UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION OF EMERGING Campylobacter SPECIES FROM BROILER CHICKEN AND CHICKEN MEAT IN SELANGOR AND THEIR ANTIBIOTIC RESISTANCE

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MASTER OF VETERINARY SCIENCE
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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Veterinary Science

May 2014

This thesis is dedicated to my Mom and Dad, bang Agung and dek Wahyu

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

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Chairperson : Professor Saleha Abdul Aziz, PhD

Faculty : Veterinary Medicine

Campylobacters have been recognized as the most common bacteria causing gastroenteritis in humans. *Campylobacter jejuni* and *C. coli* are the most isolated species from animals and cases of human campylobacteriosis. However, the common isolation technique used in laboratories usually does not support the growth of other, potentially pathogenic non-*jejuni*, non-*coli Campylobacter* species. The application of an isolation technique such as the Cape Town protocol developed by Le Roux and Lastovica (1998) can lead to the isolation of other *Campylobacter* species. The members of these non-*jejuni* and non-*coli Campylobacter* species discovered are termed as emerging *Campylobacter* species due to increase in incidence in humans since its first introduction; they consisted of five species and have been recognized as important pathogens in humans and animals. In Malaysia, most of the studies were on isolated *C. jejuni* and *C. coli* and almost no study on isolated emerging *Campylobacter* species have been done. Thus, this present study was undertaken to determine the occurrence of emerging *Campylobacter* species in broiler chicken and chicken meat and their antibiotic resistance patterns.

Two hundred and ten cloacal swab samples were taken from broiler chickens from seven different farms within Selangor. The samples were subjected to Cape Town protocol which combines membrane filtration onto antibiotic free blood agar plates and incubated at 37^{o}C in hydrogen enriched micro-aerobic atmosphere. *Campylobacter* were presumptively identified by biochemical tests and confirmed by using multiplex PCR. A hundred and seven of the chickens (50.9%) were positive for *Campylobacter*. Among the isolates, *C. jejuni* was the most isolated species (69.5%) followed by *C. coli* (16.2%). *Campylobacter fetus* and *C. upsaliensis* were the only emerging *Campylobacter* species isolated in this study, 11 (9.3%) and three isolates (2.5%) respectively. The prevalence of *Campylobacter* in broiler chicken raised in open house system farms were found higher (70%) compared to broiler chicken raised in close house system farms (3.3%). Close house system farms apply strict bio

security measure and hygiene practice that able to prevent *Campylobacter* transmission from environment to broiler flocks.

To determine the occurrence of emerging *Campylobacter* species in chicken meat, 109 chicken meat samples were collected from 11 wet markets and five supermarkets in Selangor. Thirty three samples (30.3%) were found positive for *Campylobacter.* Twenty of *Campylobacter*-positive isolates (60.6%) were identified as *C. jejuni*, while five isolates (15.1%) were *C. coli*. *Campylobacter fetus* was the only emerging *Campylobacter* species isolated in this study, at 24.3% or eight isolates. The prevalence of *Campylobacter* isolated from supermarkets was higher (68%) than those from wet markets (14.3%). This may happen due to the different way of displaying of chicken meat; where in the wet market, chicken meat were only displayed in room temperature that provokes *Campylobacter* to enter viable but non culturable (VBNC) condition that made them not able to be isolated by plating method used in this study.

An antibiotic susceptibility test was conducted to determine the antibiotic resistance pattern of these *Campylobacter* isolates. A total of 140 *Campylobacter* isolates were tested against 12 antibiotics by disc diffusion method. A total of 115 (97.4%) of *Campylobacter* isolated from broiler chicken and 33 (100%) of *Campylobacter* isolated from chicken meat were found resistant to one to ten antibiotics. In broiler chicken, more than 50% of *Campylobacter* isolates were found resistant to ampicillin, tetracycline, ciprofloxacin, nalidixic acid, enrofloxacin with the highest resistance was to nalidixic acid (79.6%) and the lowest resistance was to chloramphenicol (0.8%). In chicken meat, more than 50% of *Campylobacter* isolated from chicken meat were found resistant to trimethoprim/sulfamethoxazole, ampicillin, erythromycin, ciprofloxacin and nalidixic acid with the highest resistance was to ampicillin (87.8%) and the lowest resistance was to gentamicin (9.0%). Multidrug resistance (MDR) *Campylobacter* was observed in this study, 77.1% and 66.6% of *Campylobacter* isolated from broiler chicken and chicken meat respectively were resistant to three or more antibiotics.

