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PREVALENCE AND CHARACTERIZATION OF CHLAMYDIA PSITTACIE FROM BIRDS IN MALAYSIA

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PREVALENCE AND CHARACTERIZATION OF *CHLAMYDIA PSITTACIE* FROM BIRDS IN MALAYSIA

By

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Thesis Submitted in Fulfilment of the Requirement for the Degree of Master of Science in Faculty of Veterinary Medicine and Animal Science Universiti Putra Malaysia

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Dedicated with love and gratitude
to :

My husband Wesley Voon and my parents,
as
my source of encouragement and support in
completion of this study.

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LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
CDC	Center For Disease Control
CO_2	Carbon Dioxide
CTM	Chlamydia Transport Medium
DCF	Direct Complement Fixation
DNA	Deoxyribonucleic Acid
EB	Elementary Body
ELISA	Enzyme Linked Immunosorbent Assay
FCS	Fetal Calf Serum
FITC	Fluorescein Isothiocyanate
GM	Growth Medium
H_2O_2	Hydrogen Peroxide
IB	Intermediate Body
IIF	Indirect Immunofluorescence
IIP	Indirect Immunoperoxidase
IMR	Institute Medical Research
KG	Kilogram
LIP	Lipopolysaccharide
MEM	Minimum Essential Media

mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
MM	Maintenance Medium
MOMP	Major Outer Membrane Proteins
nm	Nanometer
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
RB	Reticulate Body
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic Acid
SBL	Sick Bird Look
SDS-PAGE	Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoresis
SPG	Sucrose Phosphate Glutamate
TEM	Transmission Electron Microscopy
UPM	Universiti Putra Malaysia
µg	Microgram
µm	Micrometer
µl	Microliter

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

PREVALENCE AND CHARACTERIZATION OF *CHLAMYDIA PSITTACIE* FROM BIRDS IN MALAYSIA

By

PHONG SU FUN
February 1998

Chairperson: **Professor Dr. Aini Ideris, Ph.D.**

Faculty: **Veterinary Medicine and Animal Science**

Chlamydia psittacie causes a range of clinical syndromes, from life threatening systemic disease to an inapparent infection in birds and is a zoonotic disease. The disease is important in many countries with infection rates ranging from 10 - 40 % and carrier rate that could exceed 90 %. In Malaysia, no study on avian chlamydiosis has been done. Therefore, this study is conducted to determine the epidemiological and biological properties of *Chlamydia psittacie* in birds from Malaysia. The surveillance on prevalence rate of *Chlamydia psittacie* in pigeons, doves and parrots was carried out in the areas of Klang Valley. One hundred and fifty two (152) fresh faecal samples were collected from different species of birds with different management background. The birds were grouped into four categories, namely, pet bird shops, parks, individual pet birds owner and

wild birds. The samples were tested with Clearview Chlamydia test kit which was the solid phase sandwich immunoassay using the chromographic principle and direct colour label. Pet bird shops had the significantly highest prevalence rate (81.1%), followed by the category owner (55.9%), park (52.0%) and birds in the wild (25.0%). Among the group of *Columbiformes* and *Psittaciformes*, they had no significant difference in their percentage. Birds kept in population had significant higher prevalence rate (78.9%) compared to birds which were kept individually in cages (40.4%). The percentage of birds showing signs of chlamydiosis was 4.6% and the carrier rate was 45.4%. Poor hygiene, sanitation, nutrition, management, crowded confinement, no specific quarantine programme and proper treatment to suspected sick birds led to the highest prevalence rate in pet bird shops. Lack of knowledge in disease control and management were major contributions to high prevalence rate in the categories of individual pet bird owners and recreational parks. Wild doves had lowest prevalence rate probably because they live in less stressful environment. The birds were from different management background and therefore species susceptibility could not be determined. Parakeets and budgerigars had the highest prevalence rates probably because they were most popularly kept as captive birds.

Chlamydia psittacie was successfully isolated from local doves which showed clinical signs of diarrhoea and poor feathering, using McCoy cells tissue culture method. The infected cells were harvested and the organism was detected by using Giemsa stain and Gimenez stain. Identification was then done by using the indirect immunofluorescent technique, indirect immunoperoxidase technique and transmission electron microscopy. The chlamydia appeared as inclusion bodies in the cytoplasm of the McCoy cells. The study therefore confirmed that *Chlamydia psittacie* is present in birds form Malaysia and thus, it should be considered in the diagnosis of sick birds, especially because it is a zoonotic disease.

