OCCURRENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF *CAMPYLOBACTER SPP*. FROM STRAY CATS IN KOTA BHARU, KELANTAN

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By

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A RESEARCH PAPER SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF VETERINARY MEDICINE

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OCCURRENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF *CAMPYLOBACTER SPP.* FROM STRAY CATS IN KELANTAN.

ABSTRACT

An abstract of the research paper presented to the Faculty of Veterinary Medicine, Universiti Malaysia Kelantan, in partial requirement for the course DVT 55204 – Research Project.

Campylobacter spp., are common in animal gastrointestinal tract, which its opportunism can cause diarrhoea and enteritis in humans and animals. Their zoonotic potential raises concerns about stray cat interactions with humans. Antimicrobial resistance (AMR) in *Campylobacter* further complicates treatment, posing a global public health threat. Therefore, the aim of this study is to investigate the occurrence and antimicrobial resistance patterns of *Campylobacter spp*. in stray cats from Kelantan, Malaysia. Out of 61 samples collected, *Campylobacter jejuni* was detected in 4 samples, while *Campylobacter coli* was not identified. No significant associations between *Campylobacter jejuni* prevalence and variables like age, gender, and breed. However, body condition score (P = 0.0154) and health status (P = 0.0039) were significantly associated with the prevalence of *Campylobacter jejuni* thus making malnourished or unhealthy cats more likely to have *Campylobacter jejuni.* Antimicrobial resistance testing on the other hand revealed complete resistance (100%) to Trimethoprim, Nalidixic Acid, Ceftazidime, and Compound Sulfonamide. Partial resistance was observed for Streptomycin (66.67%), while other antibiotics, including Ciprofloxacin and Enrofloxacin, showed varied resistance levels (33.33% resistant, intermediate, and susceptible). Overall in conclusion, this study found a low occurrence rate of *Campylobacter jejuni* from stray cats in Kota Bharu, Kelantan, with malnourished and sick cats at higher risk of infection and improvement of the animal welfare plus enhancement of public health measures are very much needed.

Keywords: *Campylobacter spp*, Antimicrobial resistance (AMR), stray cats, zoonotic potential, Public health

KEJADIAN DAN POLA RINTANGAN ANTIMIKROBA *CAMPYLOBACTER SPP.* DI KALANGAN KUCING TERBIAR DI KELANTAN.

ABSTRAK

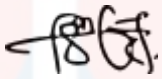
Abstrak kertas penyelidikan yang dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Malaysia Kelantan, sebagai sebahagian daripada keperluan kursus DVT 55204 – Projek Penyelidikan.

Campylobacter spp. sering ditemui dalam sistem pencernaan haiwan, di mana sifat oportunisnya boleh menyebabkan cirit-birit dan enteritis pada manusia dan haiwan. Potensi zoonotiknya menimbulkan kebimbangan tentang interaksi kucing jalanan dengan manusia. Ketahanan antimikrob (AMR) dalam *Campylobacter* pula menyukarkan rawatan, menjadikannya ancaman kesihatan awam global. Oleh itu, kajian ini bertujuan menyiasat kehadiran dan corak ketahanan antimikrob *Campylobacter* spp. dalam kucing jalanan di Kelantan, Malaysia. Daripada 61 sampel yang dikumpulkan, *Campylobacter jejuni* dikesan dalam 4 sampel, manakala *Campylobacter coli* tidak dikenal pasti. Tiada hubungan signifikan antara kelaziman *Campylobacter jejuni* dengan pembolehubah seperti umur, jantina, dan baka. Namun, skor keadaan badan (P = 0.0154) dan status kesihatan (P = 0.0039) menunjukkan hubungan signifikan dengan kelaziman *Campylobacter jejuni*, di mana kucing yang kekurangan zat atau tidak sihat lebih berisiko dijangkiti. Ujian ketahanan antimikrob mendapati rintangan sepenuhnya (100%) terhadap Trimethoprim, Nalidixic Acid, Ceftazidime, dan Compound Sulfonamide. Rintangan separa diperhatikan untuk Streptomycin (66.67%), manakala antibiotik lain seperti Ciprofloxacin dan Enrofloxacin menunjukkan tahap rintangan yang pelbagai (33.33% tahan, sederhana, dan sensitif). Kesimpulannya, kajian ini mendapati kadar kejadian *Campylobacter jejuni* yang rendah dalam kucing jalanan di Kota Bharu, Kelantan, dengan kucing yang kurang zat dan sakit lebih berisiko dijangkiti. Penambahbaikan kebajikan haiwan serta langkah kesihatan awam yang lebih baik amat diperlukan.