Overall, the finding from this study indicated that Cape Town protocol is useful to isolate not only *C. jejuni* and *C. coli* but also other *Campylobacter* species. *Campylobacter jejuni* and *C. coli* were still dominant *Campylobacter* species in broiler chicken and chicken meat. Although the method is somewhat laborious, it however enables to isolate some emerging *Campylobacter* species (*C. fetus* and *C. upsaliensis*) which may cause the occurrence of diseases in humans. The public health significance of this study is the finding of other *Campylobacter* species in chickens and chicken meat that may cause gastroenteritis and other infections in humans and the presence of high MDR *Campylobacter* species which could compromise treatment in humans if required. Also from this study it is observed that there is a need to create awareness among farmers on the increase in antibiotic resistance and the prudent use of antibiotics in chickens among farmers.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk Master Sains Veterinar

PENCIRIAN SPESIS *Campylobacter* MUNCUL DARIPADA AYAM PEDAGING DAN DAGING AYAM DI SELANGOR DAN KERINTANGAN ANTIBIOTIK

Oleh

TEGUH SURANTA SINULINGGA

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Campylobacter telah diiktiraf sebagai bakteria yang paling lazim menyebabkan gastroenteritis pada manusia. *Campylobacter jejuni* dan *C. coli* adalah spesis yang paling kerap diasingkan daripada haiwan dan kes-kes kampilobakteriosis pada manusia. Walau bagaimanapun, teknik pengasingan yang biasa digunakan di makmal tidak menyokong pertumbuhan *Campylobacter* lain yang bukan spesis *C. jejuni* dan *C. coli*, yang berpotensi patogenik. Penggunaan teknik pengasingan seperti protokol Cape Town yang dibangunkan oleh Le Roux dan Lastovica (1998) membawa kepada pengasingan spesis *Campylobacter* lain. Spesis *Campylobacter* bukan *jejuni* dan bukan *coli* ini digelar sebagai spesis *Campylobacter* muncul kerana insidens yang meningkat pada manusia sejak mula dikenali; mereka meliputi lima spesis dan telah diiktiraf sebagai patogen penting pada manusia dan haiwan. Di Malaysia, kebanyakan kajian dapat mengasing hanya *C. jejuni* dan *C. coli* dan hampir tiada dapat mengasing *Campylobacter* muncul. Oleh kerana itu, kajian ini dijalankan untuk menentukan kehadiran spesis *Campylobacter* muncul pada ayam pedaging dan daging ayam dan corak kerintangan antibiotik keatas isolat *Campylobacter.*

Dua ratus sepuluh sampel swab kloaka diambil daripada ayam pedaging di tujuh ladang yang berbeza di Selangor. Sampel dikultur dengan mengguna protokol Cape Town yang menggabungkan penapisan membran keatas agar darah tanpa antibiotik dan pengeraman pada suhu 37^{o}C dalam suasana mikroaerobik yang diperkaya hidrogen. *Campylobacter* dikenalpasti dengan menggunakan ujian biokimia kemudian disahkan dengan menggunakan multipleks PCR. Satu ratus tujuh ayam (50.9%) adalah positif terhadap *Campylobacter*. Diantara isolat positif, *C. jejuni* adalah spesis yang paling kerap diasingkan (69.5%) diikuti oleh *C. coli* (16.2%). *Campylobacter fetus* dan *C. upsaliensis* adalah spesis *Campylobacter* muncul yang berjaya diasingkan dalam kajian ini, masing-masing sebanyak 11 isolat (9.3%) dan tiga isolat (2.5%). Ayam pedaging yang dibesarkan dalam sistem kandang terbuka didapati lebih tinggi (70%) terdedah kepada *Campylobacter* berbanding dengan ayam pedaging yang dibesarkan di ladang sistem kandang tertutup (3.3%). Ladang

sistem kandang tertututup menerapkan secara ketat langkah keselamatan bio dan amalan kebersihan yang dapat mencegah penyebaran *Campylobacter* daripada persekitaran untuk ternakan ayam pedaging.

Untuk menentukan kehadiran spesis *Campylobacter* muncul pada daging ayam, 109 sampel daging ayam diperoleh daripada 11 pasar basah dan lima pasar raya di Selangor. Tiga puluh tiga sampel daging ayam (30.3%) didapati positif terhadap *Campylobacter*. Dua puluh daripada isolat positif *Campylobacter* (60.6%) dikenal pasti sebagai *C. jejuni*, manakala lima isolat (15.1%) adalah *C. coli*. *Campylobacter fetus* merupakan satu-satunya spesis *Campylobacter* muncul yang diasingkan dalam kajian ini yaitu sebanyak lapan isolat (24.3%). Kelaziman *Campylobacter* terpencil daripada daging ayam yang dijual di pasar raya adalah lebih tinggi (68%) dibandingkan dengan daging ayam yang dijual di pasar basah (14.3%). Ini mungkin berlaku kerana cara yang berbeza memaparkan daging ayam; di mana di pasar basah, daging ayam hanya dipaparkan dalam suhu bilik yang menimbulkan *Campylobacter* masuk pada keadaan berdaya maju tetapi tidak dapat diasingkan (VBNC) yang menjadikan mereka tidak berupaya untuk diasingkan dengan kaedah yang digunakan dalam kajian ini.

