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OCCURRENCE AND ANTIBIOTIC RESISTANCE OF Campylobacter AND Arcobacter spp. IN DOGS AND CATS

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By

MOHAMMED DAUDA GONI

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Master of Science

August 2014

DEDICATIONS

I would like to dedicate this work to my parents

Alh. Mohammed Goni and **Hajiya Aishatu Goni**

Who are my inspiration and for their affection, love, continuous support and prayers

My stepmother **Hajiya Fatsuma Goni**

And my siblings

For their continuous support and prayers

In memory of my Late Uncle

Alh. Ibrahim Abubakar Danbauchi

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

OCCURRENCE AND ANTIBIOTIC RESISTANCE OF *Campylobacter* AND *Arcobacter* spp. IN DOGS AND CATS

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August 2014

Chairman: Professor Saleha Abdul Aziz, PhD

Faculty: Veterinary Medicine

Campylobacter and *Arcobacter* are becoming more recognised because of their detection in a wide range of hosts and food of animal origin. *Campylobacter* is considered one of the most common causes while *Arcobacter* has emerged as a cause of gastro-enteritis in humans and both are of public health concern. They are gram negative, curved, spiral or S- shaped and are members of the order *Campylobacterales*, class *Epsilon* and phylum *Proteobacteria.* Several studies have been conducted in developed countries on their occurrence and characterisation in dogs and cats but such studies are lacking in most developing nations like Malaysia. Due to this present scenario, this study was conducted to determine the presence of *Campylobacter* and *Arcobacter* in dogs and cats and to also determine antibiotic resistance patterns of the isolates.

The presence of these organisms was determined using conventional and molecular techniques. For *Arcobacter*, rectal and buccal swab samples were collected from owned dogs (40) and cats (40) presented to the University Veterinary Hospital (UVH), Universiti Putra Malaysia (UPM) and a private veterinary clinic within Kuala Lumpur, and stray dogs (61) and cats (46) from an animal shelter and Dewan Bandaraya Kuala Lumpur (DBKL) dog pound. Rectal swabs were also taken for the detection of *Campylobacter* in these animals. Suspected colonies of the two organisms were subcultured and subjected to biochemical tests which included catalase, oxidase, hippurate hydrolysis and indoxyl acetate hydrolysis tests. Multiplex polymerase chain reaction (mPCR) was employed for the confirmation of the suspected isolates and differentiation of species. Overall, the results showed carriage rates of 32.6% (15/46) and 12.5% (5/40) of *Campylobacter* in stray and client owned cats respectively, while *Arcobacter* was detected in 34.78% (16/46) and 45% (18/40) in stray and client owned cats respectively. In stray dogs, *Campylobacter* and *Arcobacter* were detected at 16.3% (10/61) and at 50% (31/61) respectively. *Arcobacter butzleri* was the only species that was isolated and

Campylobacter upsaliensis (60%), *C. helviticus* (20%) and *C. jejuni* (11.4%) were the species of *Campylobacter* isolated.

Risk factors for *Campylobacter* and *Arcobacter* infections in dogs and cats were determined through questionnaires filled by pet owners. Among the factors that were looked at included age, sex, breed category, single or multi-pet household, recent treatment with antibiotic, housing of the dogs and cats, source of drinking water, contact with other animals, consumption of raw meat and fish and place of residence of the owner. Factors found to significantly increase the risk for *Arcobacter* infections were multi-pets household type and source of drinking water in cats while none were significant in the case of dogs. None of the factors analyzed was significant in terms of the occurrence of *Campylobacter* in both dogs and cats.