Kata kunci: *Campylobacter* spp., Rintangan antimikrob (AMR), kucing terbiar, potensi zoonotik, Kesihatan awam

CERTIFICATION

This is to certify that we have read this research paper entitled **'Occurrence And Antimicrobial Resistance Patterns Of *Campylobacter Spp.* From Stray Cats In Kelantan.'** by **Effa Ilyana Binti Zaffrulla**, and in our opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirements for the course DVT 55204 – Research Project.



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DEDICATIONS

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LIST OF ABBREVIATIONS

PCR	Polymerase Chain Reaction
DNA	Deoxyribose Nucleic Acid
mCCDA	Modified Charcoal Cefoperazone Deoxycholate Agar
AST	Antibiotic Sensitivity Test
AMR	Antimicrobial resistance
MHA	Mueller Hinton Agar
CAT	Cefoperazone, Amphotericin And Teicoplanin
DSH	Domestic Short Hair
DLH	Domestic Long Hair

LIST OF SYMBOLS

%	Percentage
°C	Celsius
Cm	Centimeter
mL	Milliliter
µL	Microlitre
w/v	Weight per volume
V	Voltage
mA	Milliampere

CHAPTER 1

INTRODUCTION

1.1 Research Background

Domestic animals that are not owned, especially pets like dogs and cats, are known as stray animals of which their characteristics include living mostly on their own, reproducing without control, overcrowding the environment, and frequently becoming an ongoing nuisance and threat to environmental and public health (Sandøe *et al*., 2019, Voslářová and Passantino, 2012, Abdulkarim *et al*., 2021). These categories of animals could pose a risk for dissemination and spread of pathogens in the environment and across different species of animals.

The *Campylobacter* species are distributed around the world and some of them are commensals in the healthy animals' gastrointestinal tracts and mucosa of the oral cavities (Markey *et al*., 2013). There are some non-pathogenic *Campylobacte*r variants present environmentally as saprophytes (Markey *et al*., 2013). The vast majority of *Campylobacter spp.* are pathogenic, causing intestinal and reproductive illness in both humans and animals (McVey *et al.*, 2022) thus making *Campylobacter* as one of the contributors towards infectious gastrointestinal diseases (Uzal, 2022) associated with enteritis, enterocolitis, irritable bowel syndrome and acute self-limiting bloody diarrhea (Markey *et al*., 2013, CDC 2021).

Globally, *Campylobacter* is one of the most prevalent foodborne pathogens that can cause foodborne illness (Bolinger & Kathariou, 2017). Certain species of *Campylobacter* are potentially zoonotic thus having a pet such as cats and dogs has been linked to an increased risk of contracting *campylobacteriosis* within human population although common source of infection are via consumption of contaminated animal products or livestock (Giacomelli *et al*., 2015, Goni *et al*., 2017) therefore making increasing incidence of *Campylobacter* enteritis as one of the public health concerns from a socio-economic perspective as the potential interactions between these stray animals and people, particularly children, that may occur in many different countries. (WHO, 2020; Slater, 2001).

Antimicrobial resistance (AMR) occurs when bacteria, viruses, fungi, and parasites stop responding to antibiotics drugs which makes those drugs to be futile and therefore treating infections increasingly become challenging or impossible thus raises the risk of infection spread,

serious illness, disability, and death, causing it to be a true challenge to modern medicine (WHO, 2021, CDC, 2021), especially when Campylobacter AMR transmissions to humans occur after consuming Campylobacter-contaminated animal products or exposure to infected animals. According to the United States Department of Health and Human Services, an estimated 1.5 million *Campylobacter* infections occur annually whereby 29% of those infections have a lower susceptibility to antibiotics such as fluoroquinolones or macrolides (Irfan *et al*., 2010, CDC, 2024). Insufficient data on the epidemiology of *Campylobacte*r cases, especially in pets and food safety norms makes it difficult to estimate and control campylobacteriosis in South-East Asian countries, including Malaysia (Premarathne *et al*., 2017, Goni *et al*., 2017). Therefore, assessment of the antimicrobial resistance pattern of *Campylobacter spp*. is crucial in Malaysia.

1.2 Problem Statement

Campylobacter isolates in cats are less studied as compared to livestock animals because consumption of contaminated, raw animal products and unpasteurized milk are the more common way of campylobacteriosis in humans rather than close association or contact with cats or pets. The occurrence of *Campylobacter* in dogs and cats in Selangor has been studied and reported by Goni *et al*., 2017, but the occurrence of *Campylobacter* in cats has not yet been studied in Kota Bharu. Besides, pathogenic *Campylobacter spp*. that are antimicrobial resistant are harder to rely on antibiotic treatment, especially in severe cases therefore the socio-economic concern, and in fact that campylobacteriosis is one of the largest contributors to social-economic loss (WHO,2021).