Ujian kerintangan antibiotik juga dijalankan untuk menentukan corak rintangan antibiotik *Campylobacter* yang telah diasingkan. Sebanyak 140 *Campylobacter* yang telah diasingkan pada kajian ini diuji untuk kerintangan antibiotik kepada 12 agen antibiotik dengan kaedah "disk diffusion". Sebanyak 115 (97.4%) daripada *Campylobacter* yang diasingkan daripada ayam pedaging dan 33 (100%) yang diasingkan daripada daging ayam didapati rintang terhadap 1-10 antibiotik. Pada ayam pedaging, lebih daripada 50 % daripada *Campylobacter* yang diasingkan didapati rintang terhadap ampicillin, tetracycline, ciprofloxacin, asid nalidixik, enrofloxacin dengan rintangan yang paling tinggi adalah terhadap asid nalidixik (79.6 %) dan rintangan yang paling rendah adalah terhadap chloramphenicol (0.8%). Pada daging ayam, lebih daripada 50% daripada *Campylobacter* didapati rintang terhadap trimethoprim/sulfamethoxazole, ampicillin, erythromycin, ciprofloxacin dan asid nalidixik dengan rintangan yang paling tinggi adalah terhadap ampicillin (87.8%) dan rintangan yang paling rendah adalah terhadap gentamicin (9.0%). Rintangan terhadap pelbagai antibiotik (MDR) pada *Campylobacter* telah didapati dalam kajian ini, sebanyak 77.1 % dan 66.6% daripada *Campylobacter* diasingkan daripada ayam pedaging dan daging ayam rintang terhadap tiga atau lebih antibiotik.

Secara keseluruhan, didapati daripada kajian ini bahawa protokol Cape Town berguna untuk mengasingkan bukan sahaja *C .jejuni* dan *C. coli* tetapi juga spesis *Campylobacter* muncul. *Campylobacter jejuni* dan *C. coli* masih merupakan spesis *Campylobacter* dominan yang ditemui pada ayam pedaging dan daging ayam. Walaupun kaedah ini agak susah, ia bagaimanapun membolehkan untuk mengasingkan beberapa spesis *Campylobacter* muncul (*C. fetus* dan *C. upsaliensis*) yang telah dilaporkan boleh menyebabkan berlakunya penyakit pada manusia. Kepentingan kesihatan awam daripada kajian ini adalah penemuan spesis "emerging" *Campylobacter* pada ayam pedaging dan daging ayam yang boleh menyebabkan gastroenteritis dan jangkitan penyakit lain pada manusia. Kehadiran MDR *Campylobacter* yang tinggi dapat menjejaskan rawatan pada manusia sekiranya diperlukan. Juga daripada kajian ini, perlu diadakan kesedaran kepada peternak

mengenai meningkatnya rintangan antibiotik dan amalan penggunaan antibiotik yang baik pada ayam.

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May God bless you always.

TEGUH SURANTA SINULINGGA

I certify that a Thesis Examination Committee has met on 26th June 2014 to conduct the final examination of Teguh Suranta Sinulingga on his thesis entitled "Antibiotic Resistance in Emerging *Campylobacter* Species Isolated from Broiler Chicken and Chicken Meat in Selangor" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Veterinary Science.

Members of the Thesis Examination Committee were as follows

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This thesis was submitted to the 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:

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CHAPTER 1

INTRODUCTION

Campylobacter species are one of the leading causes of zoonotic enteric infections in developed and developing countries and the incidence are reported to be increasing (Anonymous, 2000). The majority of incidence occurs sporadically and the highest risk is associated with consumption of poultry, contact with infected animals, drinking contaminated water or travel (Everest and Ketley, 2001).

