caused by *Mycoplasma gallisepticum* (MG). Infected birds show respiratory and reproductive problems which lead to severe production losses in poultry industry. *Mycoplasma gallisepticum* has been isolated in chickens and free flying birds (FFB) in several parts of the world. Therefore the current study was carried out to determine the prevalence of MG infection, also to molecularly characterize MG isolated from commercial chickens (broilers and layers), multiflock birds (indigenous chickens, ducks, turkeys and guinea fowls) and FFB in Selangor.

This study showed that using fresh yeast extract in preparation of mycoplasma agar and taking samples from choanal site of birds have made isolation of MG possible especially in layer chickens and indigenous chickens. Twenty-seven (27) MG isolates were isolated

from layer birds and indigenous chickens. The recovery rate by culture method was lower compared to the detection rate by DNA profiling using Polymerase chain reaction (PCR). Excessive usage of antibiotics in broiler farms may have contributed to the failure to isolate MG from broiler chickens by culturing method. The attempt to isolate MG from FFB was unsuccessful due to the small anatomical structure of choanal cleft and high contamination of oral cavity of FFB. The latter may have hindered the growth of MG artificially. Therefore, this study also proved that PCR is a better tool for epidemiological study compared to culturing method.

Broiler chickens and FFB at farms showed a high prevalence of MG infection based on serology and DNA detection but isolation could not be isolated. Crows from non-farm area or were not in close contact with infected birds or farms, did not show evidence of MG infection. This study also shows that only clinically ill infected birds, excreted and spread the organism to other flocks or species, as observed in the crows from the infected farm. However, sub-clinically infected birds as in indigenous chickens did not transmit the organism to other chickens or birds.

Characterisations of the MG isolates were conducted to investigate the presence of one or more types of MG strain in this study. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and restriction endonuclease analysis (REA) were used as characterisation tools. All MG isolates from commercial chickens showed a unique band at 75 kilo-Dalton in SDS-PAGE, which was an important characteristic of MG F strain, which suggested that the isolates from layer farms could have derived from MG F strain, whereas the isolates from indigenous chickens were not similar to MG F strain. Different environmental exposures by MG strain may have caused alternation in genotype and/or phenotype, which might vary from one farm to another farm. Thus, these MG strains

might reveal different patterns in REA and SDS-PAGE. It is possible that the genotypic and phenotypic heterogeneity of MG demonstrated in the present study may have adversely influenced the outcome of the serum agglutination serology and may be important to consider optimizing antibody and organism detection systems. However, the unique characteristic of MG strain was reported to be stable when tested by SDS-PAGE. Whereas, based on REA, only one strain of MG will circulate among the flock of a farm.

This study therefore shows a high evidence of MG infection in commercial birds and FFB in farm. All MG isolates recovered from layer chickens were identical and possess a unique 75 kilo-Dalton protein band which was specific for MG F. However, the MG isolates obtained from the indigenous chickens were different from MG F strain .Their origin could not be determined from this study.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Master Sains Veterinar

PREVALENS OLEH *MYCOPLASMA GALLISEPTICUM* PADA AYAM TERNAKAN DOMESTIK DAN BURUNG-BURUNG TERBANG BEBAS SERTA PENCIRIAN MOLEKUL BAGI ISOLATNYA

Oleh

MAHADEVAN JAGANATHAN, DVM

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Penyakit pernafasan kronik (CRD) dan penyakit komplikasi pernafasan kronik (CCRD) adalah disebabkan oleh *Mycoplasma gallisepticum* (MG). Ayam yang berinfeksi menunjukkan tanda-tanda masalah pernafasan dan reproduktif, yang memberi kerugian yang besar pada industri ayam. *Mycoplasma gallisepticum* telah diasingkan daripada ayam dan burung-burung terbang bebas di beberapa tempat di dunia. Oleh itu, kajian ini adalah untuk menentukan prevalens, dan membuat pencirian molekul bagi MG yang diasingkan daripada ayam komersial (ayam pedaging dan penelur), ladang ternakan kumpulan poltri berbagai (ayam kampung, itik, ayam belanda dan ayam mutiara) dan burung-burung terbang bebas di Selangor.

Kajian ini menunjukkan bahawa pengunaan yis ekstrak yang disediakan sebaiksebelum dalam penyediaan agar mycoplasma and pengambilan sampel dari bahagian choanal

telah berjaya mengasingkan MG, terutama dari ayam penelur and ayam kampung.Dua puluh tujuh (27) isolat MG telah berjaya diperolehi daripada ayam penelur dan ayam kampung. Kadar pengasingan organisma ini melalui pengkulturan adalah lebih rendah berbanding dengan kadar pengesanan melalui analisis profil DNA dengan menggunakan *Polymerase chain reaction* (PCR) .Penggunaan antibiotic yang tidak menentu pada ayam pedaging mungkin telah menghindari pertumbuhan MG melalui pengkulturan. Manakala, bagi FFB, bentuk anatomi choanal kecil and ruang mulut yang tercemar merupakan faktor-faktor yang menyebabkan kegagalan pengasingan MG secara pengkulturan. Oleh yang demikian, untuk kajian epidemiogi, PCR telah membuktikan sebagai kaedah saintifik yang lebih sesuai berbanding dengan pengkulturan.