1.3 Research Questions

I. What is the rate of occurrence of *Campylobacter spp*. in stray cats?

II. What are the *Campylobacter* species isolated in stray cats?

III. Which antibiotics are resistant to *Campylobacter spp.* Isolated from stray cats?

1.4 Research Hypothesis

I. *Campylobacter spp.* can be isolated from stray cats.

II. There are multiple isolates of *Campylobacter spp*. that can be found in cats.

III. Different antibiotics pose different susceptibility to *Campylobacter spp.* isolate in cats.

1.5 Significance of the Study

The significance of this study is to determine the antibiotics that are resistant to *Campylobacter* isolates in stray cats within Kota Bharu diameter. On the other hand, with an increasing population of stray cats, this study will provide the data on the presence of *Campylobacter* in cats and their roles in the dissemination of these pathogen and zoonotic potentials. *Campylobacter* isolated such as *Campylobacter jejuni* and *Campylobacter coli* may pose minor symptoms or even subclinical in cats but rather enterocolitis in humans (McVey *et al.*, 2022). These research findings might be able to assist in raising awareness and management of food-borne illnesses of campylobacteriosis linked with close contact with cats in Kota Bharu, especially within wet market compounds. Data obtained pertaining to this study may strengthen the core responsibilities of one health constitution.

1.6 Research Objectives

1. To determine the occurrence of *Campylobacter spp.* in stray cats.
2. To identify *Campylobacter spp*. isolated from stray cats.
3. To identify the antibiotic resistance patterns of *Campylobacter* species isolates.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of *Campylobacter spp.*

Significant revisions have been made in several studies on taxonomy of the genus *Campylobacter*, it shows that the family *Campylobacteraceae,* order *Campylobacterales,* class *Epsilonproteobacteria,* phylum *Proteobacteria* and consisting of three genera which are *Campylobacter, Arcobacte*r and *Helicobacter* species (Markey *et al*., 2013, Facciolà *et al*., 2017, Fitzgerald *et al*., 2011) due to similarities on genotypes and phenotypes of those three genera (Acke, 2018, Vandamme *et al.* 2005). The genus *Campylobacter* consists of nine species overall with two species further classified into subspecies, and one species classified into three biovars (McVey *et al.*, 2022), although there are extensive changes that make Ngulukun , (2017) reviewed and suggested that there are 27 species, and 8 subspecies of the genus *Campylobacter* identified currently. The species such as *Campylobacter fetus, Campylobacter jejuni ssp. jejuni, Campylobacter coli, Campylobacter upsaliensis, Campylobacter lari, Campylobacter hyointestinalis, Campylobacter helveticus*, *Campylobacter sputorum*, and *Campylobacter mucosalis* are the species of veterinary importance where over 90% of gastroenteritis cases in humans are caused by *C. jejuni* and *C. coli,* to a lesser extend but mild to subclinical signs in cats and dogs. (McVey *et al.*, 2022, Marks *et al.* 2011, Bolton , 2015). The method of direct membrane filtration onto agar medium containing many different antibiotics was the first successful isolation of *Campylobacter* from stool specimens of patients with diarrhea which was achieved in Belgium in 1968 and described in 1972 (Tellez-Isaias *et al.*, 2022).

2.2 Morphology and Characteristics of *Campylobacter spp.*

Campylobacter spp. are microaerophilic Gram negative, non-spore-forming, capsulated, non-saccharolytic bacteria. *Campylobacters* thrive well in an environment with a low oxygen tension of 5% oxygen, 10% carbon dioxide, 85% nitrogen, and media supplemented with 5-10% blood

