



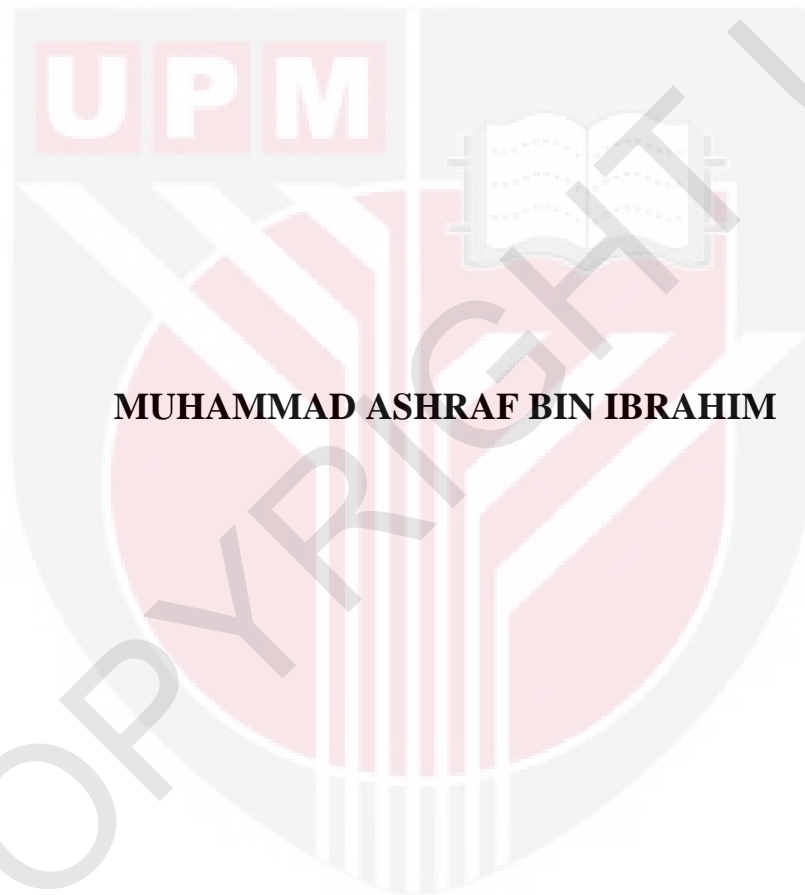
**UNIVERSITI PUTRA MALAYSIA**

***OCCURRENCE OF SALMONELLA SPP. AND CAMPYLOBACTER SPP.  
IN EXOTIC BIRDS IN WETLAND, PUTRAJAYA***

**MUHAMMAD ASHRAF BIN IBRAHIM**

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FPV 2015 49**

**OCCURRENCE OF *SALMONELLA* SPP. AND  
*CAMPYLOBACTER* SPP. IN EXOTIC BIRDS IN WETLAND,  
PUTRAJAYA**



**MUHAMMAD ASHRAF BIN IBRAHIM**

**FACULTY OF VETERINARY MEDICINE,  
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SERDANG, SELANGOR.**

**2015**



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IN EXOTIC BIRDS IN WETLAND, PUTRAJAYA**

**MUHAMMAD ASHRAF BIN IBRAHIM**

A project paper submitted to the  
Faculty of Veterinary Medicine, Universiti Putra Malaysia

In partial fulfilment of the requirement for the  
**DEGREE OF DOCTOR OF VETERINARY MEDICINE**

Universiti Putra Malaysia  
Serdang, Selangor Darul Ehsan.

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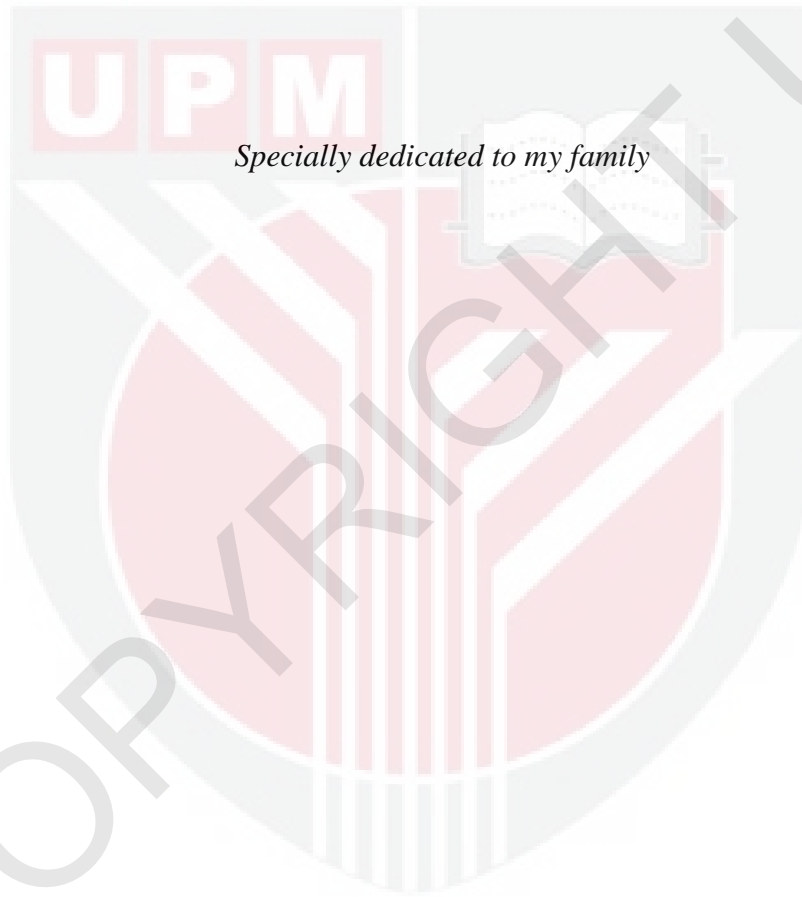
It is hereby certified that we have read this project paper entitled “Occurrence of *Salmonella* spp. and *Campylobacter* spp. in Exotic Birds in Wetland, Putrajaya” by Muhammad Ashraf bin Ibrahim and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.

