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OCCURRENCE OF CAMPYLOBACTER IN WILD BIRD, AND CHICKENS AND DUCKS IN SELECTED MALAYSIAN FARMS

MOHAMED YOUSIF IBRAHIM MOHAMED

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By

MOHAMED YOUSIF IBRAHIM MOHAMED

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MOHAMED YOUSIF IBRAHIM MOHAMED

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Chairman: Professor Dr. Saleha Abdul Aziz, PhD

Faculty: Veterinary Medicine

Poultry, in particular chickens and wild birds are reported to be frequently infected with *Campylobacter*. It is well recognized that *Campylobacter jejuni* is one of the main causes of gastroenteritis in humans, and poultry meat is reported to be the main source. A number of studies in the country had shown the occurrence of *Campylobacter* in chicken and chicken meat. Wild birds were observed in abundance at market places and farms. It was reported that wild birds may harbor and transmit *Campylobacter* to farm animals. However, very few studies had been carried out on *Campylobacter* in wild birds in Malaysia and the antibiotic resistance status of *Campylobacter* in these birds was not known. Thus, this study had three main objectives: the first objective was to determine the occurrence of *Campylobacter* in the wild birds in open environment away from poultry farms and those near poultry farms; secondly, to determine the prevalence of *Campylobacter* in poultry (chickens and ducks) in the farms and thirdly, to determine the antibiotic resistance patterns among *Campylobacter* isolates. The birds in open environment in five locations (>5 km away from poultry farms) (92) and near six poultry farms (66) were humanely trapped using mist net. The photograph of each bird was taken and the birds were given an identification mark; once the cloacal swab was taken, the birds were released. Six poultry farms were visited and cloacal swabs were taken from 101 chickens from three farms and 103 were taken from ducks in three duck farms. In the laboratory, the swabs were directly streaked onto selective agar media and incubated under microaerophilic condition for isolation of *Campylobacter*. Suspected colonies of *Campylobacter* were subjected to biochemical tests for phenotypic identification. The confirmation of the isolates was done using multiplex polymerase chain reaction (mPCR) assay. From 66 birds belonging to nine species trapped near the farms environment, 20% were positive for *Campylobacter* and out of these, 92.3% was *C. jejuni*. Eurasian Tree Sparrow (39.3%) followed by Rock Pigeon (34.8%) were frequently trapped. A total of 92 birds belonging to 12 species were caught in the open environment. The most frequent bird species identified positive to *C. jejuni* and *C. coli* was Eurasian Tree Sparrow (62%), followed by Rock Pigeon (25.8%). Twenty seven percent (27%) of the birds in open environment were found positive for *Campylobacter* and most of the isolates were identified as *C. jejuni* (60%). In the

farms, a total of 40% of poultry were *Campylobacter*-positive; of these 29% of the ducks were positive for *Campylobacter* spp. of which 80% were *C. jejuni*, while 20% were *C. coli*. In chickens, a high prevalence of *Campylobacter* spp. was isolated at 60.3% and all 100% were *C. jejuni*. The *Campylobacter* isolates were subjected to antibiotic susceptibility test using disc diffusion method. The isolates were tested against 12 antibiotics, namely clindamycin, erythromycin, tetracycline, streptomycin, cefotaxime, ampicillin, gentamicin, nalidixic acid, enrofloxacin, ciprofloxacin, sulfamethoxazole-trimethoprim and chloramphenicol. The *Campylobacter* isolates from wild birds in open environment showed resistance to 2-9 antibiotics. The highest resistance was to cefotaxime and clindamycin (92.9% each), followed by nalidixic acid and streptomycin (78.6% each), tetracycline (64.3%) and chloramphenicol (57.1%). Those birds around poultry farms showed resistance to 1-4 antibiotics and the highest resistance was to clindamycin (66.7%) and less than 50% to the other four antibiotics. The isolates from poultry showed resistance to 1-9 antibiotics and highest resistance was to clindamycin (87.7%), followed by erythromycin (69.2%) and tetracycline (63.1%). From this study, it was found that *Campylobacter* was prevalent in wild birds in the open environment and near poultry farms and in chickens and ducks in the farms. The *Campylobacter* showed high resistance to antibiotics. Therefore, the birds could play a role in the dispersal and spread of antibiotic resistant *Campylobacter* in the environment and poultry farms. Thus, the presence of *Campylobacter* in wild birds, poultry and the environment may cause health hazard to human upon exposure to the organisms.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains Veterinar.