(McVey *et al.*, 2022, Garénaux A *et al.*, 2008). The shape ranges from slender curved rods, or as spirals with a width of 0.2-0.5 μm and length of 0.5-5 μm (Markey *et al*., 2013, McVey *et al.*, 2022). Sometimes, in bacterial cultures that are left for a long period or exposed to oxygen, the shape of the bacteria will vary between spherical or coccoid bodies (Ngulukun , 2017). Most *Campylobacter* species are motile with the presence of single polar flagellum, except *Campylobacter gracilis* and *Campylobacter showae* that are immobile with the absence of flagella and have multiple flagella respectively (Debruyne L *et al.*, 2008). Certain *Campylobacter spp.* are thermophilic and grow optimally at 42°C with a minimum temperature for growth of 32°C (Acke, 2018; Tellez-Isaias et al., 2022). Apart from that, the pH values range from 4.9, 6.5 to 7.5, and 9.5 for minimum, optimum and maximum values respectively for *Campylobacter spp.* growth (Park Sf., 2002, Silva J *et al*., 2011, Tellez-Isaias *et al*. 2022). *Campylobacter* are considered as non-saccharolytic bacteria because they do not oxidize carbohydrates due to the fact that they have an incomplete glycolytic pathway known as Embden-Meyerhof-Parnas (EMP) as a result of lacking in hexose catabolism enzymes such as phosphofructokinase and glucokinase but rather amino acids and intermediates of the tricarboxylic acid cycle are the sources of energy for *Campylobacter spp*.. (Vandamme *et al.* 1991, Yeow *et al*., 2020, McVey *et al.*, 2022) . The DNA of *Campylobacter* is approximately 1.6 to 1.7 Mbps and consists of high adenine and thymine with GC ratio at about 30% (Owen *et al.* 1981, A. Facciolà *et al.*, 2017).

2.3 Transmission of *Campylobacter spp.*

Transmission of *Campylobacter* can occur via fecal-oral route (WHO, 2020). Typically, campylobacteriosis is obtained via ingestion of contaminated food and water, either with the fecal material of the infected animal itself or contaminated environment. Kothary MH *et al.* , 2001 described that an infective dose of 500 of *C. jejuni* can cause clinical manifestations in humans. In humans, consumption of raw or contaminated poultry has been linked to higher risk exposure of obtaining campylobacteriosis in humans as chickens with an infection may have as much as 105–108 CFU/g of microorganisms in their faeces whereby this elevated levels allow bacteria to proliferate readily in the surroundings, enabling the contamination (Keener KM *et.al*,

2004). In cats on the other hand, similarly transmission is via fecal-oral route. However, some cats and dogs are known to be hosts for certain *Campylobacter spp.* (A. Facciolà *et al*., 2017).

2.4 Prevalence of Campylobacteriosis in cats

The reported prevalence, particularly in cats and dogs, depends on the diversification of several risk factors as discussed in (*6.5*), the geographic location, and most importantly, the study methodology which overall can affect the reported prevalence (Marks *et al.*, 2011, Mustafa Yildiz *et al*., 2023). Study comparisons of campylobacteriosis are challenging due to the variability of results based on the diagnostic techniques used but nevertheless, incorporating multiple culture techniques has been shown to increase the recovery of various species and increase overall prevalence (Frasao *et al*., 2017). Goni *et al*., 2017 reported in their cross-sectional study of the occurrence of *Campylobacte*r in dogs and cats in Selangor, Malaysia that in dogs, the total prevalence of *Campylobacter* was 14.85% whereas 23.25% in cats which in both cats and dogs, *C.upsaliensis* predominated then followed by *C.helveticus.*

2.5 Risk factors of occurrence of Campylobacteriosis in cats

Campylobacteriosis in cats can be caused by several reasons, including age, housing, the presence of coexisting diseases or infections with other enteropathogenic organisms, animal signalment, season, and previous recent antibiotic therapy. (Goni *et al*., 2017, E Acke, 2018). The occurrence of infections in immature cats is higher than in adult cats, indicating that young animals are more vulnerable to infection since their immune systems have not been exposed to the pathogen before (Selwet *et al.*, 2015, Acke, 2018). Animals that are kept in extensive housing have a higher risk of horizontal *Campylobacter* transmission, given their limited living spaces, stress as well as altered diets (Leahy *et al.* 2017). Apart from that, multiple studies have verified that animals with diarrhea had a greater frequency of Campylobacteriosis than animals without diarrhea (Carbonero *et al.* 2012; Verma *et al.* 2014, Acke, 2018). Compared to cats, dogs exhibit a higher prevalence of campylobacteriosis (Marks *et al.* 2011). A cross-sectional study in Iran has shown that the prevalence of cats infected with *Campylobacter* is higher in summer while lower in spring (Torkan *et al*. 2018). Nonetheless, compared to pets without a

history of antibiotic use, those with a recent record of antibiotic administration had reduced *Campylobacter* infection (Goni *et al*., 2017).

