UNIVERSITI PUTRA MALAYSIA

THE BACTERIAL FLORA OF THE UPPER RESPIRATORY TRACT IN BUDGERIGARS AND PEACEFUL DOVES IN CAPTIVITY

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FPV 1997 8

THE BACTERIAL FLORA OF THE UPPER RESPIRATORY TRACT IN BUDGERIGARS AND PEACEFUL DOVES IN CAPTIVITY

By

LIAU CHAI BONG

A Project Paper Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Veterinary Medicine in the Faculty of Veterinary Medicine and Animal Science Universiti Putra Malaysia

June 1997

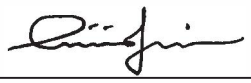
The Lord is my shepherd:
I have everything I need.
He lets me rest in fields of green grass
and leads me to quiet pools of fresh water.
He gives me new strength.
He guides me in the right paths.
as he promised.
Even though if I go through the deepest
darkness.
I will not be afraid, LORD,
for you are with me.
Your shepherd's rod and staff protect me.

Psalms 23: 1- 4.

Dedicated especially for my most loving parents.
To everyone who loves me and whom I love.
May the Lord protect and bless all of you.

CERTIFICATION

It is hereby certified that we have read this project paper entitled " The Bacterial Flora of the Upper Respiratory Tract in Budgerigars and Peaceful Doves in Captivity" by Liau Chai Bong and in our opinions it is satisfactory in terms of scope, quality and presentation as a partial fulfilment for the requirements of the course VPD 598 - Project.



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ACKNOWLEDGEMENTS

I wish to express my most sincere thanks and appreciation to my supervisor, Professor Dr. Aini Ideris, my co-supervisors, Dr. Nadzri Salim and Dr. Mohd. Shah Abdul Majid for their invaluable guidance and advice.

Special thanks to the owners of the five pet shops for participating in this study, and to Bayer (Malaysia) Sdn. Bhd. for sponsoring the enrofloxacin antibiotic sensitivity discs.

Sincere gratitude and appreciation also forwarded to the staffs of Bacteriological Laboratory especially En. Basri and En. Hajaraih for their patience, kind assistance and cooperation. Kindly thanks also go to Dr. Wahid for enabling the use of audio visual aid system during my presentation and En. Fauzi for his assistance in the photography section.

Last but not least, my most sincere thanks go to my colleagues, H.C. Chen, K.W. Wong, M. Vijandra, Siti Barkeh, C.N. Tan, Zunita, my house-mates Seri Mahal and Nithiah, and to everyone else who has helped or contributed in one way or another towards the completion of this study.

LIAU CHAI BONG

June, 1997.

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ABSTRACT

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THE BACTERIAL FLORA OF THE UPPER RESPIRATORY TRACT IN BUDGERIGARS AND PEACEFUL DOVES IN CAPTIVITY

By

LIAU CHAI BONG

1997

Supervisor: **Professor Dr. Aini Ideris**
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A bacteriological investigation of the upper respiratory tract of clinically healthy and sick budgerigars and peaceful doves from five pet shops within the Klang Valley as well as the antibiotic sensitivity tests of potentially pathogenic microorganisms isolated from the sick bird samples were reported. In the healthy budgerigar group, gram-positive microorganisms were more commonly isolated (80%) than gram-negative microorganisms (20%). In the sick budgerigar group, isolation of gram-negative microorganisms mainly *E. coli* was increased to 41% as compared to 20% in healthy budgerigars. In the healthy and sick dove groups, about 50% of gram-negative microorganisms were isolated in each group. *Mycoplasma gallinarum* and other untyped *Mycoplama spp*. were found mainly in the doves in both the healthy and sick groups with almost similar number of microorganisms. Antibiotic sensitivity tests revealed that most gram-negative

microorganisms tested were sensitive to enrofloxacin, polymycin B and newer third generation of aminoglycosides such as amikacin and gentamicin.

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Kedoktoran Veterinar dan Sains Penternakan untuk memenuhi sebahagian daripada keperluan Kursus VPD 598 – Projek.