KEHADIRAN *CAMPYLOBACTER* PADA BURUNG LIAR DAN AYAM ITIK DI LADANG

Oleh

MOHAMED YOUSIF IBRAHIM MOHAMED

Oktober 2014

Pengerusi: Profesor Saleha abdul Aziz , PhD

Fakulti: Perubatan Veterinar

Unggas, khususnya ayam, dan burung liar telah dilaporkan kerap dijangkiti *Campylobacter*. Adalah diakui bahawa *Campylobacter jejuni* adalah salah satu punca utama gastroenteritis pada manusia, dan daging ayam dilaporkan menjadi punca utama. Banyak kajian dalam negara menunjukkan kehadiran *Campylobacter* pada ayam dan daging ayam. Banyak bururg liar dijumpai di sekitar kawasan pasar dan ladang. Telah dilaporkan bahawa terdapat *Campylobacter* pada burung liar yang dapat disebarkan kepada haiwan ternakan. Terdapat sedikit sekali kajian mengenai kehadiran *Campylobacter* pada burung liar dan status kerintangan antibiotik *Campylobacter* tersebut tidak diketahui. Oleh itu, kajian ini mempunyai tiga objektif utama: Objektif pertama adalah untuk menentukan kehadiran *Campylobacter* pada burung liar di persekitaran terbuka jauh dari ladang ternakan; kedua, untuk mengesan kehadiran *Campylobacter* pada unggas liar di sekitar ladang ternakan dan daging ayam dan ketiga, untuk menuju pola rintangan antibiotik di kalangan pencilan *Campylobacter*. Burung liar dari lima persekitaran terbuka (>5 km jauh dari ladang ayam) (92) dan berhampiran enam ladang ternakan (66) telah diperangkap secara berperikemanusiaan menggunakan “mist net”. Gambar setiap burung diambil dan burung-burung diberi tanda pengenalan; setelah swab kloaka diambil, burung-burung dibebaskan. Enam ladang ternakan dilawati dan swab kloaka telah diambil daripada 101 ayam daripada tiga ladang dan 103 telah diambil daripada itik dalam tiga ladang itik. Dalam makmal, swab disaput terus ke atas media agar terpilih dan dieram di bawah keadaan mikroaerofilik untuk pengasingan *Campylobacter*. Ujian biokimia telah dijalankan ke atas isolat yang disyaki *Campylobacter* untuk pengenalpastian fenotip. Pengesahan kepada pencilan dilakukan dengan menggunakan PCR asei multipleks. Daripada 66 burung dalam kalangan sembilan spesies yang didapati berhampiran ladang, 20% adalah *Campylobacter* spp. yang mana 92.3% terdiri daripada *C. jejuni*. Eurasian Tree Sparrow (39.3%) diikuti oleh Rock Pigeon (34.8%) telah kerap terperangkap. Sebanyak 104 burung daripada 14 spesies telah terperangkap dalam persekitaran terbuka. Spesies burung yang paling kerap dikenalpasti adalah Eurasian Tree Sparrow (62%), diikuti oleh Rock Pigeon (25.8%). Dua puluh tujuh peratus (27%) burung dalam persekitaran terbuka didapati positif untuk *Campylobacter* dan kebanyakan pencilan telah dikenalpasti sebagai *C. jejuni*

