UNIVERSITI PUTRA MALAYSIA

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

MOHAMMED DAUDA GONI

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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
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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

By

MOHAMMED DAUDA GONI

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

MOHAMMED DAUDA GONI

Ogos 2014

Pengerusi : Professor Saleha Abdul Aziz, PhD

Fakulti : Perubatan Veterinar


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 13.75% (5/37) 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 diasangkan dan Campylobacter upsaliensis (60%), C. helveticus (20%) dan C. jejuni (11.4%) adalah spesis Campylobacter yang telah diasangkan.

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 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 ; penisinil G (P), 10 μg ; streptomycin (S), 10 μg ; 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%), penisinil G (32.1%), tetracycline (32.1%), ampicillin (42.8%), erythromycin (42.8%), streptomycin (50%), asid nalidixic (46.4%), amoxicillin / asid klavulanik (46.4%), 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%), penisinil G dan ampicillin (98.9%), tetracycline (33.6%), erythromycin (53.6%), streptomycin (85.2%), asid nalidixic (61.0%), amoxicillin / 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 dan 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 kedu-dua manusia dan haiwan malah dapat menyebarkan gen kerintangan antibiotik.
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My special gratitude and thanks go to my family, relatives and all well wishers for their prayers and support.
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.

Members of the Thesis Examination Committee were as follows:

**Latiffah binti Hassan, PhD**  
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Abdul Rahim bin Abdul Mutalib, PhD**  
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Internal Examiner)

**Malaika Watanabe, PhD**  
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Internal Examiner)

**Kasing Apun, PhD**  
Associate Professor  
Faculty of Resource Science and Technology  
Universiti Malaysia Sarawak  
(External Examiner)

---

**ZULKARNAIN ZAINAL, PhD**  
Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 23 January 2015
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:

**Saleha Abdul Aziz, PhD**  
Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Chairman)

**Zunita Zakaria, PhD**  
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Gurmeet Dhaliwal, PhD**  
Associate Professor  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
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<tr>
<td>°C</td>
<td>Degree celcius</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic</td>
<td></td>
</tr>
<tr>
<td>flaA</td>
<td>Flagellin A gene</td>
<td></td>
</tr>
<tr>
<td>glyA</td>
<td>Serine hydroxyl methyl transferase gene</td>
<td></td>
</tr>
<tr>
<td>g</td>
<td>Gram(s)</td>
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<tr>
<td>h</td>
<td>Hour(s)</td>
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<tr>
<td>hip</td>
<td>Hippuricase gene</td>
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<tr>
<td>Kb</td>
<td>Kilobase</td>
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<tr>
<td>ml</td>
<td>Millilitre</td>
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<td>min</td>
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<tr>
<td>MDR</td>
<td>Multidrug resistance</td>
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<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
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<tr>
<td>MICE</td>
<td>Minimum Inhibitory Concentration Evaluator</td>
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<tr>
<td>mPCR</td>
<td>Multiplex Polymerase Chain Reaction</td>
<td></td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
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</tr>
<tr>
<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
<td></td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed Field Gel Electrophoresis</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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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°C and 37°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.,
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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