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*Specially dedicated to my family*

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**ABSTRAK**

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir.

**KEHADIRAN *SALMONELLA* SPP. DAN *CAMPYLOBACTER* SPP. PADA  
BURUNG EKSOTIK DI WETLAND PUTRAJAYA**

Oleh

**MUHAMMAD ASHRAF BIN IBRAHIM**

**2015**

**Penyelia: Prof. Madya Dr. Jalila Binti Abu**

**Penyelia Bersama: Prof. Dr. Saleha Binti Abdul Aziz**

Manusia dan alam, saling memerlukan. Itulah gambaran yang terbaik untuk menggambarkan Putrajaya Wetlands. Putrajaya Wetland merupakan tanah lembap air tawar yang pertama dibina dan terbesar di kawasan tropika. Walau bagaimanapun, Putrajaya Wetland bukan sahaja berfungsi sebagai penapis air yang cekap, tetapi juga sebagai habitat yang sesuai untuk hidupan liar dan eksotik hidup di tanah lembap dan berair yang sesuai untuk didiami. Walau bagaimanapun, burung-burung eksotik boleh menjadi pembawa patogen yang mungkin mempunyai risiko zoonotik.

Oleh itu, kajian ini dijalankan untuk memeriksa kehadiran dan rintangan antibiotik *Salmonella* spp. dan *Campylobacter* spp. dalam burung eksotik. Sampel swab Cloaca diambil dari 50 burung yang kelihatannya sihat dari empat kawasan dalam kuarantin, satu kolam flamingo dan satu kawasan Pelantar. Tiada *Salmonella* spp. telah diasingkan daripada 50 sampel yang terdiri daripada spesies burung air seperti Angsa hitam, Angsa mesir, Rajah shell duck, Greater flamingo, Burung pelican putih, Pink backed pelican dan Swan putih. *Campylobacter coli* telah diasingkan sebanyak 7 (14.0%) daripada 50 sampel dari Angsa hitam, Burung pelican putih dan Swan putih. *Campylobacter lari* telah diasingkan daripada 2 (4.0%) daripada 50 sampel dari Angsa hitam sahaja. Tidak ada ujian kepekaan antibiotik telah dijalankan ke atas *Salmonella* spp. kerana tidak ada pertumbuhan jajahan terpencil. *Campylobacter coli* dan *Campylobacter lari* tidak terkesan kepada clindamycin (100%) dan sensitif kepada gentamycin (100%). 55.5% asingan tahan kepada satu antibiotik manakala 22% asingan tahan kepada dua antibiotik dan tidak ada asingan tahan kepada tiga antibiotik. Walau bagaimanapun, terdapat 22.2% asingan tahan kepada empat antibiotik, yang menunjukkan bahawa terdapat tahap MDR yang tinggi dari *Campylobacter* spp. diasingkan daripada burung eksotik di Wetland, Putrajaya.

Kata Kunci: Burung eksotik, *Salmonella* spp, *Campylobacter* spp, kejadian, rintangan antibiotik

**ABSTRACT**

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

**OCCURRENCE OF *SALMONELLA* SPP. AND *CAMPYLOBACTER* SPP. IN WETLAND EXOTIC BIRDS IN WETLAND, PUTRAJAYA**

By

**MUHAMMAD ASHRAF BIN IBRAHIM**

**2015**

**Supervisor: Assoc. Prof. Dr. Jalila Binti Abu**

**Co-supervisor: Prof. Dr. Saleha Binti Abdul Aziz**

Man and nature, side by side. That is the best words to describe Putrajaya Wetlands the first constructed freshwater wetland and the largest in the tropics. Nevertheless, Putrajaya Wetlands is much more than just an efficient water filter. It has become to some extent the real thing, a functioning wetland, a site with available water to varied exotic bird species and wildlife. However, the exotic birds as carrier of a number of pathogen may have possible zoonotic risk. Therefore, this study was

carried out to examine the presence and antibiotic resistance of *Salmonella* spp. and *Campylobacter* spp. in exotic birds. Cloaca swab sample was taken from 50 apparently healthy birds from four compartment in quarantine areas, one flamingo pond and one view deck areas. There is no *Salmonella* spp. was isolated out of 50 samples which were from water birds species which included Black swan, Egyptian goose, Radjah shelduck, Greater flamingo, White pelican, Pink backed pelican and White swan. *Campylobacter coli* was isolated from 7 (14.0 %) out of 50 cloaca swabs samples from Black Swan, White Pelican and White Swan. *Campylobacter lari* was isolated from 2(4.0 %) out of 50 cloaca swabs samples from Black Swan only. There was no antibiotic sensitivity test were done on *Salmonella* spp. because there are no isolated colonies growth. *Campylobacter coli* and *Campylobacter lari* isolates were resistance to clindamycin (100%) and sensitive to gentamycin (100%). 55.5% isolates were resistant to one antibiotics while 22% were resistant to 2 antibiotics and there are no resistance to 3 antibiotics. However, there are 22.2% isolates resistant to four antibiotics, which indicate that there is high level of MDR from *Campylobacter* spp. isolated from exotic birds in Wetland, Putrajaya.

Keywords: Exotic birds, *Salmonella* spp., *Campylobacter* spp., occurrence, antibiotic resistance

## 1.0 INTRODUCTION

Putrajaya, the new Federal Government Administrative Center of Malaysia was developed by Putrajaya Holdings Berhad in 1996 and covers the area of 4,931 ha. The master plan for the development incorporates comprehensive policies and guidelines for landscaped areas for its estimated 330,000 inhabitants. Among the main green spaces provided in Putrajaya are Putrajaya Lake and Wetlands (600 ha). It is one of the largest fully constructed freshwater wetland in the tropics (Lim *et al.*, 1998). The swamp forest bordering entire Putrajaya wetland system connects habitats along the Putrajaya waterways.