FLORA BAKTERIA PADA SALURAN PERNAFASAN ATAS DI BURUNG SERINDIK DAN BURUNG MERBOK DALAM KURUNGAN

Oleh

LIAU CHAI BONG

1997

Penyelia: **Profesor Dr. Aini Ideris**
Penyelia Sampingan**: Dr. Nazri Salim**
Dr. Mohd. Shah Majid

Satu investigasi bakteriologikal terhadap saluran pernafasan atas burung serindik dan merbok yang sihat dari segi klinikal dan yang sakit, dari lima kedai burung di sekitar kawasan Lembah Kelang serta ujian sensitiviti antibiotik ke atas mikroorganisma yang berpotensi menyebabkan penyakit, yang diasingkan dari burung yang sakit telah dilaporkan. Dalam kumpulan burung serindik yang sihat, mikroorganisma gram-positif terutamanya cocci dan rod paling biasa diasingkan (80%) berbanding dengan mikroorganisma gram-negatif (20%). Dalam kumpulan burung serindik yang sakit, pengasingan mikroorganisma gram-negatif terutamanya *E. coli* telah meningkat kepada 41% berbanding dengan 20% pada burung serindik yang sihat. Dalam kumpulan burung merbok yang sihat dan sakit, kira-kira 50% mikroorganisma gram-negatif telah diasingkan

dari setiap kumpulan. *Mycoplasma gallinarum* dan lain *Mycoplasma spp*. yang tidak dapat ditentukan dijumpai kebanyakannya dalam kumpulan merbok yang sihat dan sakit, dengan nombor mikroorganisma yang lebih kurang sama di kedua-dua kumpulan. Ujian sensitiviti antibiotik menunjukkan kebanyakan mikroorganisma gram-negatif masih sensitif terhadap enrofloxacin, polymycin B dan generasi baru ketiga aminoglikosida umpamanya amikacin dan gentamicin.

1.0 INTRODUCTION

Bird keeping has been a traditional hobby in Malaysia especially among the village folks for the past few decades. In the recent years, more and more people are turning their attention to bird keeping and conservation, due to the level of interest and awareness. With the increasing popularity of birds as pets, so is the need for competent and quality veterinary care. However, little work has been done on providing local data regarding the health and disease status of the birds. It is with this point in mind that the need for the assembly of certain parameters of the local birds, initiate the commencement of this project.

Of the diagnostic modalities available (other than haematology and blood chemistry evaluation), bacteriology is the most important test in avian medicine (Woerpel and Rosskopf, 1984). Gram staining, culture and antibiotic sensitivity testing should be included as a routine diagnosis procedure in clinically healthy birds and as a diagnostic aid before the onset of therapy in clinically ill birds (Woerpel and Rosskopf, 1984).

Studies showed that gram-positive bacteria (cocci, bacilli) predominate in the psittacine gastrointestinal and respiratory systems (Sailstorfer, 1979; Paul-Murphy, 1992; Tully and Harrison, 1994). The presence of gram-negative bacteria is generally considered to be potentially pathogenic (Woerpel and Rosskopf, 1984; Rosskopf *et al.*, 1985; Olsen, 1989; Oglesbee, 1991; Bauck *et al.*, 1992; Paul-Murphy, 1992; Fudge *et al.*, 1993; Graham, 1994; Tully and Harrison, 1994). These are viewed as opportunists, capable of causing disease in individuals that are experiencing acute or chronic stress.

Gram-negative bacteria belonging to the Family of *Enterobacteriaceae* are most often the cause of diseases of pet birds (Woerpel and Rosskopf, 1984). This makes the techniques of gram staining and culturing with antibiotic sensitivity testing extremely useful in avian diagnostic medicine (Woerpel and Rosskopf, 1984).

Companion birds are commonly exposed to infectious microorganisms through the environment, hand-feeding techniques, food, and contact with other birds (Gerlach, 1994; Spenser, 1991). In an immune-compromised state such as in neonatal or hand-fed birds, young birds with nutritional deficiency or concurrent infectious process, avian species become susceptible to these ubiquitous infectious microorganisms.