Berdasarkan pada ujian serologi dan pengesanan DNA, ayam komersial dan burung-burung terbang bebas (FFB) yang berada di kawasan ladang menunjukkan kadar prevalens yang tinggi, tetapi MG tidak berjaya diasingkan. Burung gagak yang bukan berada di ladang ayam, ataupun tidak mempunyai kontak dekat dengan ayam berpenyakit di ladang-ladang, tidak menunjukkan tanda infeksi oleh MG. Kajian ini juga menunjukkan hanya ayam-ayam yang berpenyakit mengeluarkan dan menyebarkan penyakit ini kepada kumpulan dan spesis lain seperti yang dilihat pada gagak daripada ladang berinfeksi. Walaubagaimanapun, burung yang berinfeksi secara sub-klinikal seperti yang dilihat pada ayam kampung, tidak menyebarkan organisma ini kepada ayam atau burung-burung lain.

Pencirian terhadap isolat-isolat MG telah dijalankan untuk mengenalpasti kehadiran satu atau lebih jenis strain dalam kajian ini. Kaedah-kaedah yang digunakan untuk teknik pencirian adalah *sodium dodecyl sulphate- polyacrylamide gel electrophoresis*

(SDS-PAGE) dan *restriction endonuclease analysis* (REA), Semua isolat MG daripada ayam komersial menunjukkan gegelang unik pada 75 kilo-Dalton (kD) dalam ujian SDS-PAGE, yang merupakan satu ciri penting bagi strain MG F, menunjukkan bahawa kemungkinan besar MG yang diisolat daripada ayam penelur berasal daripada strain MG F, manakala isolat daripada ayam kampung tidak sama dengan strain MG F. Pendedahan persekitaran yang berlainan oleh strain MG boleh menyebabkan pertukaran dalam genotip dan/atau fenotip yang mungkin berlainan antara satu ladang dengan ladang yang lain. Oleh itu, strain MG ini berkemungkinan mendedahkan corak REA dan SDS-PAGE yang berbeza. Barangkali,kewujudan perbezaan dalam genotip dan fenotip MG dalam kajian ini secara tak lansung boleh mempengaruhi keputusan ujian serologi *serum agglutination test* dan adalah dicadangkan agar untuk mengoptimasikan antibodi dan sistem pengesan orgnisma. Walaubagaimana pun, ciri strain MG yang unik dilaporkan stabil bila diuji dengan menggunakan SDS-PAGE. Manakala ,berdasarkan dari keputusan REA , hanya satu jenis strain MG akan berlegar di kelompok ayam dalam ladang tertentu.

Kajian ini telah menunjukkan prevalen infeksi MG yang tinggi pada ayam komersial dan FFB di ladang ayam. Semua isolat dari ayam penelur adalah sama dan memiliki satu gegelang di 75 kilo-Dalton yang merupakan ciri spesifik bagi MG F, manakala bagi ayam kampung ia adalah berbeza dari MG F. Asal isolat ini tidak dapat ditentukan dalam kajian ini.

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I certify that an Examination Committee has met on 5th May 2006 to conduct the final examination of Mahadevan Jaganathan on his Master of Veterinary Science thesis entitled "Prevalence of *Mycoplasma gallisepticum* in Domestic Chickens and Free Flying Birds and Molecular Characterisation of the Isolates" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutes.

MAHADEVAN JAGANATHAN

Date: 10 th AUGUST 2006

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LIST OF ABBREVIATIONS

B cell	Bursal cell
BRL	Bethesda Research Laboratories
CFU	Colony forming units
CRD	Chronic respiratory disease
CCRD	Complicated chronic respiratory disease
CPZ	Chlorpromazine
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetra acetic acid
ELISA	Enzyme-link immunosorbent assay
FLHS	Fatty liver hemorrhagic syndrome
HA	Hemagglutination
HI	Hemagglutination inhibition
IFA	Indirect Fluorescence Antibody
Ig A	Immunoglobulin A
IBD	Infectious bursal disease
Ig M	Immunoglobulin M
Ig G	Immunoglobulin G
IM	Intra-muscularly
IT	Intra-tracheally
kD	kilo Daltons
M	Molar
MG	*Mycoplasma gallisepticum*
MS	*Mycoplasma synoviae*
mm	millimeter

ml	milliliter
nm	nanometer
PCR	Polymerase chain reaction
pH	Logarithm 10 {H}
RAPD	Random amplified polymerase DNA
REA	Restriction Endonuclease Analysis
SDS-PAGE	Sodium dodecyl sulphate- polyacrylamide gel electrophoresis
SPA	Serum plate agglutination
T cell	Thymus cell
TBE	Tris-Borate
USA	United States of America
s	Seconds
ºC	Degree Celsius
%	Percentage
μm	micrometer