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PREVALENS DAN PENCIRIAN *CHLAMYDIA PSITTACIE* PADA BURUNG-BURUNG DI MALAYSIA

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Chlamydia psittacie boleh menyebabkan pelbagai tanda klinikal daripada jangkitan sistem yang mengancam nyawa kepada yang tidak nyata pada burung dan ia juga adalah satu penyakit zoonotik. Penyakit ini adalah penting di negara-negara lain, seperti mana yang telah dilaporkan oleh Fudge (1990) bahawa kadar jangkitan chlamydia pada burung kesayangan adalah antara 10 hingga 40 % dan kadar pembawa boleh melebihi 90%. Di Malaysia, tiada kajian dijalankan terhadap penyakit chlamydia avian. Oleh itu, satu kajian telah dijalankan untuk menentukan ciri-ciri epidemiologi dan biologi *Chlamydia psittacie* pada burung-burung di Malaysia. Tinjauan terhadap kadar jangkitan yang disebabkan oleh *Chlamydia psittacie* pada burung-burung merpati, tekukur dan nuri telah dijalankan di sekitar kawasan Lembah Kelang. Sebanyak 152 sampel najis segar burung telah diambil dari kumpulan burung yang berlainan dari segi spesis dan pengurusannya.

Burung-burung itu dikumpulkan kepada 4 kategori, iaitu dari kedai-kedai burung, taman burung, burung yang dipelihara oleh orang perseorangan dan burung-burung liar. Sampel tersebut kemudiannya diuji dengan kit ujian Clearview Chlamydia yang berdasarkan prinsip kromografi dan label perwarnaan terus. Burung-burung dari kedai-kedai burung didapati mempunyai kadar jangkitan keertian tertinggi (81.1%), diikuti oleh kumpulan pemilik perseorangan (55.9%), taman burung (52.0%) dan burung liar (25.0%). Didapati burung *Columbiformes* dan *Psittaciformes*, tidak mempunyai kadar perbezaan yang tererti dalam kadar jangkitan. Burung-burung yang dipelihara secara kelompok (78.9%) mempunyai kadar jangkitan yang lebih tinggi dan tererti berbanding dengan burung-burung yang dipelihara secara berasingan di dalam sangkar (40.4%). Pada keseluruhannya, hanya 4.6 % burung yang menunjukkan tanda-tanda chlamydiosis dan kadar pembawa pula adalah sebanyak 45.4 %. Faktor-faktor kebersihan, pemakanan dan pengurusan yang tidak memuaskan, sangkar yang sesak, tiada pengamalan spesifik dalam program kuarantin dan rawatan yang betul, menyebabkan kadar jangkitan yang tinggi di kedai-kedai burung. Kekurangan pengetahuan dalam pengawalan penyakit dan pengurusan adalah paling mungkin menyebabkan kadar jangkitan yang tinggi di kumpulan pemilik perseorangan dan di taman burung. Burung-burung liar mempunyai kadar jangkitan yang terendah mungkin disebabkan kehidupan

yang kurang tekanan. Disebabkan kesemua burung adalah dari latarbelakang pengurusan yang berbeza, jadi kadar kejangkitan antara spesis tidak dapat ditentukan. Burung parakeet dan budgerigar mempunyai kadar jangkitan yang tinggi mungkin disebabkan burung-burung tersebut lebih diminati untuk dipelihara.

Chlamydia psittacie telah berjaya diasingkan dari burung-burung tekukur yang menunjukkan tanda-tanda klinikal penyakit chlamydiosis seperti cirit-beret dan bulu kusut dengan menggunakan teknik tisu kultur sel McCoy. Sel-sel yang dijangkiti dikumpulkan dan diuji untuk mengesan kehadiran chlamydia dengan menggunakan perwarna Giemsa dan Gimenez. Identifikasi chlamydia dijalankan dengan menggunakan teknik immunoberpendaflouran tidak langsung, immunoperoksidase tidak langsung dan mikroskop elektron pemancaran. Chlamydia kelihatan sebagai jasad di dalam sitoplasma sel McCoy. Dengan itu, kajian ini telah mengesahkan kehadiran chlamydia di Malaysia. Jadi, penyakit ini patut dipertimbangkan semasa membuat diagnosis pada burung yang sakit, terutamanya kerana ia merupakan penyakit zoonotik.