2.6 Antimicrobial resistance of Campylobacter strains

In many clinical laboratories, antimicrobial susceptibility testing (AST) for *Campylobacter spp*. is not typically done regularly. The emergence of isolates that are resistant, however, highlights the significance of AST (Ge *et al.*, 2013). Antimicrobial susceptibility tests can be done by using several methods and techniques. Some of the examples of laboratory techniques for this test are the Minimal Inhibitory Concentration (MIC) and Kirby-Bauer Disk Diffusion Susceptibility Test. MIC can be done with the broth microdilution technique (Azrad *et al.*, 2018). Mustafa Yildiz *et al.*, (2023) reported that upon accommodating the MIC method with broth microdilution technique, his study revealed that the percentage of *C. jejuni* isolates that were resistant to ciprofloxacin and nalidixic acid, was 7.7% and 19.2%, respectively whereas 3.8% for both tetracycline and gentamicin.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Study Design

This study implemented a cross-sectional study design in which randomly collected samples were processed and analyzed for the presence or absence of *Campylobacter spp.* infection from a stray cat population within Kota Bharu district at one point of time.

3.2 Study Population

Based on calculator.net (Figure1), the minimum sample size for the study was supposed to be 370 with an estimation of the total population of 10,000 stray cats in Kelantan. However, the sample size was reduced to 60 cats only due to limited feasibility.

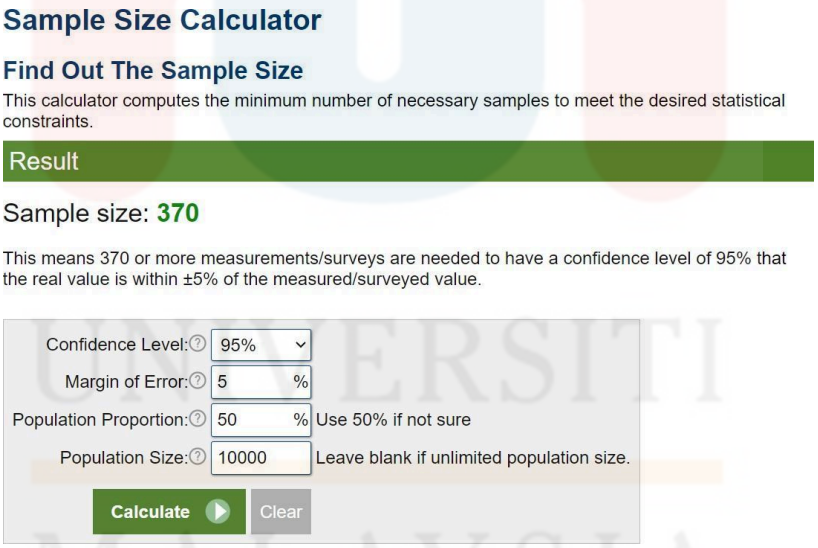

Figure 1. Sample Size Estimation, (Maple Tech. International LLC., 2008).

3.3 Study Criteria

3.3.1 Inclusion criteria

The inclusion criteria for this study were stray cats that were randomly selected with no bias pertaining to age, sex, health status, and body weight.

3.3.2 Exclusion criteria

Cats that were owned were excluded from this study.

3.4 Sample collection

A convenient sampling of stray cats through fecal samples by rectal swabs were collected. Prior to rectal swab sampling, the cats were examined for the signalment and health status and the data obtained from the physical examination were recorded. Firstly, the temperament of the cats were observed, and aggressive cats were deselected or handled with care. Beforehand, the transport medium, box, and nitrile gloves were readily available nearby. The transport medium that was used in this study was the Cary-Blair and Amies swab transport medium (Figure 2) which was chosen due to its suitability for clinical samples involving rectal swabs and stool samples. The sampling involved two personnel, one as a sample collector and the other as the restrainer. The cats were attracted with wet food, and then were restrained by either lateral recumbency or sternal recumbency. Depending on the temperament of the cat, scruffing or towel restraints were implemented. After restraining, the swab was removed from the pouch and the tip of the swab was ensured to not be touched or contaminated with the surrounding objects. The swab was then inserted through the anal sphincter (roughly 2 to 3 cm in, depending on the size and age of the cat) and rotated gently. Then, the swab was removed and checked for visible feces on the tip of the swab. The swab was then transferred into the Cary-Blair medium (Oxoid, England) or Amies swab (LTC, Malaysia), labelled promptly, and placed in the ice box to be transported to the laboratory. The samples were cultured within 2 to 4 hours of collection.

Figure 2 : Amies swab (Blue cap), ice box, and cat food.