Antibiotic resistance pattern using minimum inhibitory concentration (M.I.C) and disc diffusion methods were carried out. Eighty nine (89) *Arcobacter butzleri* and 28 *Campylobacter* isolates were tested against 12 antibiotics using the disc diffusion method namely ciprofloxacin (Cip) 5µg; ampicillin (Amp), 10 µg; tetracycline (Te), 30 µg; erythromycin (E), 15 µg; gentamicin (CN), 10 µg; cefotaxime (CTX), 30 µg; penicillin G (P), µg; streptomycin (S), µg; nalidixic acid (NA), µg; enrofloxacin (Enr), µg; amoxicillin/clavulanic acid (AMC), µg and ceftazidine (CAZ), µg. Four antibiotics, namely ampicillin, tetracycline, erythromycin and ciprofloxacin were used against *Campylobacter* and *Arcobacter butzleri* isolates for the M.I.C. Overall, the isolates were found resistant to at least one antibiotic using both techniques. For *Campylobacter* isolates, the resistance to the antibiotics using the disc diffusion method was as follows: ciprofloxacin (17.8%), gentamycin (32.1%), cefotaxime (42.8%), penicillin G (53.5%), tetracycline (32.1%), ampicillin (42.8%), erythromycin (50%), streptomycin (42.8%), nalidixic acid (46.4%), amoxycillin/clavulanic acid (17.8%), ceftazidine (50%) and enrofloxacin (21.4%). Using the M.I.C.E. strip (Oxoid), *Campylobacter* and *A. butzleri* isolates showed exception in the resistance to ciprofloxacin. For *Arcobacter* isolates the resistance to antibiotics using the disc diffusion was as follows: ciprofloxacin (2.1%), gentamicin (63.1%), cefotaxime (69.4%), penicillin G and ampicillin (98.9%), tetracycline (33.6%), erythromycin (53.6%), streptomycin (85.2%), nalidixic acid (61.0%), amoxycillin/clavulanic acid (43.1%), ceftazidine (3.6%) and enrofloxacin (31.5%). In comparison, the resistance rates between the disc diffusion and M.I.C. were not significantly different. The antibiotic resistance showed and 35 patterns for *Campylobacter* and *Arcobacter* isolates respectively. *Campylobacter* isolates were found resistant to nine (9) antibiotics while *Arcobacter* showed resistance to ten (10) antibiotics. Multi drug resistance (MDR) was reported among 50% and 78.9% of *Campylobacter* and *Arcobacter* isolates respectively.

It can be concluded that the occurrence of *Campylobacter* and *Arcobacter* species in dogs and cats is of great public health significance as pets are in close contact with humans. Good management and controlling the population of stray dogs and cats are key factors in preventing the spread of *Campylobacter* and *Arcobacter* in these animal species. Antibiotic resistance in *Campylobacter* and *Arcobacter* not only increases the risk of treatment failure in both humans and animals but also spreads antibiotic resistance genes.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KEHADIRAN DAN KERINTANGAN ANTIBIOTIK *Campylobacter* DAN *Arcobacter* spp. PADA ANJING DAN KUCING

Oleh
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MOHAMMED DAUDA GONI

Ogos 2014

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Campylobacter dan *Arcobacter* menjadi lebih dikenali kerana pengesanan mereka pada pelbagai perumah dan makanan berasaskan haiwan. *Campylobacter* dianggap sebagai salah satu penyebab yang paling lazim dan *Arcobacter* telah muncul sebagai penyebab gastroenteritis pada manusia dan kedua-duanya membimbangkan kesihatan awam. Bakteria ini bersifat gram negatif, berbentuk melengkung, lingkaran atau berbentuk S dan merupakan anggota dalam *order Campylobacterales*, kelas *Epsilon* dan filum *Proteobacteria*. Beberapa kajian telah dijalankan di negara maju mengenai kejadian dan pencirian organisma tersebut pada anjing dan kucing tetapi kajian seumpama ini sangat kurang di kebanyakan negara sedang membangun seperti Malaysia. Berikutan daripada senario tersebut, kajian ini telah dijalankan untuk menentukan kehadiran *Campylobacter* dan *Arcobacter* pada anjing dan kucing dan juga menentukan corak kerintangan antibiotik isolat.

Kehadiran organisma ini telah ditentukan dengan menggunakan kaedah konvensional dan molekular. Untuk *Arcobacter*, sampel swab rektum dan bukka telah diambil daripada anjing (40) dan kucing (40) peliharaan yang dibawa ke Hospital Veterinar Universiti (UVH), Universiti Putra Malaysia (UPM) dan satu klinik veterinar swasta di Kuala Lumpur, serta anjing liar (61) dan kucing liar (46) di pusat perlindungan haiwan dan tempat simpanan anjing di Dewan Bandaraya Kuala Lumpur (DBKL). Swab rektum juga diambil bagi pengesanan *Campylobacter* pada haiwan tersebut. Koloni bakteria yang disyaki disubkultur, dan menjalani ujian biokimia termasuk *catalase*, *oxidase*, *hippurate hydrolysis* dan *indoxyl acetate hydrolysis*. *Multiplex polymerase chain reaction* (mPCR) telah dilakukan bagi pengesahan dan menentukan spesis isolat. Secara keseluruhannya, keputusan menunjukkan kadar peratus *Campylobacter* adalah 32.6% (15/46) pada kucing liar dan 12.5% (5/40) pada kucing kesayangan, manakala *Arcobacter* dikesan sebanyak 34.78% (16 /46) pada kucing liar dan 45% (18 /40) pada kucing peliharaan. Pada anjing liar,