Wetlands are one of the Earth's most valuable resources per unit area (Costanza *et al.* 1997). The wetland ecosystem offers a diversity of habitats to attract wildlife. It also functions as breeding grounds and nurseries for, invertebrates, mammals, reptiles, amphibians and fish (Perbadanan Putrajaya, 1999). The Wetlands Park of Putrajaya is the home of many aquatic faunas. There are 12 species of exotic birds such as Radjah Shelduck, Black swan, Egyptian goose, White pelican, Pink-backed pelican, Greater flamingo, White swan, Canadian goose, Muscovy, Pekin and Mandarin ducks. The term "water bird" refers to bird species dependent on aquatic habitats to complete portions of their life cycles (Sarker *et al.*, 2012).

*Campylobacter* spp and *Salmonella* spp. are the leading causes of zoonotic enteric infections in developed and developing countries, and their incidence is increasing even in countries with adequate public health surveillance (Ramos *et al.*, 2010). Well-known modes of transmission to humans include physical contact with

domestic animals, person-to-person spread, and consumption of contaminated food and water.

According to Berrang (2013), clinical infections of *Campylobacter* in humans are particularly common in immunosuppressed adults. The symptoms may include watery or sticky diarrhoea, fever, nausea, vomiting, abdominal pain, headache, muscle pain and the faeces may contain blood. Salmonellosis is an extremely common disease among humans. Following a 12 to 36 hour incubation period, symptoms of fever, headache, diarrhoea, abdominal pain, nausea and dehydration develop, which may lead to septicaemia or endotoxemia.

The justification of this project was there was no research done on the prevalence of *Salmonella* spp. and *Campylobacter* spp. in exotic birds in wetland area. This study site was chosen based on places frequented visited by the public, such as recreational parks for families and tourist attractions. Therefore, the hypothesis for this project was the presence of *Salmonella* spp. and *Campylobacter* spp. in exotic birds was low. Therefore, the objectives of this study were to detect the occurrence of *Salmonella* spp. and *Campylobacter* spp. in a group of exotic birds and to determine the antibiotic susceptibility of the isolates.

## 2.0 LITERATURE REVIEW

### 2.1 Exotic Water Birds in Wetland Putrajaya

The term "water bird" refers to bird species dependent on aquatic habitats to complete portions of their life cycles (Sarker *et al.*, 2012). While the term "exotic" refer to something that is exotic is unusual and interesting, usually because it comes from or is related to a distant country (Collins, 2003). The wetland ecosystem offers a diversity of habitats to attract wildlife. There are 12 species of exotic birds such as Radjah Shelduck, Black swan, Egyptian goose, White pelican, Pink-backed pelican, Flamingo, White swan, Canadian goose, Muscovy, Pekin and Mandarin duck.

Black swan or the scientific names *Cygnus atratus* has the longest neck of any swan species. This long, slender neck is often arched in an 'S' shape or held erect, and this species often carries its wings raised in an aggressive display. The black swan's bill is a bright, waxy red with a pale bar and tip, and a patch of bare, red skin extends back from the bill to the reddish eyes. The legs and feet of the black swan are black or greyish-black (Delany *et al.*, 2006).

White swan or the scientific names *Cygnus Olor* are one of the largest waterfowl in North America weighing up to 13kg. Adults grow to 1.50 meter in length with wingspans up to 2.4 meter. Their orange beak with the black basal knob and terminal nail distinguishes white swans from all other species of swans. White swans have webbed feet that range in colour from black to grey-pink. While swimming, the birds hold their necks in a characteristic "S" shaped curve. Juvenile white swans, or cygnets, have grey or white coloured down (Delany *et al.*, 2006).

Greater flamingos are the only members of the family Phoenicopteridae. Distinguishing characteristics of flamingo include long legs, curved neck; and a goose-like voice (Delany *et al.*, 2006).

The Pink-backed pelican is one of the smallest of the eight pelican species. Its plumage is white, grey and pink in colour with dark grey wing tips. The top of the beak is yellow and the inside of the pouch pink. Mature individuals have long feather plumes on the back of the head. 2-3 large white eggs are laid in a large stick nest and are incubated for 30 days (Delany *et al.*, 2006).

The White pelican about four feet tall and has a wingspan of about nine feet. It is entirely white except for its black-edged wings that are visible when the white pelican is in flight. It has a long neck, a long orange bill with an expandable pouch and short orange legs with big webbed feet (Delany *et al.*, 2006).

The Radjah Shelduck or white-headed shelduck (*Tadorna radjah*) is a species of shelduck. It is more commonly known as a Burdekin Duck. Both the male and female of the species are mostly white, with dark wingtips and a distinctive "collar" of dark feathers (Delany *et al.*, 2006).

The Egyptian goose (*Alopochen aegyptiacus*) is a large, distinctive, pale-coloured water bird which is easily recognised by the conspicuous dark chocolate-brown patch around its eye. The head and neck are pale buff, and there is another dark brown patch around the base of the beak, usually joined to the eye patch by a narrow line (Delany *et al.*, 2006).

## 2.2 *Salmonella* spp.

*Salmonella* spp is an enterobacteriaceae, gram negative facultative anaerobic, non-lactose fermenters and rod-shaped zoonotic bacteria that can infect people, birds, reptiles, and other animals. They can survive in dried faeces from 8 months up to 2 years while water stable about 3 weeks (Blood *et al.*, 2007). This genus includes approximately 2000 species divided into five subgenera where two subgenera, *subgenus I* and *subgenus III*, can be found in birds. *Subgenus I* contain the species of *salmonella* that most commonly infect birds and *Subgenus III*, contains the species *Salmonella arizonae* and *arizona hinshawii*, which have occasionally been reported in birds, particularly those that are in contact with, or close proximity to reptiles (Kapperud and Rosef, 1983). Most vertebrates can be infected with *Salmonella* spp however the host susceptibility and development of carrier states vary widely among species. Birds can be sub-clinical carriers and serve as a reservoir of bacteria. There is limited study of prevalence of salmonella in water birds species.

However, Sarker *et al.* (2012) reported that zoonotic gram-negative bacteria previously isolated from seabird species include, but are not limited to *Salmonella* spp., *Campylobacter* spp., and *Yersinia* spp. Birds can act as vectors for spreading diseases such as psittacosis, salmonellosis, campylobacteriosis, and mycobacteriosis.