Companion birds suffering from respiratory diseases are frequently admitted as patients to veterinary hospitals (AAV Practice Survey, 1987). Correct diagnosis and treatment of a respiratory problem will prevent secondary disease, long-term health problems, and possibly death (Tully, 1995).

In Malaysia, the most popularly kept birds as pets include doves and other song birds, various types of parrots commonly budgerigars, some mynahs, finches and many more. The use of antibiotics is also widespread among the local pet shops and some individual owners (through personal interviews and clinical experiences). It is important to know the status of susceptibility of the potential pathogens towards these antibiotics, as the findings would be able to assist us to know the types of antibiotics that are still effective for treatment and those that the bacteria has developed resistance.

There has been no published report on the nasal flora of healthy or sick birds in captivity in Malaysia. Thus, the objective of this study is to provide current data on the bacterial flora of the upper respiratory tract in both clinically healthy and sick budgerigars and peaceful doves, and their antibiotic sensitivity tests.

The objectives of this study are summarized as below: -

i) to study the common bacterial flora found in the upper respiratory tract of clinically healthy and sick budgerigars (*Melopsittacus undulatus*) and peaceful doves (*Geopelia striata*).

ii) to compare the predominant organisms found in healthy and sick budgerigars and peaceful doves.

iii) to perform antibiotic sensitivity test on the potential pathogens isolated from clinically sick birds.

2.0 LITERATURE REVIEW

2.1 Anatomical placement, structure and some abnormal signs of choanal

Knowledge of the anatomy of the avian respiratory system is important for determining the site and nature of the respiratory distress. The upper respiratory system of the bird includes the external nares, operculum, nasal concha, infraorbital sinus, and choanal slit (Tully and Harrison, 1994). The choanal slit is a median elongated triangular opening in the roof of the mouth.

The upper respiratory tract ends at the choana. Aerobic culture from the choanal is thought to represent the upper respiratory tract flora (Joyner, 1991). The choanal slit may be viewed on the dorsal aspect of the oral cavity forming a V shape, with the apex at the rostrum position. Epithelial projection (papilla) extends to the centre of the choanal cleft in most avian species. The appearance of the papilla at the time of physical examination can give the veterinarian an idea of the chronicity of the condition or the nutritional status of the bird. Blunted, edematous papilla often indicates chronic irritation or inflammation (Tully and Harrison, 1994). Foreign bodies and abscesses can occur in the rostrum aspect of the choana and are associated with dyspnoea or infection.

2.2 Bacteria isolated in the respiratory tract of psittacine birds

i) Gram-positive cocci

In the study of nasal flora of some healthy *Psittaciformes* species, Sailstorfer (1979) found that gram-positive organisms were predominant. *Staphylococci spp.* were found among lactobacilli and bacilli but there were marked differences between the bird species. Although *Staphylococcus aureus* is an infrequent isolate and is infrequently obtained, clinical problem associated with this organism was notable in Amazon parrots. Certain *Staphylococcus spp.* and *Streptococcus spp.* may create clinical circumstances especially in budgerigars and cockatiels (Woerpel and Rosskopf, 1984). Paul-Murphy (1992) stated that normal choanal floras comprise of *Staphylococcus spp.* and *Streptococcus spp.* although at times both *S. aureus* and *Streptococcus spp.* were found involved in rhinitis and sinusitis. *Staphylococcus spp.* was also found to be involved in causing rhinitis and sinusitis (Gerlach, 1986; Tully and Harrison, 1994).

ii) Gram-positive rods

Study by Sailstorfer (1979) revealed the presence of lactobacilli, bacilli and staphylococci as the predominant gram-positive organisms. *Mycobacterium tuberculosis*, and *Norcadia asteroides* are gram-positive bacteria that have been isolated from birds exhibiting rhinitis and sinusitis (Long *et al.*, 1983; Gerlach, 1986; Clubb *et al.*, 1994; Breadner, 1994; Van Der Heyden, 1994; Tully and Harrison, 1994). *Bacillus spp.*, *Corynebacterium spp.* and *Lactobacillus spp.* are common

nonpathogenic bacteria isolated from the upper respiratory system (Paul-Murphy, 1992; Tully and Harrison, 1994).