CHAPTER 1

INTRODUCTION

Caged and aviary birds such as parrots, pigeons and doves are increasingly popular in Malaysia. They are mostly kept as pet birds by individual owners or in recreational parks. Knowledge in birds management, breeding, feeding and disease control are still lacking and most birds die without proper investigation and diagnosis of disease. Among the common diseases encountered in the pet birds in many countries is chlamydiosis, which is caused by *Chlamydia psittacie* (Fudge, 1990). In Malaysia, no research has been done on chlamydiosis in the birds, therefore Malaysian isolates of *Chlamydia spp.* and the prevalence rate are unknown. Furthermore its importance in relation to human and bird health in Malaysia is also unknown although in other countries, chlamydiosis is a threat to their pet birds industry (Fudge, 1989 ; 1990).

Chlamydia psittacie is an obligate intracellular coccoid Gram - negative organism. Chlamydia infection in birds, mammals, or other animals is generally referred to as chlamydiosis. The disease in human caused by

chlamydia contracted from psittacine birds was called psittacosis (Cullen, 1993; Grimes and Wyrick, 1991 ; Salisch et al, 1996). Parrot fever, is another commonly used synonym. Ornithosis, a term first used in 1941 (Meyer, 1941) was introduced to describe chlamydia infection in humans contracted from non-psittacine birds.

Chlamydiosis can occur with a range of clinical syndromes from life threatening systemic disease to an inapparent infection (Grimes, 1985; Grimes and Wyrick, 1991). It is a very common chronic infection of psittacine birds and is of public health significance because of the popularity of psittacine birds kept as pets and the increased placement of these birds in home for the aged. Many of the birds become chronically infected but show no clinical signs until stressed. These birds often shed chlamydial organism intermittently and serve as source of infection for human and other birds.

Clinically, *Chlamydia psittacie* causes a wide range of disease, including, conjunctivitis, pneumonitis, infertility, abortion, enteric infection, polyarthritis and meningitis (Timms, 1989). Psittacine birds with active chlamydiosis can exhibit a variety of clinical signs including somnolence, respiratory distress, diarrhoea, weight loss and inactive. These clinical signs are non-specific and may be called a 'cold' by the client or the veterinarian

(Fudge, 1990). The survivor will become an asymptomatic carrier. Carrier birds are less active, show poor feathering and poor reproductive performance.

'Chlamydiosis is also a common chronic infection in pigeons (Andersen and Tappe, 1989). Gross lesions of uncomplicated chlamydiosis in pigeons are fibrinous exudates on thickened air sacs, peritoneal serosa and occasionally on epicardium. The liver is usually swollen, soft and discoloured. The spleen may be enlarged, soft and dark. The amount of urate in cloacal contents is higher than normal if catarrhal enteritis occurs. In less severe infection it may involve only the liver or air sacs. Some heavily infected shedders however may show no lesion at all (Grimes and Wyrick, 1991).

Chlamydiosis is of great interest and concern to avian veterinarians because it: 1) is difficult to diagnose and treat; 2) causes significant mortality and morbidity in birds, especially exotic birds which are highly valued, both monetarily and as companion animals; and 3) is potentially transmissible to human (Harrison, 1989). Since this disease is known to be a zoonotic disease (Andrewes and Walton, 1976; Salisch et al, 1996).

The disease in human has an incubation period of one to two weeks, sometimes longer. The onset may be sudden, with chills, or it may be insidious. Patchy bronchopneumonia is a characteristic of the disease, with an irritating, usually non-productive cough. Some myocardial damage is common.

In severe cases there may be nausea, vomiting and other symptoms. The disease may run a short course or last for several months. Infected but apparently normal birds may also transmit infection to man. In one incident, 26 people who entered a room containing apparently healthy parrots contracted psittacosis and five of them died (Andrewes and Walton, 1976). The organism has also been responsible for numerous laboratory infections (Andrewes and Walton, 1976). People who are in constant contact with birds are the pet bird breeders, pet bird owners, workers in recreational park with birds and pet shops workers.

The objectives of this study are therefore ;

1. to study the epidemiological aspects of avian chlamydiosis in three common species of birds in the Klang Valley (parrots, pigeons and doves).

The epidemiological aspects are :

a. The prevalence rate of chlamydia infected birds from different source (pet shops, recreational areas, pet birds household and wild) in the Klang Valley and from two (2) orders of birds, the *Columbiformes* and *Psittaciformes*.

b. The frequency distribution of chlamydia infected birds in asymptomatic carriers and clinically infected birds; birds kept in crowded confinement, not

crowded confinement, more than one birds in one cage (population) and single bird in one cage (single) and finally in various species of birds.

2. to culture, isolate and identify the Chlamydia from positive samples.