Campylobacter jejuni is the most frequently studied member of the *Campylobacter* genus followed by *C. coli* and both organisms are zoonotic, causing infections in human. There are also a number of other *Campylobacter* species of relevance in human diseases (Crushell et al., 2004). However, the role of *Campylobacter* species other than *C. jejuni* and *C. coli* in clinical diseases in human are far less well understood; this is because relatively few strains of these organisms have been isolated and not all laboratories use the same method to detect these species (Anonymous, 2000; Lastovica, 2006). Furthermore, current laboratory methods usually allow only the growth of *C. jejuni* and *C. coli* and not other *Campylobacter* species. Most diagnostic laboratories use 42 ^{o}C as primary incubation temperature, which is suitable for *C. jejuni* and *C. coli* but not other species, such as *C. fetus* or *C. hyointestinalis*, which grow at 37 ^{o}C (Lastovica, 2006). The usage of antibiotics in the formulation of the selective media may also inhibit the growth of some *Campylobacter* species (Corry et al., 1995).

One method that is currently used for isolation of almost all known species of *Campylobacter* is the Cape Town protocol (Diegaardt et al., 2003). It uses filtration through a membrane filter onto antibiotic free blood agar plate and subsequent incubation at 37 ^{o}C in hydrogen enriched microaerobic atmosphere. It was reported that culture positive for *Campylobacter* in faeces rose to 21.8% after implementation of this protocol (Lastovica, 2006). However, to date there is no standard method for isolation of *Campylobacter* species from food or faecal samples.

Campylobacter species are frequently found in many domestic and wild mammalian, including cattle, swine, goats, horses, dogs, cats, and rodents, and avian species, including poultry and birds (Man, 2011). *Campylobacter* have also been isolated from marine animals such as shellfish and dolphins. Among all these animals, poultry is considered as the main reservoir of *Campylobacter*. The prevalence of *Campylobacter* in poultry may vary according to age and the type of farm. *Campylobacter* are rarely detected in broiler chicken less than 2-3 weeks old and in those raised in close house system (Sahin et al., 2002). Once a broiler chicken becomes infected, *Campylobacter* spread rapidly to other broiler chickens in that flock, which remain colonized up to slaughter age. In processing plant, such colonized chickens may cause carcass contamination during processing (Jacobs-Reitsma et al., 1994).

Up to 100 % broiler chickens brought to the slaughter houses may harbour *Campylobacter* (Jacobs-Reitsma et al., 1994). The contaminated chicken meat acts as a potential risk of campylobacteriosis in human. According to European Food Safety Authority (2010), 20 – 30 % of human cases of campylobacteriosis may be associated with handling, preparation and consumption of chicken meat. *Campylobacter* are able to survive on soiled food contact and even some clean surfaces for more than 4 hours at 27 ^{o}C and 60 – 62 % relative humidity (De Cesare et al., 2003); they can remain viable at -20 and -70 ^{o}C and are able to replicate at 4 ^{o}C (Lee et al., 1998).

Rapid increases in the proportion of *Campylobacter* resistance to antibiotics have been reported in many countries worldwide. The improper usage of antibiotics in food animals is one of the possible ways that can lead to the increase in antibiotic resistance. *Campylobacter* have been reported resistant to a number of antibiotics including ciprofloxacin and other fluoroquinolones, macrolides and licosamides, chloramphenicols, aminoglycosides, tetracyclines, ampicillins and other β-lactams, cotrimoxazole, and tylosin (Padungton and Kaneene, 2003; Moore et al., 2006).

According to Woolhouse (2002), emerging pathogen can be defined as an organism which show increasing incidence following its first introduction into a new host population. In this case, there is an increasing number of *Campylobacter* species other than *C. jejuni* and *C. coli* that have been recognized as human and animal pathogens. For instance, *C. upsaliensis* and *C. concisus* are now recognized causing diarrhoea and intestinal inflammation in humans (Patton et al., 1988; Bourke, 1998; Labarca, 2002; Man, 2011), and *C. rectus* is considered to be associated with human periodontal disease (Rams et al., 1993). These *Campylobacter* species are termed as emerging *Campylobacter* species.

In Malaysia, most of studies on *Campylobacter* practice the common isolation techniques which are mainly able to isolate *C. jejuni* and *C. coli*. However they were not able to detect the presence of other *Campylobacter* species including emerging *Campylobacter* species. The improper use of antibiotics in farm animals still occurs until today. Therefore, the hypotheses of this study were:

1. There is the presence of emerging *Campylobacter* in broiler chicken and chicken meat.

2. The occurrence of antibiotic resistant in *Campylobacter* isolated from broiler chickens and chicken meat is high.

The objectives of this study were:

1. To determine the occurrence of emerging *Campylobacter* in broiler chickens and chicken meat

2. To determine the species *Campylobacter* isolated

3. To determine the antibiotic resistant pattern of the emerging and common *Campylobacter* species isolates

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