Infected birds may shed *Salmonella* bacteria in their faeces for weeks or months. Crowding, stress, contamination of feed with faeces, and the presence of carriers of *Salmonella* bacteria promote the regular occurrence of salmonellosis.

The disease can affect all species of domestic animals; young animals and pregnant and lactating animals are the most susceptible. Enteric disease is the commonest clinical manifestation, but a wide range of clinical signs, which include acute septicaemia, abortion, arthritis and respiratory disease, may be seen. Many animals, especially pigs and poultry, may also be infected but show no clinical illness. Such animals may be important in relation to the spread of infection between flocks and herds and as sources of food contamination and human infection (OIE, 2010).

Disease is usually referred to as salmonellosis, although the term paratyphoid may be used, e.g. swine paratyphoid. In poultry, pullorum disease or bacillary white diarrhoea and fowl typhoid are often used to describe infections caused by *S. Pullorum* and *S. Gallinarum*, respectively (OIE, 2010).

### 2.3 *Campylobacter* spp.

The genus *Campylobacter* comprises sixteen species and six subspecies and more recently, two additional species have been proposed (Vandamme, 2008). Members of the genus are typically gram-negative, non-spore forming, S-shaped or spiral shaped bacteria (0.2–0.8  $\mu\text{m}$  wide and 0.5–5  $\mu\text{m}$  long), with single polar flagella at one or both ends, conferring a characteristic corkscrew-like motility. These bacteria require microaerobic conditions, but some strains also grow aerobically or anaerobically. They neither ferment nor oxidise carbohydrates. Some species, particularly *C. jejuni*, *C. coli* and *C. lari*, are thermophilic, growing optimally at 42°C.

In birds, detection of intestinal colonisation is based on the isolation of the organism from faeces, rectal swabs or caecal contents. Agar media containing selective antibiotics are required to isolate these bacteria from faecal or intestinal samples. Preliminary confirmation of isolates can be made by light microscopy where the organisms have a characteristic rapid corkscrew-like motility. Biochemical and molecular tests can be used to confirm various *Campylobacter* species.

*Campylobacter jejuni* and *Campylobacter coli*, have been recognized as a major cause of human gastroenteritis throughout the world (Stoyanchev, 2004). *C. jejuni* is the predominant species associated with foodborne infection derived from poultry. *C. coli* and *C. lari* can also be recovered from the intestinal tract of poultry and have also been implicated in foodborne infection (OIE *Terrestrial Manual*, 2008). *Campylobacter jejuni* is commonly isolated from chicken and cattle, and chicken is expected to be one of the major sources of infection for humans. Pigs

commonly carry *Campylobacter coli*. *Campylobacter* food poisoning occurs in most cases sporadically affecting individuals. Outbreaks due to *Campylobacter* infections are rare. Outbreaks due to contaminated milk and drinking water are described more often than food borne outbreaks. In some countries where large quantities of pork are consumed *Campylobacter coli* infections frequently occur (Hendriksen *et al.*, 2003).

*Campylobacter* spp. can be easily spread from bird to bird through a common water source or through contact with infected faeces. When an infected bird is slaughtered, *Campylobacter* organisms can be transferred from the intestines to the meat. In 2011, *Campylobacter* was found on 47% of raw chicken samples bought in grocery stores and tested through the National Antimicrobial Resistance Monitoring System (CDC, 2014). Surface water and mountain streams can become contaminated from infected faeces from wild birds.

Within bird populations such as mature poultry and similar avian food animals such as ducks and turkeys, *Campylobacter* infections are non-pathogenic, where they do not cause disease and thus avian species are simply carriers. This also holds true for wild birds carrying the bacterium where the disease is silent and the animal does not exhibit any symptoms (Keller, 2009). This especially makes detection of the bacterium challenging and also there is limited study of the bacterium in exotic water birds due to its limitation.

## 2.4 Carriage of Pathogen by Water Birds

Birds can act as vectors for spreading diseases such as Psittacosis, Salmonellosis, Campylobacteriosis, Mycobacteriosis, Avian Influenza, and Cryptosporidiosis over long distances. Some of these are zoonotic diseases that can also be transmitted to humans (Sarker *et al.*, 2012). *Campylobacter* spp. is the main cause of human bacterial intestinal disease in many industrialised countries. Well-known modes of transmission to humans include physical contact with domestic animals, person-to-person spread, and consumption of contaminated food and water.

Contact with pets and livestock, the consumption of contaminated water or raw milk and travelling in high prevalence areas are also considered risks factors in human disease (OIE, 2008). Salmonellosis are well recognized in humans, livestock, companion and zoo animals where it can result in morbidity, mortality, and significant economic losses (Khethiwe *et al.*, 2012). Countless studies reported human campylobacteriosis and salmonellosis are frequently due to consumption of poultry and poultry products as the main risk factor; other foods involved include consuming raw or unpasteurised milk, drinking untreated drinking water and handling of animals and pets may also be sources of infections in humans (Faiza *et al.*, 2013). In a recent study by French *et al.* (2009), wild bird faecal samples were collected on and around children's playgrounds in a New Zealand city, yielding *Campylobacter jejuni* in 12.5% of the 192 samples which become public health concerns.

### 3.0 MATERIAL AND METHODS

#### 3.1 Sample Collection

Samples from 50 exotic birds of 16 species were collected from 12 Black swans, 12 Egyptian goose and 8 Rajdah Shellduck in quarantine area, 10 Flamingo from flamingo pond, five White Pelicans, two Pink-backed pelican and one White swan from plantar area.

**Table 1 : Samples collected from exotic birds in Wetland, Putrajaya.**

Species	Scientific names	No. of samples
Black swan	<i>Cygnus atratus</i>	12
Egyptian goose	<i>Alopochenaeegyptiaca</i>	12
Greater flamingo	<i>Phoenicopterus roseus</i>	10
Radjah shelduck	<i>Tadornaradjah</i>	8
White pelican	<i>Pelecanusonocrotalus</i>	5
Pink-backed pelican	<i>Pelecanusrufescens</i>	2
White swan	<i>Cygnus olor</i>	1
<b>Total</b>		<b>50</b>

The sampling areas located at the wetland Putrajaya, near the main road of Putrajaya. The sampling location is divided into quarantine 1,2,3,4, view deck area and flamingo area. Table 1 presents the detailed information number of sample collection from each species.