Campylobacter dikesan sebanyak 16.3% (10/61) dan 50% (31/61) untuk *Arcobacter*. *Arcobacter butzleri* adalah satu-satunya spesis yang diasingkan dan *Campylobacter upsaliensis* (60%), *C. helveticus* (20%) dan *C. jejuni* (11.4%) adalah spesis *Campylobacter* yang telah diasingkan.

Faktor berisiko bagi jangkitan *Campylobacter* dan *Arcobacter* pada anjing dan kucing ditentukan secara statistik melalui soal-selidik yang diisi oleh pemilik haiwan kesayangan. Antara faktor yang dilihat termasuk umur, jantina, jenis baka, haiwan kesayangan yang terdapat dalam isi rumah sama ada tunggal atau pelbagai, rawatan terkini haiwan yang disampel dengan antibiotik, tempat tinggal anjing dan kucing, sumber air minuman, sentuhan dengan haiwan lain, pengambilan daging dan ikan mentah dan tempat kediaman pemilik. Faktor berisiko yang signifikan ke atas jangkitan *Arcobacter* adalah pelbagai jenis haiwan kesayangan di rumah dan sumber air minuman bagi kucing manakala tidak signifikan bagi anjing. Faktor yang di analisis bagi kehadiran Campylobacter pada anjing dan kucing didapati tidak signifikan.

Corak kerintangan antibiotik dilakukan menggunakan kaedah *Minimum Inhibitory Concentration* (M.I.C) dan *disk diffusion*. Lapan puluh sembilan (89) isolat *Arcobacter butzleri* dan 28 isolat *Campylobacter* telah diuji terhadap 12 antibiotik menggunakan kaedah *disk diffusion* iaitu *ciprofloxacin* (Cip) 5µg ; *ampicillin* (Amp), 10 µg ; *tetracycline* (Te), 30 µg ; *erythromycin* (E), 15 µg ; *gentamicin* (CN), 10 µg ; *cefotaxime* (CTX), 30 µg ; penisilin G (P), 10µg ; *streptomycin* (S), 10µg ; asid nalidixik (NA), 5µg ; *enrofloxacin* (ENR), 5µg ; *amoxicillin* / asid klavulanik (AMC), 10µg dan *ceftazidine* (Caz), 30µg. Empat antibiotik iaitu *ampicillin*, *tetracycline*, *erythromycin* dan *ciprofloxacin* telah digunakan terhadap 16 isolat *Campylobacter* dan *A. butzleri* untuk kaedah M.I.C. Secara keseluruhannya, isolat didapati rintang terhadap salah satu antibiotik menggunakan kedua-dua kaedah. Untuk isolat *Campylobacter*, kerintangan terhadap antibiotik menggunakan kaedah *disk diffusion* adalah seperti berikut: *ciprofloxacin* (17.8%), *gentamicin* (32.1%), *cefotaxime* (42.8%), penisilin G (53.5%), *tetracycline* (32.1%), *ampicillin* (42.8%), *erythromycin* (50%), *streptomycin* (42.8%), asid nalidixic (46.4%), *amoxycillin* / asid klavulanik (17.8%), *ceftazidine* (50%) dan *enrofloxacin* (21.4%). Dengan menggunakan strip M.I.C.E (Oxoid), isolat *Campylobacter* dan *Arcobacter butzleri* tidak menunjukkan kerintangan terhadap *ciprofloxacin*. Untuk isolat *Arcobacter*, kerintangan antibiotik dengan menggunakan *disk diffusion* adalah seperti berikut: *ciprofloxacin* (2.1%), *gentamicin* (63.1%), *cefotaxime* (69.4%), penisilin G dan *ampicillin* (98.9%), *tetracycline* (33.6%), *erythromycin* (53.6%), *streptomycin* (85.2%), asid nalidixik (61.0%), *amoxycillin* / asid klavulanik (43.1%), *ceftazidine* (3.6%) dan *enrofloxacin* (31.5%). Secara perbandingan, tiada perbezaan signifikan bagi kadar kerintangan antibiotik di antara *disk diffusion* dan MICE. *Campylobacter* menunjukkan 18 corak kerintangan dan *Arcobacter* menunjukkan 35 corak. Isolat *Campylobacter* didapati menunjukkan kerintangan terhadap sembilan antibiotik manakala *Arcobacter* menunjukkan kerintangan terhadap sepuluh antibiotik. Kerintangan multi-drug dilaporkan di kalangan 50% isolat *Campylobacter* dan di kalangan 78.9% isolat *Arcobacter*.