3.5 Laboratory Procedure

3.5.1 Bacterial isolation & identification

The rectal swab obtained from sampling were plated by streaking onto Modified Charcoal Cefoperazone. Deoxycholate Agar (mCCDA), (Oxoid, England). The cultures were then incubated at 42°C for 48 hours under microaerophilic conditions. Microaerophilic conditions were achieved by using candles in an anaerobic jar (Figure 3). After that, to acquire pure colonies, suspected *Campylobacter* colonies from the mCCDA cultures were then subcultured onto another mCCDA agar using 4 quadrants streaking method. The colonies were taken to undergo Gram staining. Gram staining was done by staining the dried and fixed culture on a glass slide with crystal violet stain, then iodine, decolorizer and then followed by safranin.

Figure 3 : Streaked mCCDA agar in anaerobic jar with microaerophilic condition achieved with using candle. White-cap tubes were samples in a cary-blair transport medium.

3.5.2 Antibiotic Susceptibility Test (AST)

Kirby-Bauer Disk Diffusion methods were used for antibiotic susceptibility tests. Test was conducted by taking a sterile cotton swab to retrieve a colony sample from confirmed isolates and was placed into a test tube with 5 ml saline which then the mixture was stirred. A densitometer was also used with a range of 0.5 McFarland units. In cases of high turbidity achieved, normal saline was added and the mixture was remeasured on a densitometer. The Mueller Hinton Agar (MHA) was labeled with numbers from 1-8. The sample solution was then inoculated on the MHA in a circle clockwise method based on the number sequence. Antibiotic discs of selection which are Trimethoprim 5 µg , Nalidixic Acid 30 mcg, Ceftazidime 30 mcg, Compound Sulfonamide 300 µg, Ciprofloxacin 10 mcg, Enrofloxacin 5 mcg, Ampicillin/Sulbactam 10 mcg, Amoxicillin 25 µg , Streptomycin 10 mcg were placed and arranged on top of the MHA by number sequence. The agar was then incubated at 42°C for 48 hours under microaerophilic conditions. The diameter of the inhibition zone was then measured and interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard.

3.5.3 DNA extraction, Polymerase Chain Reaction (PCR) and Gel Electrophoresis

Conventional PCR were used in this study. Firstly, DNA extraction was performed by using a boiling method for suspected colonies from the culture including a known positive strain obtained from the Department of Medical Microbiology and Parasitology of Universiti Sains Malaysia as per manufacturer's instructions. The mastermix concoctions were made which were inclusive of 5 µl template DNA, 1 µl of forward and reverse primers, and 12.5 µl of PCR Master Mix (PCR Master Mix Kit, Qiagen®, USA) and 5.5 µl RNase free water were used to get a final volume of 25 µl.

Table 1 : The primers used for PCR amplification for Campylobacter detection

Target Organism	Target DNA Region	Forward Primer (5' → 3')	Reverse Primer (5' → 3')	Amplicon Size (bp)	Reference
C. jejuni	cj0414	C-1 (5'-CAAATAAAGTTAGAGGTAGAATGT-3')	C-3 (5'-CCATAAGCACTAGCTAGCTGAT-3')	161	Wang *et al.* (2002)
C. coli	cueE	CC18F (5'-GGTATGATTTCTACAAAGCGAG-3')	CC519R (5'-ATAAAAGACTATCGTCGCGTG-3')	502	Linton *et al.* (1997)

Thermocyclers (Bio-Rad T100™ Thermal cycler) were used for thermocycling with optimized primers. Whereby the protocol started with the initial denaturation for 15 minutes at 95 °C. Thereafter, 25 cycles of denaturation for 30 seconds at 95 °C. Annealing for 1.5 minutes at 58°C, 1 minute of extension at 72 °C, and finally 7 minutes of final extension at 72 °C Campylobacter.*coli* (ATCC 33559) and *C. jejuni* (ATCC 33560) were utilized as the positive control, while distilled water was used as the negative control. Agarose gel 1.5% (w/v) was prepared using 80 ml TAE buffer to 1.2 gram of agarose and 1µl of midori green (SYBR safe) . Afterwards, 10 µl of the PCR amplified end products were inserted into the wells of a 1.5% (w/v) agarose gel for gel electrophoresis at 100 V, 400 mA, for 45 minutes. Afterwards, DNA fragments were visualized with UV illuminator (UVP GelMax 125 Imager, USA) after staining with ethidium bromide. The targeted DNA then was compared and verified with the reference DNA ladder markers (100 bp).

CHAPTER 4

RESULTS AND DISCUSSION

The total number of samples collected was 61 in total of which the total samples collected from each location in Kelantan vary due to several limitations encountered during sampling. According to the results obtained, there were 4 positive samples for *Campylobacter jejuni* whereas no detection was found for *Campylobacter coli* among the samples collected as shown in Figure 4, Figure 5, Figure 6, and Figure 7.