Sterile swabs were used to collect samples. Two sets of swabs were obtained. One set was inserted into Oxoid Buffered Peptone Water (BPW) for the isolation of *Salmonella* spp, whereas another set was put into Oxoid Cary Blair transport medium for the isolation of *Campylobacter* spp. Then, the swabs were transported in ice-packed cool box to the Veterinary Public Health Laboratory in Faculty of Veterinary Medicine, University Putra Malaysia within two to three hours (Refer to plate 1 and 2).

### **3.2 ISOLATION AND IDENTIFICATION PROCEDURES**

#### **3.2.1 *Salmonella* spp.**

The swabs collected in Oxoid Buffered Water (BPW) were pre-enriched by incubating at 37°C for 24 hours under aerobic condition. It was put in room temperature for a while after taking it out from the ice-packed cool box before putting it into the incubator. One ml of each pre-enriched broth samples was transferred into 10 ml of Oxoid Rappaport Vassidalis (RV) enrichment broth. It was then incubated at 42°C for 24 hours as enrichment stage. One loopful of the culture from RV enrichment broth was streaked onto Merck Xylose-Lysine-Tergitol-4® (XLT4) agar. It was then incubated at 37°C for 24 to 48 hours under aerobic condition.

Presumptive *Salmonella* spp. colonies which appeared as red to yellow colonies with black centers on XLT4 agar were picked to proceed with biochemical tests which include Triple Sugar Iron (TSI), Lysine Iron Agar (LIA) and urease tests. For TSI test, positive for *Salmonella* spp. will have acid (yellow) butt and alkaline

(red) slant and possible H<sub>2</sub>S production while for LIA test, it will have alkaline (purple) butt and alkaline (purple) slant and possible H<sub>2</sub>S production. *Salmonella* spp. are urease-negative thus no color change in urea agar. It was then confirmed serologically by using Slide Agglutination Test (SAT) with *Salmonella* polyvalent “O” and “H” antisera A-S.

### **3.2.2 *Campylobacter* spp.**

The swabs collected in the Oxoid Cary Blair transport medium was streaked directly onto Oxoid *Campylobacter* blood-free selective agar base (modified CCDA-Preston) incorporated with Oxoid CCDA selective supplement. The plates were incubated at 42°C for 48 hours under microaerophilic condition (5% oxygen, 10% carbon dioxide and 85% nitrogen) using an aerobic jar containing a gas generating packs (BD Campy Pak).

The presumptive *Campylobacter* colonies appear as irregular shape, moist, slightly raised, grey, mucoid and flat spreading colonies. For confirmation of *Campylobacter* spp., ‘hanging drop’ was used to identify *Campylobacter* and it was seen as curved, S- or gull-wing shaped with corkscrew or darting movement under 100X magnifications. Each presumptive colony was then subjected to Gram-staining and these organisms appear as Gram-negative, slender rods, comma shaped curved bacteria. Subculture was done on Columbia blood agar with 5% defibrinated horse blood added, and incubated at 42°C for 48 hours to obtain pure culture for biochemical tests.

Pure colonies suspected for *Campylobacter* spp. were subjected to 'hanging drop' technique or wet mount and Gram-staining were repeated on each pure colony before proceeding to biochemical tests. The biochemical tests include catalase, oxidase, urease, indoxyl acetate hydrolysis and hippurate hydrolysis tests. The species of *Campylobacter* spp. isolated corresponding to the biochemical tests are listed in Table 2 (Refer to plate 3).

**Table 2: *Campylobacter* spp. identification test**

<b>Biochemical test</b>	<b><i>C. jejuni</i></b>	<b><i>C. coli</i></b>	<b><i>C. lari</i></b>
<b>Oxidase</b>	+	+	+
<b>Catalase</b>	+	+	+
<b>Urease</b>	-	-	+
<b>Indoxyl acetate hydrolysis</b>	+	+	-
<b>Hippurate hydrolysis</b>	+	-	-

Key: + = positive, - = negative

### 3.3 ANTIBIOTIC SUSCEPTIBILITY TEST

#### 3.3.1 *Campylobacter* spp.

The isolates of *Campylobacter* spp. were subjected to antibiotic susceptibility test on Muller Hinton agar with 5% defibrinated horse blood added. The 6 antibiotics used were Ampicillin, Ciprofloxacin, Clindamycin, Erythromycin, Gentamycin, and Tetracycline. The diameter for zone of inhibition was measured by using vernier caliper and interpreted as sensitive, intermediate, or resistant based on the table below (Refer to Plate 4).

**Table 3 : Zone diameter interpretive standards and equivalent minimal inhibitory concentration (MIC) breakpoints for *Campylobacter* spp.**

Antimicrobial agent	Disc concentration (µg)	Zone diameter breakpoint (mm)		
		Sensitive	Intermediate	Resistant
Ampicillin	10	≥ 17	14-16	≤ 13
Ciprofloxacin	5	≥ 21	16-20	≤ 15
Clindamycin	2	≥ 21	15-20	≤ 14
Erythromycin	15	≥ 23	14-22	≤ 13
Gentamycin	10	≥ 15	13-14	≤ 12
Tetracycline	30	≥ 15	12-14	≤ 11

*Note.* From Clinical and Laboratory Standards Institute (CLSI), 2013

## 4.0 RESULTS

### 4.1 *Salmonella* spp. Isolation

A total of 50 samples birds were examined for *Salmonella* spp. in this study. However there was no *Salmonella* spp. isolated.