iii) Gram-negative bacteria

Gram negative bacteria frequently isolated from birds with upper respiratory tract infection are *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Pasteurella multocida, Yersinia pseudotuberculosis* and *Salmonella spp.,* Other less common bacterial that may cause signs of upper respiratory disease include *Proteus spp.*, *Haemophilus spp.*, *Bordetella avium, Mycoplasma spp., Chlamydia psittaci* and *Rhinosporidium spp*. (Rosskopf *et al.*, 1985; Olsen, 1989; Paul-Murphy, 1992; Tully and Harrison, 1994).

McDonald and Watts (1981) cultured the choana in clinically healthy birds and birds with upper respiratory infection. They found a shift from a predominantly gram-positive population to a gram-negative population comprised of *Pseudomonas spp.*, *Pasteurella sp.*, *E. coli*, *K. pneumoniae*, and *Enterobacter cloacae*. Whitford and Jones (1978) reviewed previous work and added their own findings which implicated *Salmonella spp,*, *Shigella spp.*, *E. coli*, *K. pneumoniae*, and *Pseudomonas sp.* as common gram-negative pathogens in both enteric and respiratory disease.

Dolphin and Olsen (1978) highlighted the involvement of *Pasteurella spp.* in the generalized infection of the nasal-oral cavity, including the pharyngeal surface and the nasal turbinate. They also found ulcers in the bottom of the oral cavity or on the tongue caused by *Pseudomonas spp.* Woerpel and Rosskopf (1984) also mentioned

about the isolation of *Pasteurella urea* in the mouth of two macaws suffering from upper respiratory infection.

In one study Bauck *et al*. (1992) suggested differential bacteriological aetiologies for rhinitis in the psittacine birds included *E. coli*, *Pseudomonas*, *Klebsiella* and others. Findings by another author stated the most frequently isolated pathogens involved in rhinitis include *E. coli*, *Enterobacter spp.*, *Pseudomonas*, *Aeromonas*, *Pasteurella and Klebsiella* (Oglesbee, 1991).

Mycoplasmas have also been isolated from pet birds. Reported cases include air sac and sinus infection of the parakeet, canary, cockatiel, cockatoo, severe macaw and Yellow-naped Amazon (Gaskin, 1987). It has been suggested that the New World psittacines such as imported Amazons and their contacts were prone to mucopurulent rhinitis caused by mycoplasmas (Clubb, 1986).

Pharyngitis is usually associated with coliforms or *Pseudomonas* but can be seen with mycobacteriosis and cocci (Fudge *et al*., 1993). In cases of avian upper respiratory tract infection, Graham (1994) had isolated *P. aeruginosa*, *K. pneumoniae*, *E. coli* and *Proteus spp.* through nasal flushing.

2.3 Bacteria involved in the respiratory tract of other wild birds

Richter (1981) studied the composition of the nasal flora in 74 hawks and 28 owls. He found 77.3 % of the isolates were gram-positive bacteria, with a predominance of staphylococci, micrococci, streptococci and bacilli. The most frequent species was *Staphylococcus xylosus* (66 strains), followed by *Streptococcus*

faecalis (61), *Staphylococcus sciuri* (37), *Bacillus coagulans* (31) and the *Staphylococcus epidermidis* group (20). *Acinetobacter calcoaceticus* (21 strains), *E. coli* (20) and *Alcaligenes faecalis* (9) represented the gram-negative flora mainly. Other *Enterobacter* occurred only sporadically. Strains of *Corynebacterium bovis* were isolated. Facultative pathogens were *A. faecalis*, *A. calcoaceticus*, *E. coli*, *K. pneumoniae*, *Bordetella bronchiseptica*, *Moraxella sp.*, *Branhamella catarrhalis*, *P. aeruginosa and Aeromonas hydrophila.*

Sambyal and Baxi (1980) studied the bacterial flora of the respiratory tract of wild birds in Ludhiana (Punjab). Among 100 wild birds, 63 harboured bacteria. Seventy-nine bacterial strains belonging to 16 species were isolated from the laryngo-tracheal region of these birds. The prevalence of the pathogenic bacteria such as *S. aureus*, *Corynebacterium*, *Erysipelothrix*, *Klebsiella*, *E. coli*, *Pseudomonas and Pasteurella* indicated that wild birds might be responsible for transmitting these microorganisms from one species to another.