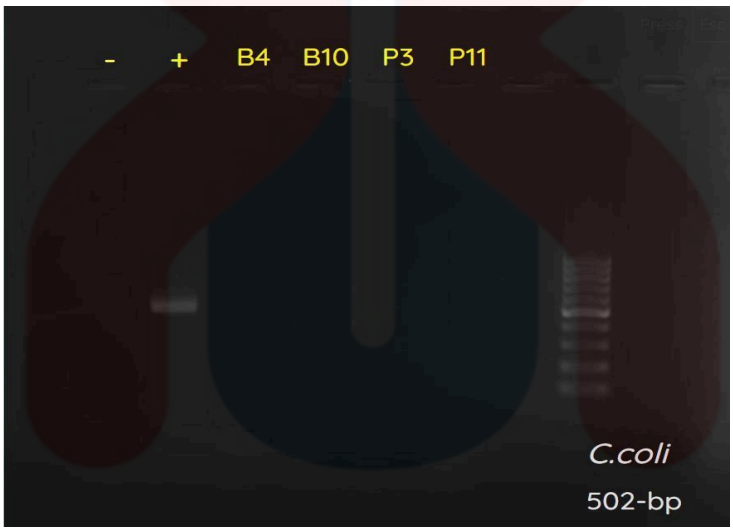

Figure 4 : PCR amplification of *Campylobacter coli* with 502-bp. Lane B4, B10, P3, P11 were the samples. Lane (+), was the positive control and Lane (-) was the negative control.

Figure 5 : PCR amplification of *Campylobacter coli* with 502-bp. Lane 1-11 were the samples. Lane (+), was the positive control and Lane (-) was the negative control.

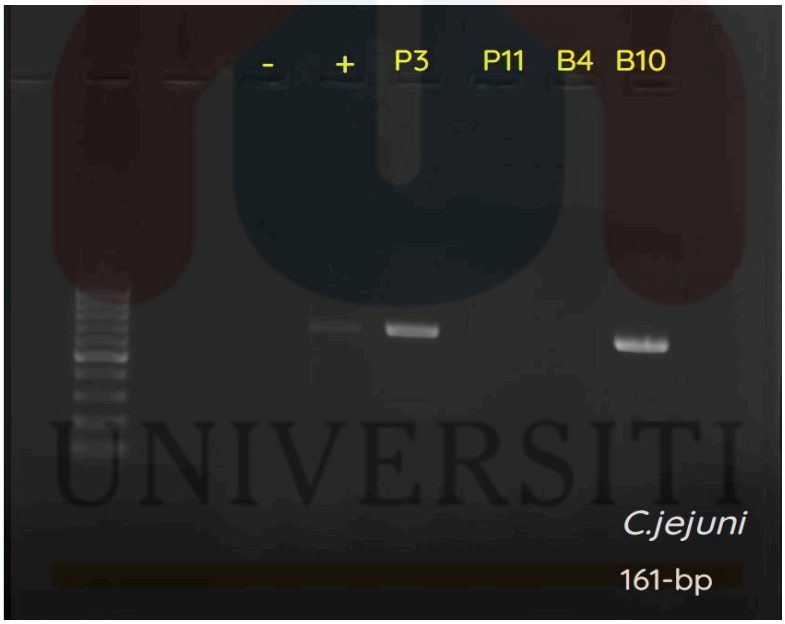

Figure 6 : PCR amplification of *Campylobacter jejuni* with 161-bp. Lane B4, B10, P3, P11 were the samples. Lane (+), was the positive control and Lane (-) was the negative control.

Figure 7 : PCR amplification of *Campylobacter jejuni* with 161-bp. Lane 1-11 were the samples. Lane (+), was the positive control and Lane (-) was the negative control.

Based on the data collected, the P values for several categorized variables such as age, gender and breed are 0.7033, 0.6335, and 0.6379, respectively, of which are considered statistically insignificant; therefore, there is no correlation between age, gender, and breed in the prevalence of *Campylobacter spp*. in stray cats. However, the body condition score and the health status revealed a P value of 0.0154 and 0.0039, respectively, which is statistically significant. As described in (Table 2).