### 4.2 *Campylobacter* spp. Isolation

A total of 50 samples were examined for *Campylobacter* spp. in this study. For isolation of *Campylobacter*, out of 12 samples taken from Black swan, six samples were tested positive, out of five samples taken from White pelican two were tested positive and one sample taken from White swan was also tested positive. There was no isolation of *Campylobacter* in Egyptian goose, Flamingo, Rajdah Shellduck and Pink-backed pelican. Although the total prevalence only 18%, but the prevalence for *Campylobacter* isolated from each species was quite high. The prevalence for *Campylobacter* in Black swan was 50%, White pelican was 40% and White swan was 100%.

For sampling so it may not the true pictures of prevalence of *Campylobacter* spp. Table 4 presents the detailed information on the isolation *Campylobacter* spp. Table 5 presents the detailed information on the biochemical test on the isolated *Campylobacter* spp.

**Table 4: Isolation of *Campylobacter* spp.**

Species	No. of samples	<i>Campylobacter</i> spp.
Black swan	12	6
Egyptian goose	12	0
Flamingo	10	0
Rajah ShelDuck	8	0
White pelican	5	2
Pink-backed pelican	2	0
White swan	1	1
Total	50	9 (18%)

**Table 5: Biochemical test on the isolated *Campylobacter* spp.**

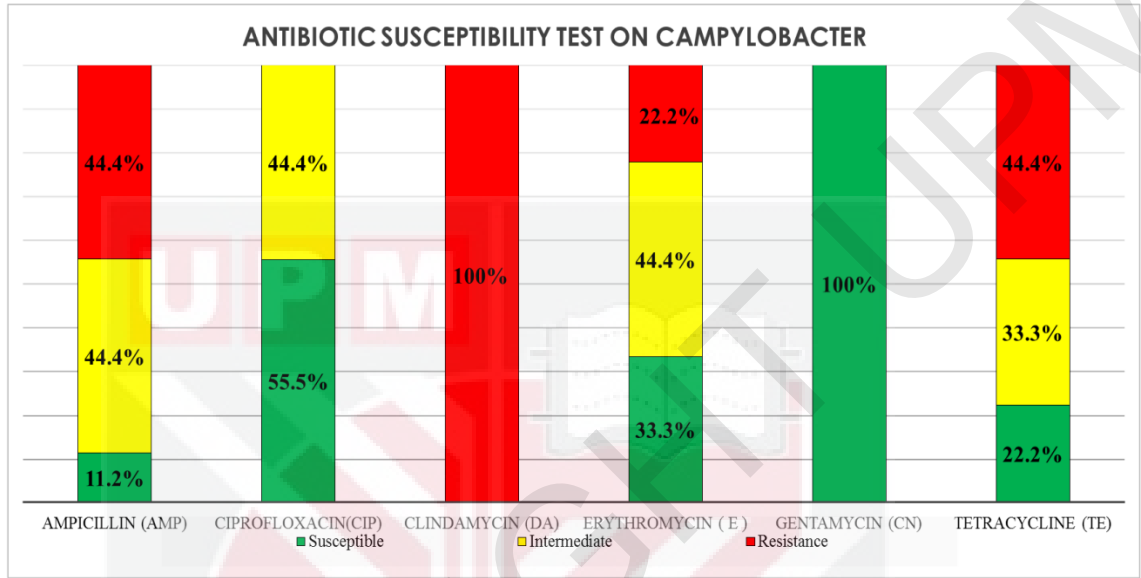
ID	Oxidase	Catalase	Urease	Indoxyl acetate	Hippurate hydrolysis	Results
Black swan 1	+	+	-	+	-	<i>C. Coli</i>
Black swan 3	+	+	-	+	-	<i>C. Coli</i>
Black swan 7	+	+	+	-	-	<i>C. lari</i>
Black swan 8	+	+	-	+	-	<i>C. Coli</i>
Black swan 10	+	+	-	+	-	<i>C. Coli</i>
Black swan 12	+	+	-	+	-	<i>C. Coli</i>
White pelican 3	+	+	-	+	-	<i>C. Coli</i>
White pelican 5	+	+	+	-	-	<i>C. lari</i>
White swan	+	+	-	+	-	<i>C. Coli</i>

### 4.3 Antibiotic susceptibility test of *Campylobacter* spp.

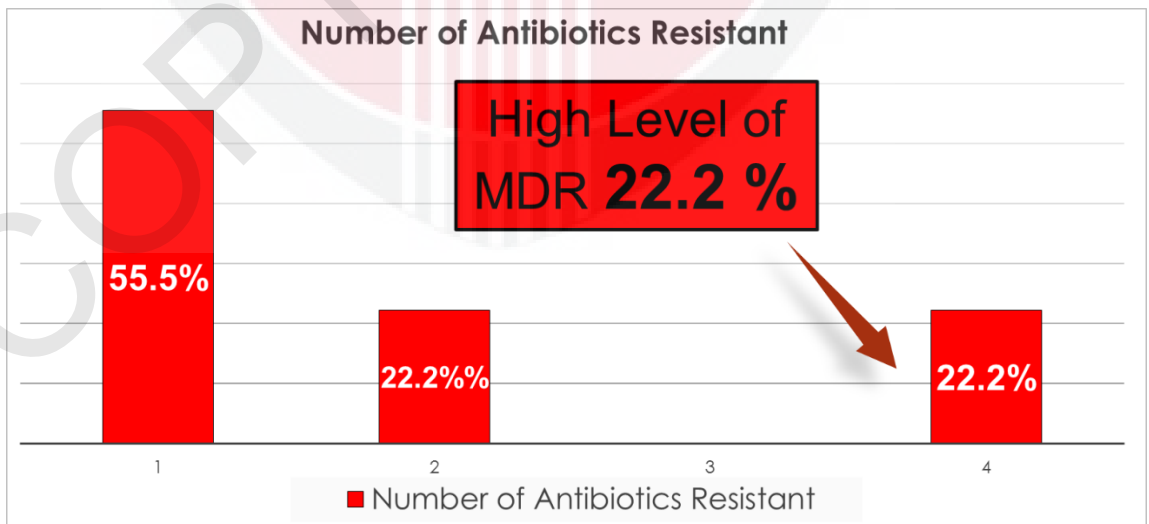
All the nine *Campylobacter* spp. isolates grew on MHA with 5% debrinated horse blood were all subjected to antibiotic susceptibility test against 6 antibiotics. A high percentage of isolates showed resistant to clindamycin (100%) ampicillin and tetracycline (44.4 %) as shown in Table 6 and Figure 1. While Figure 2 presents the detailed information on the percentage of *Campylobacter* spp. that resistant to number of antibiotics. Table 7 showed the number of *Campylobacter coli* and *Campylobacter lari* isolates that resistant to number of antibiotics.