(60%). Di ladang, sebanyak 40% daripada ayam adalah *Campylobacter* positif; dua puluh sembilan peratus 29% daripada itik didapati positif untuk *Campylobacter* spp. yang mana 80% adalah *C. jejuni*, manakala 20% adalah *Campylobacter coli*. Pada ayam, *Campylobacter* spp telah diasingkan sebanyak 60.3%, dan semua 100% adalah *C. jejuni*. Isolat *Campylobacter* dijalani ujian kerintangan antibiotik menggunakan kaedah cakera penyebaran. Isolat telah diuji terhadap 12 antibiotik, iaitu clindamycin, erythromycin, tetracycline, streptomycin, cefotaxime, ampicillin, gentamicin, asid nalidixic, enrofloxacin, ciprofloxacin, sulfamethoxazole-trimethoprim dan chloramphenicol. *Campylobacter* yang diasingkan daripada burung liar di persekitaran terbuka menunjukkan kerintangan terhadap 2-9 antibiotik. Rintangan tertinggi adalah untuk cefotaxime dan clindamycin (92.9% setiap satu), diikuti oleh asid nalidixic (78.6%), tetracycline (64.3%) dan chloramphenicol (57.1%). Unggas di sekitar ladang ternakan menunjukkan kerintangan kepada 1-4 antibiotik dan rintangan yang paling tinggi adalah untuk clindamycin (66.7%) dan kurang daripada 50% kepada empat antibiotik yang lain. Isolat *Campylobacter* yang diasingkan daripada ayam menunjukkan kerintangan kepada 1-9 antibiotik dan rintangan tertinggi adalah untuk clindamycin (87.7%), diikuti dengan erythromycin (69.2%) dan tetracycline (63.1%). Daripada kajian ini, didapati bahawa *Campylobacter* tersebar luas pada burung yang terbang di pesekitaran terbuka dan berhampiran ladang ayam dan itik. *Campylobacter* menunjukkan rintangan yang tinggi terhadap antibiotik. Oleh itu, burung-burung boleh memainkan peranan dalam penyebaran *Campylobacter* tahan antibiotik dalam persekitaran dan ladang ayam. Oleh itu, kehadiran *Campylobacter* pada burung liar, ayam dan itik dan persekitaran boleh menimbulkan kemudharatan kepada manusia akibat terdedah kepada organisma tersebut.

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: _____	Signature: _____
Name of Chairman of Supervisory Committee:	Name of Member of Supervisory Committee:
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Name of Member of Supervisory Committee:	Name of Member of Supervisory Committee:

This thesis was submitted to Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Veterinary Science. The members of supervisory committee were as follows:

Saleha Bt Abdul Aziz, PhD
Professer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Siti Khairani Binti Bejo, Phd
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Jalila Abu, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Puan Chong Leong PhD
Lecturer
Faculty of Forestry
Universiti Putra Malaysia
(Member)

BUJANG BIN Kim HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

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LIST OF ABBREVIATIONS

ATCC : American Type Culture Collection

^{0}C : Degree Celsius

CCUG : Culture Collection, University of Goteborg

CDC : Centers for Disease Control and Prevention

DNA : Deoxyribonucleic acid

ECDC : European Centre for Disease Prevention and Control

EFSA : Community Zoonoses Reports of the European Food Safety Authority

FAO : Food and Agriculture Organization

FoodNet : Foodborne Diseases Active Surveillance Network

km : Kilometer

MDR : Multidrug drug resistance

ml : Milliliter

m-PCR : Multiplex polymerase chain reaction

PCR : Polymerase chain reaction

PFGE : Pulsed field gel electrophoresis

rRNA : Ribosomal ribonucleic acid

V : Volt

DEDICATION

This thesis is specially dedicated to:

My beloved parents,

YOUSIF IBRAHIM MOHAMED ELNOHI

and

SIHAM TAHA SALIM HAMED

My beloved siblings,

AHLAM YOUSIF IBRAHIM MOHAMED,

ESRAA YOUSIF IBRAHIM MOHAMED,

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and

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Who always supported and encourage me to do the best.

CHAPTER 1

INTRODUCTION

Campylobacteriosis is a foodborne disease in humans caused by thermophilic *Campylobacter* spp., mainly *Campylobacter jejuni* and *Campylobacter coli* (Bahrndorff et al., 2013). *Campylobacter* are slender, spirally curved, gram-negative rods with a characteristic corkscrew-like darting motility. In comparison with other food-borne bacterial pathogens, *Campylobacter* are more fragile and require microaerobic conditions for growth (Park, 2002). The two species of *Campylobacter* are widespread among livestock and poultry, usually colonizing the intestines without causing clinical diseases (Doyle and Erickson, 2006). Animals harboring *Campylobacter* consistently shed the bacteria through feces, spreading within and among animal species on farms (Doyle and Erickson, 2006). Wild mammals and wild birds have been identified as possible reservoirs for *Campylobacter* transmission to agricultural animals because they carry *Campylobacter* spp. in their gut and also, these animals could be possible potential reservoirs for human (Horrocks et al., 2009; Kwan et al., 2008; Colles et al., 2003; Broman et al., 2002; Craven et al., 2000). Thus far, compylobacterosis has been reported in wild birds and mammals.