Dapat disimpulkan bahawa pengesanan spesis *Campylobacter* dan *Arcobacter* pada anjing dan kucing mempunyai kepentingan kesihatan awam disebabkan ianya berhubungan rapat dengan manusia. Pengurusan yang baik dan kawalan populasi anjing dan kucing liar adalah faktor utama dalam mencegah penyebaran

Campylobacter dan *Arcobacter* pada spesis haiwan tersebut. Kerintangan antibiotik pada *Campylobacter* dan *Arcobacter* tidak hanya boleh meningkatkan risiko kegagalan rawatan pada kedua-dua manusia dan haiwan malah dapat menyebarkan gen kerintangan antibiotik.

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I certify that a Thesis Examination Committee has met on 21 August 2014 to conduct the final examination of Mohammed Dauda Goni on his thesis entitled "Occurrence and Antibiotic Resistance of *Campylobacter* and *Arcobacter* spp. In Dogs and Cats" 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 Science.

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

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LIST OF ABBREVIATIONS

ATCC	American Type Culture Collection
bp	Base pairs
CCUG	Culture Collection of the University of Goteborg
cueE	Siderophoreenterochelin
CLSI	Clinical Laboratory Standard Institute
^{0}C	Degree celcius
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic
flaA	Flagellin A gene
glyA	Serine hydroxyl methyl transferase gene
g	Gram(s)
h	Hour(s)
hip	Hippuricase gene
Kb	Kilobase
ml	Mililitre
mg	Milligram
mg	Milligram(s)
min	Minute(s)
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration
MICE	Minimum Inhibitory Concentration Evaluator
mPCR	Multiplex Polymerase Chain Reaction
NaCl	Sodium chloride
NCCLS	National Committee for Clinical Laboratory Standards
PFGE	Pulsed Field Gel Electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid

rRNA	Ribosomal RNA
s	Second(s)
Spp.	Species
TAE	Tris-acetate-EDTA
TBE	Tris-borate EDTA
UV	Ultraviolet
V	Volt
Taq	Thermophilus aquaticus
WHO	World Health Organization
µL	Micro liter
µg	Micro Gram
µM	Micro molar

CHAPTER 1

INTRODUCTION

Arcobacter is widely regarded as an emerging food-borne pathogen because of its relationship with food production and human health. *Arcobacter* was initially recognized as 'aerotolerant *Campylobacter'* belonging to the family *Campylobacteraceae,* genus *Campylobacter* due to its phenotypic and phylogenetic resemblance with *Campylobacter*. However, the ability to grow at 15°C and its aerotolerance distinguishes it from *Campylobacter* (Vandamme and De Ley, 1991). From the discovery of *Arcobacter* in 1977 to date, various species have been identified and discovered in various animals which include domestic animals, pets, wild animals, birds and food products originating from domestic animals and may result in diseases such as mastitis, abortion and diarrhoea in animals (Collado and Figueras, 2011). Ten species have so far been identified of which seven are regarded as emerging food-borne pathogens namely: *A. butzleri*, *A. skirrowii*, *A. cryaerophilus*, *A. cibarius*, *A. mytili*, *A. thereius*, and *A. trophiarum*. *Arcobacter butzleri*, *A. skirrowii*, and *A. cryaerophilus* have been isolated from faecal samples of human beings and healthy farm animals (Driessche *et al*., 2005; Merga *et al*., 2011). However, the current identification and detection method of *Arcobacter* species is difficult and cumbersome thereby the incidence is most likely underestimated (Vandenberg *et al.*, 2004)