**Table 6: Antibiotic susceptibility of *Campylobacter* spp. isolates against 6 antibiotics**

<b>Antibiotics</b>	<b>Percentage of Susceptible isolates (%)</b>	<b>Percentage of Intermediate isolates (%)</b>	<b>Percentage of Resistant isolates (%)</b>
<b>Ampicillin</b>	11.2	44.4	44.4
<b>Ciprofloxacin</b>	55.5	44.5	0
<b>Clindamycin</b>	0	0	100
<b>Erythromycin</b>	33.3	44.4	22.2
<b>Gentamycin</b>	100	0	0
<b>Tetracycline</b>	22.2	33.3	44.4



**Figure 1: Chart of Antibiotic Susceptibility Test on *Campylobacter* spp**



**Figure 2: Percentage of *Campylobacter* spp. Resistant to number of antibiotics**

**Table 7: Number of *Campylobacter coli* and *Campylobacter lari* isolates resistant to number of antibiotics**

<b>Number of antibiotics</b>	<b>Number and percentage (%) of isolate resistant to antibiotics</b>
<b>1 antibiotic</b>	5 (55.5%)
<b>2 antibiotics</b>	2 (22.2%)
<b>3 antibiotics</b>	0 (0.0%)
<b>4 antibiotics</b>	2 (22.2%)