In human, *Campylobacter* infection could either be severe or mild depending on the individuals infected; for example, in young, old or people with weak immunity, it is known to have serious prolonged effect while in the middle aged people with strong immunity it is easily controlled without antibiotic administration (Smith and Fratamico, 2010). The common symptoms of *Campylobacter* infection include fever, diarrhea and abdominal pain (Butzler, 2004). In few cases, the infection can lead to severe complications, such as the Guillain-Barré syndrome which is an acute autoimmune neuropathy with ascending paralysis (Zilbauer et al., 2008). Human may acquire *Campylobacter* from sources such as untreated raw milk (Heuvelink et al., 2009) or water (Abe et al., 2008), but the consumption of poultry meat, particularly raw or inadequately cooked meat, constitutes the main risk factor for infection (Wingstrand et al., 2006). Potential sources and vectors for environmental contamination are infected livestock and free-living animals (Zweifel et al., 2008; Ellis-Iversen et al., 2009), rodents and flies (Hazeleger et al., 2008), contaminated surface water (Messens et al., 2009) and personnel and farm equipment (Ramabu et al., 2004) at the farm.

There is scarcity of information on the epidemiology of *Campylobacter* in wild birds compared to poultry. A study on *Campylobacter* spp. in black-headed gull found the prevalence ranged from 27.9 to 95.5%, depending on the species sampled (Broman et al., 2002). Studies on *Campylobacter* spp. in broiler chickens found the prevalence ranged from 40 to 60% (Broman et al., 2002).

For clinical therapy of campylobacteriosis, erythromycin (a macrolide) is adjudged the drug of preference, but fluoroquinolones (e.g., ciprofloxacin) are also commonly used

owing to their expansive continuum of activity against enteric pathogens (Luangtongkum et al., 2009). The results from a study on *Campylobacter* by Chen et al. (2010) showed that a vast majority of the isolates from birds appeared to be resistant to fluoroquinolones by more than 98%. In the same study, the *C. jejuni* was resistant to gentamicin at 27.2%. Importantly, previous study from The Netherlands revealed a substantial increase in fluoroquinolone resistance among human cases since the advent of enrofloxacin in veterinary medicine (Endtz et al., 1991). According to Zendehbad et al. (2013), *Campylobacter* spp. isolates from chicken, quail, partridge, and turkey meat in Mashhad (Iran) showed low resistance to streptomycin at 4.9%, 7.4% and 5.4%, respectively.

Alternative drugs used in cases of systemic infection with *Campylobacter* include tetracyclines and gentamicin (Ge et al., 2002). However, the growing resistance to *Campylobacter* is of a serious concern globally. *Campylobacter* is continuously exposed to antibiotics used in animal production and human medicine thus, causing it to become resistant to antibiotics which can create limited therapeutic options (Luangtongkum et al., 2009).

A number of studies in the country had shown the occurrence of *Campylobacter* in chicken and chicken meat. Wild birds were observed in abundance at market places and farms. Stald et al (2003) reported that wild birds may harbor and transmit *Campylobacter* to farm animals. However, very few studies had been carried out on *Campylobacter* in wild birds in Malaysia and the antibiotic resistance status of *Campylobacter* in these birds was not known.

The hypotheses of this study were:

i) There is a high occurrence of *Campylobacter* in wild birds, chickens and ducks.
ii) *Campylobacter* isolates showed high resistance to a number of antibiotics.

Thus, the objectives of this study were:

i. to determine the occurrence of *Campylobacter* in wild birds in open environment, poultry farms and in poultry (chickens and ducks), and
ii. to determine the antibiotic resistance patterns among *Campylobacter* isolates from wild birds and poultry.

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