Marlier *et al*. (1994) sampled 48 pigeons with symptoms of acute or chronic respiratory disease. Out of 36 pigeons, a total of 85 isolates were obtained of which 46 were potential pathogens. The potential pathogens comprised of *S. intermedius* (72%), *P. multocida* (17%), *E. coli* (9%) and beta-haemolytic *Streptococcus spp.* (2%).

2.4 Bacteria isolated in the respiratory tract of domestic fowl

Dho and Mouline (1983) studied the aerobic flora of chicks from 17-38 days old and found presence of predominantly *E. coli*, *Streptococcaceae*, *Micrococcaceae* and members of the genus *Lactobacillus*. The constant presence of lactobacilli and *E. coli* in the trachea suggested that these organisms could play a role in the bacterial ecosystem of the trachea.

Reddy *et al.* (1979) conducted a study on the normal bacterial flora of respiratory tract of ducks. Swab samples were taken from the nose, throat, trachea, bronchi and lungs of 30 healthy Desi and Khaki Campbell ducks. The results revealed 250 isolates which comprised *Bacillus spp.* (35), *S. aureus* (18), *S. epidemidis* (93), *Micrococcus spp.* (4), *Corynebacterium spp.* (37), *E. coli* (17), *Enterobacter spp.* (13), *Proteus spp.* (11), *Citrobacter spp.* (10), *Alcaligenes spp.* (9), and one each of *Klebsiella sp.*, *Listeria sp.* and *Erysipelothrix rhusiopathiae*. *Corynebacterium spp.* were non-pathogenic except for 5 isolates of *C. renale*. All 17 *E. coli* isolates were non-pathogenic to poultry.

Poornima and Upadhye (1995) conducted a study on the bacterial flora of respiratory tract of healthy and sick poultry. Samples were collected from 25 apparently normal and 100 infected birds from 20 different broiler farms in and around Bangalore. A total of 64 and 238 bacterial isolates were obtained from the 2 groups of fowl respectively. The gram-negative isolates accounted for 16% of isolates in healthy birds and 56% in affected birds. The most prevalent isolates in the 2 groups were *E. coli*, *S. aureus*, *S. epidermis*, *Corynebacterium sp.* and *P. multocida*.

Phalen and Wigle (1994) reported a chronic severe sinusitis resembling that seen in turkeys with *Mycoplasma gallisepticum* infection in five rheas. *E. coli*, a species of *Staphylococcus sp.* and a *Bacillus sp.* were isolated from the sinus aspirate of one bird.

2.5 Antibiotic sensitivity test

Rosskopf *et al*. (1985) conducted a survey of antibiotic efficacy for gram negative bacterial isolates from pet psittacine birds from May 1984 to Feb 1985. They found that through their clinical experience there was an excellent correlation between *in vitro* sensitivity results and response to therapy achieved by using antibiotics or combinations dictated by the results. They tried out 14 types of antibiotics and revealed percentage of sensitivity for piperacillin (81.0%), amikacin (78.5%), cefotaxime (75.7%), gentamicin (75.4%), tobramycin (71.1%), ticarcillin (56.0%), carbenicillin (54.2%), chloramphenicol (53.2%), vetasulid (47.5%), trimethoprim sulfa (39.1%), doxycycline (22.2%), spectinomycin (16.9%), cephalothin (12.0%) and ampicillin (6.3%).

Flammer (1992) conducted an antimicrobial susceptibility pattern of six species of gram-negative bacteria isolated from the cloaca of psittacine birds. The antibiotics with the greatest predicted efficacy were advanced generation penicillins such as carbenicillin and piperacillin, cephalosporins, aminoglycosides, fluoroquinolones, and trimethoprim/sulfa. Many *Pseudomonas* isolates were resistant to multiple antibiotics.