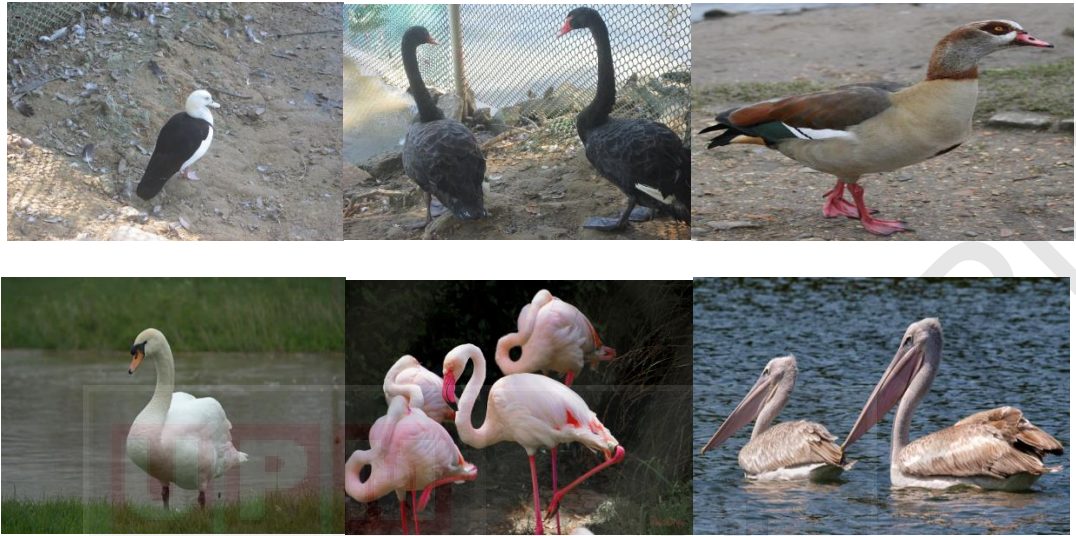


Plate 1: Subject of Study



Plate 2: Sample Collection Techniques

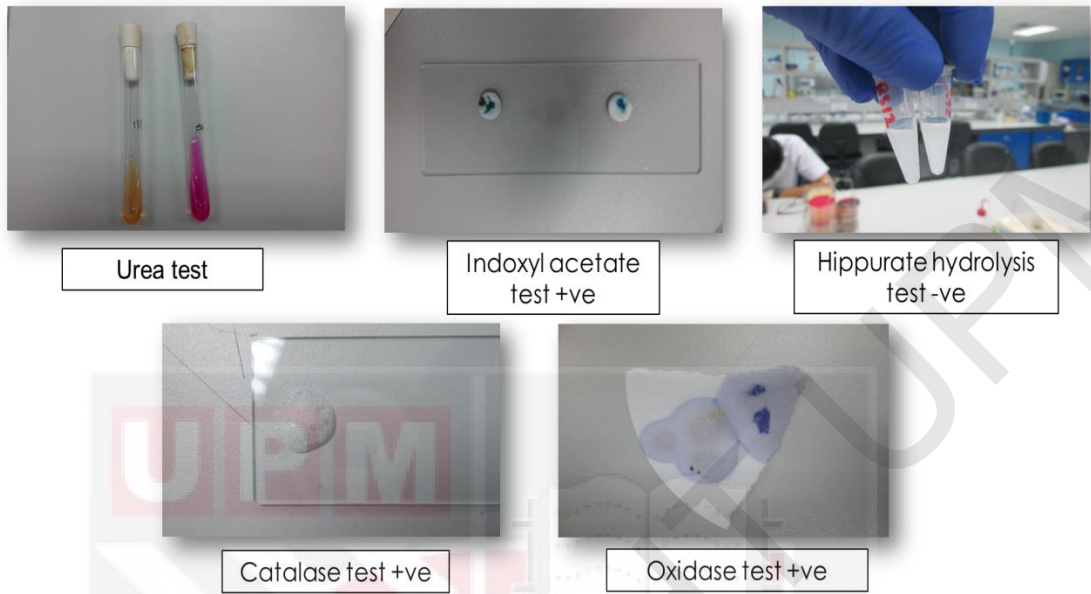


Plate 3 : *Campylobacter* spp. Identification Test

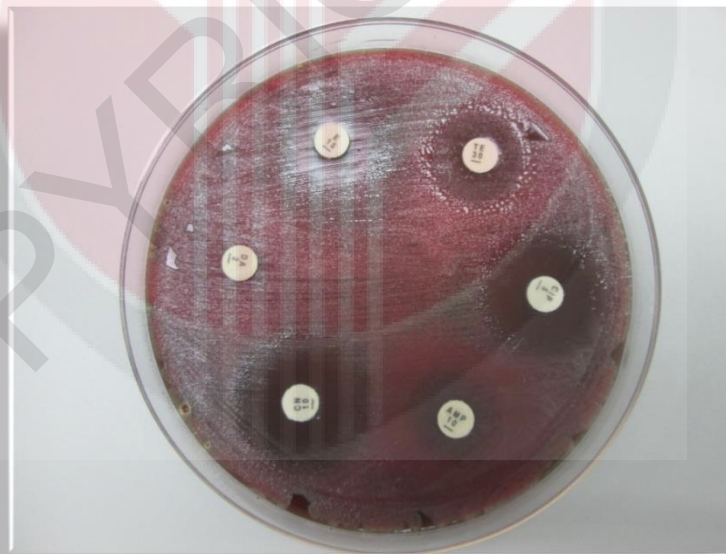


Plate 4: Antibiotic Susceptibility Test on *Campylobacter* spp. Isolates

## 5.0 DISCUSSION

In this study, the detection of *Salmonella* spp. in exotic birds showed that there was no *Salmonella* spp. isolated from the cloaca swab samples of Black swan, Egyptian goose, Rajdah Shelduck, White swan, White pelican, Pink-backed pelican and Flamingo.

There was no presence of *Salmonella* spp. could be due to several possible reasons. This includes the two main reasons which are host and environmental factor and methodology used in this study. The low occurrence already shared in previous studies in some areas. Only one sample was positive for *Salmonella* spp. out of 236 samples taken in migrating birds in Sweden (Jorge *et al.*, 2003). Of 125 birds sampled taken in Barnegat Bay, New Jersey, USA, none carried *Salmonella* spp. in seabird (Rivera *et al.*, 2012). According to Tizard (2004), the prevalence of *Salmonella* infection in healthy birds was expected to be low. Wild birds are often asymptomatic carriers of *Salmonella* spp. The prevalence of infection ranges from 1.9% in falconiformes birds to 8.7% in Ring billed gulls (Quessy *et al.*, 1992). From 1992 to 1997, six cases of *Salmonella typhimurium* infection have been diagnosed by the Quebec branch of the Canadian Cooperative Wildlife Health Centre from a total of 632 submissions of wild birds (Wilson *et al.*, 1967). Thus, low prevalence of salmonella in this study is similar to other research although there is limited study in exotic water birds.

The occurrence of *Campylobacter* spp. in this study was low. According to the study conducted by Faiza *et al.* (2012), the prevalence of *Campylobacter* spp. in duck farms in Selangor was 12% from 75 cloaca swab samples which is similar to this study. The presence of *Campylobacter* spp. in ducks which were apparently healthy showed that ducks can act as reservoirs for these organisms and spread them in the environment (Faiza *et al.*, 2012). Previous research also showed that there was no *Campylobacter* spp isolated from intestines of 54 seabirds in New Jersey USA (Rivera *et al.*, 2012). Similar study done by Ramos *et al.*, (2010) on seagull water birds at Mediterranean colonies showing low prevalence of *Campylobacter* spp. isolated from Medes Islands, Ebro Delta and Columbretes Islands.

The occurrence of *Campylobacter* isolates from duck farm in Selangor consisted of 22% *Campylobacter jejuni* and 88% *Campylobacter coli* from 12 positive samples (Faiza *et al.*, 2012). Throughout the world, researchers have found different *Campylobacter* species in wild birds, specifically isolating *C. jejuni*, *C. lari*, and *C. coli* from certain gull and thrush species among others (Waldenström *et al.*, 2002). The same families were tested in this study, yet only revealed to be positive for *C. coli* and *C. lari* but not *C. jejuni*.

MDR is multi drug resistance, define as acquired to at least one agent in three or more antimicrobial categories (Magiorokos *et al.*, 2011) In this study, there was high level of MDR from *Campylobacter* spp. isolated from exotic birds in Wetland, Putrajaya. There was limited study of *Campylobacter* spp. resistance in exotic water bird thus it is hard to compare the high level of MDR in this study to any other research.

Three conditions must be met for an antibiotic to be effective against bacteria which is a susceptible antibiotic target must exist in the cell, the antibiotic must reach the target in sufficient quantity, and the antibiotic must not be inactivated or modified (Dzidic *et al.*, 2008). Bacteria can develop resistance to antibiotics by mutating existing genes (vertical evolution) or by acquiring new genes from other strains or species (horizontal gene transfer). The sharing of genes between bacteria by horizontal gene transfer occurs by many different mechanisms. Mobile genetic elements, including phages, plasmids and transposons mediate this transfer, and in some circumstances the presence of low levels of the antibiotic in the environment is the key signal that promotes gene transfer, perhaps ensuring that the whole microbial community is protected from the antibiotic. Resistance to antibiotics can be caused by four general mechanisms which is the inactivation or modification of the antibiotic, an alteration in the target site of the antibiotic that reduces its binding capacity, the modification of metabolic pathways to circumvent the antibiotic effect and the reduced intracellular antibiotic accumulation by decreasing permeability and/or increasing active efflux of the antibiotic.

## 6.0 Conclusion

There was no occurrence of *Salmonella* spp. in exotic birds in Putrajaya Wetland. Prevalence of *Campylobacter* spp. isolated from exotic birds in Putrajaya Wetland was 18%. *Campylobacter* isolates identified were *C. coli* (78%) and *C. lari* (22%) where both are zoonotic. From this preliminary data, threats to human/wetland visitors by these organisms are considered at low risk. Twenty-two percent of the *C. coli* and *C. lari* isolates had a high level of multi-drug resistance. It is important to control antibiotic resistance before multidrug resistance in *Campylobacter* emerged into extensively drug resistance.

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