Arcobacter transmission is usually through the consumption of contaminated food and drinking water; infection can also occur through direct contact with infected animals and humans (Corry and Atabay, 2001). The organism is ubiquitous and can be found in sewage, surface water, sea water, ground water and drinking water which suggests their wider presence in the environment which can serve as an alternative means of exposure and transmission of infection to animals and humans (Fera *et al.*, 2009; Fera *et al.*, 2004; Moreno *et al.*, 2003). There are a substantial number of studies on the epidemiology of *Arcobacter* globally, but these are limited to livestock animals and not much has been done on their presence in dogs and cats in South East Asia in general and Malaysia in particular.

Campylobacter is the most widely reported bacterial agent that causes enteric disease in human beings across the world with millions of cases recorded yearly (WHO 2012). It is the most reported zoonotic enteric disease in Europe exceeding salmonellosis (Fosse *et al.*, 2007). There are about 37 species and sub species of *Campylobacter* which can be classified as thermophilic and non thermophilic *Campylobacter* based on their ability to grow at 42^{0}C and 37^{0}C (Weese, 2011). *Campylobacter* enteritis is a zoonosis of significant public health concern and it has indeed been shown to be a greater problem than *Salmonella* infection in some countries (Rosef *et al.*, 1983). The first human isolate of *Campylobacter* was from blood samples of patients suffering from diarrhoea in 1938. It was reported that there was difficulty isolating from faecal samples due to the fastidious nature of *Campylobacter* and contamination by the faecal flora (Kulkarni *et al.*, 2002). The pathogenic effect produced by *Campylobacter* in dogs is less common (Koene *et al.*,

2004). However, gastroenteritis caused by *Campylobacter* has been reported in healthy animals and they have been shown to carry the organisms (Baker *et al.*, 2008; Hald and Madsen, 1997).

Healthy dogs and cats have been shown to be carriers with the carriage rate as high as 50% for *C. upsaliensis* and *C. jejuni* as the most predominant species isolated. In human, *C. jejuni* is the most dominant species isolated followed by *C. coli* (Carbonero *et al.*, 2012b; Hald *et al.*, 2004; Jaime *et al.*, 2002).

The consumption of undercooked poultry, handling of raw poultry, drinking of untreated water, drinking unpasteurised milk or dairy products produced from non-thermo treated milk, and international travel are all considered to pose a risk to *Campylobacter* infection (Danis *et al.*, 2009). *Campylobacter* and *Arcobacter* infection in humans can be due to exposure and frequent contact with dogs and cats having diarrhea. This infection in man is reported to be due to pets ownership and has been identified and reported as a risk factor for its transmission to humans (Tenkate and Stafford, 2001). The increase in number of dogs and cats kept as pets may thus lead to the increase in *Campylobacter* and *Arcobacter* infection in human.

Campylobacteriosis is often self limiting in humans but is also associated with complications at the later stage of the infection such as Guillain-Barre Syndrome (neurological) and Reiter's Syndrome (reactive arthritis) (Yan *et al.*, 2005). *Arcobacter* infections in man are more frequently associated with persistent and watery diarrhoea and less associated with bloody diarrhoea (Vandenberg *et al.*, 2004).

The use of antibiotics in domestic animals has been widely reported to cause the development of antibiotic resistance. In pet animals, this problem is of public health significance due to close contact with human resulting in transmission of antibiotic resistant organisms.

A very limited study on *Arcobacter* has been carried out in Malaysia but none in dogs and cats. However the prevalence of *Arcobacter* in various sources ranging from chicken, beef, fresh raw dairy milk, and treated water in cattle farms have been reported in Malaysia (Shah *et al.*, 2012; Amare *et al.*, 2011;). Several studies on *Campylobacter* have been reported in chicken and other domestic animals but only one study in cats and dogs.

The hypotheses of this study were:

1. There is a low presence of *Campylobacter* and *Arcobacter* in dogs and cats.
2. The occurrence of antibiotic resistance in *Campylobacter and Arcobacter* isolated from dogs and cats is high.

Thus, the objectives of the study were:

1. To determine the prevalence of *Campylobacter* and *Arcobacter* in dogs and cats and associated risk factors
2. To determine the species of *Campylobacter* and *Arcobacter* isolates
3. To determine the antibiotic resistance of the isolates.

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