The prevalence of the condition in adults is estimated to be between 0.36% and 13.19%, indicating that it is relatively rare in this group. In contrast, no cases were observed in kittens, and the confidence interval confirms a prevalence of 0%. For males, the prevalence ranges from 0% to 13.25%, showing some uncertainty due to the small sample size, while for females, the range is 0% to 16.06%, with no significant difference compared to males. In Domestic Short-Haired (DSH) cats, the prevalence is estimated to be between 0.38% and 13.42%, suggesting it is uncommon but possible, while no cases were observed in Domestic Long-Haired (DLH) cats. Among cats with a body condition score of 2/5, the prevalence ranges from 0% to 40.24%, reflecting high uncertainty, possibly due to a small sample size. For cats with a body condition score of 3/5, the prevalence is between 0% and 6.39%, indicating a lower likelihood of the condition compared to 2/5. In healthy cats, the prevalence is between 0% and 6%, suggesting the condition is rare and shows little variability. However, in unhealthy cats, the prevalence ranges from 0.5% to 49.5%, indicating considerable uncertainty but a potential for higher prevalence in this group as shown in Table 2.

Table 2 : Prevalence and Associated Factors. N= 61.

Variables		N	Prevalence	P-Value	95% CI
Age	Adult	59	6.78	0.7033	(0.36,13.19)
	Kitten	2	0		(0,0)
Gender	Male	22	4.54	0.6335	(0,13.25)
	Female	39	7.69		(0,16.06)
Breed	DSH	58	6.89	0.6379	(0.38, 13.42)
	DLH	3	0		(0,0)
Body Score Condition (BCS)	2 out of 5	15	20.00	0.0154	(0, 40.24)
	3 out of 5	46	2.17		(0, 6.39)
Health status	Healthy	49	2.04	0.0039	(0, 6)
	Unhealthy	12	25.00		(0.5, 49.5)

Antimicrobial susceptibility testing revealed significant resistance patterns across the antibiotics tested. Trimethoprim, Nalidixic Acid, Ceftazidime, and Compound Sulfonamide demonstrated complete resistance (100%) with no intermediate or susceptible isolates detected. Streptomycin exhibited moderate resistance, with 66.67% of isolates being resistant and 33.33% showing intermediate susceptibility, while no isolates were susceptible. Ciprofloxacin, Enrofloxacin, and Ampicillin/Sulbactam displayed a balanced distribution of responses, with 33.33% of isolates classified as susceptible, 33.33% as intermediate, and 33.33% as resistant. In contrast, Amoxicillin showed 33.33% resistance and 66.67% intermediate susceptibility, with no susceptible isolates recorded. (Table 3).

Table 3 : Frequency of AST pattern in several antibiotics stated.

Species	Antibiotic	Antibiotic Sensitivity Test (AST) pattern frequency (%)		
		Susceptible	Intermediate	Resistance
C. jejuni	**Trimethoprim** (W5)	0	0	100
	Nalidixic Acid (N/A30)	0	0	100
	Ceftazidime (CAZ 30)	0	0	100
	Streptomycin (S10)	0	33.33	66.67
	Ciprofloxacin (Cip10)	33.33	33.33	33.33
	Enrofloxacin (EX5)	33.33	33.33	33.33
	Ampicillin/ Sulbactam (A/S)	33.33	33.33	33.33
	Amoxicillin (AML25)	0	66.67	33.33
	Compound Sulphonamide (S3 300)	0	0	100

Campylobacter jejuni and *Campylobacter coli* continue to be leading causes of bacterial gastroenteritis in humans globally (Sheppard & Maiden, 2015) therefore this study provides valuable insights into the occurrence and antimicrobial resistance patterns of *Campylobacter spp.* Particularly *C.jejuni* and *C.coli* among stray cats in Kelantan. From the 61 samples collected across various locations in Kelantan, only 4 (6.56%) tested positive for *Campylobacter jejuni*. Interestingly, *Campylobacter coli* was not detected in any of the samples due to the fact that *C.coli* can be found in cats but they're less common than *C. upsaliensis*, *C. jejuni*, and *C. helveticus*. (Bojanić *et al*., 2016). The limited occurrence of *Campylobacter spp.* in stray cats might be attributed to environmental factors, dietary habits, or regional differences in the prevalence of the *Campylobacter* itself (McVey *et al*., 2022) plus the low detection rate could also be influenced by sampling challenges such as the variability in sample sizes across locations and limitations during collection like aggressiveness of the strays, inadequate depth during rectal swabs, and transporting period of more than 4 hours as *Campylobacter spp*. Is fastidious (Tellez-Isaias *et al.* 2022). These results align with previous studies that reported varying

prevalence rates of Campylobacter spp. among domestic and stray animals, often influenced by geographical and ecological factors (Goni *et al*, 2017).

According to this study on the P-value findings, demographic factors like age, breed, and sex do not play a major role in the occurrence of *Campylobacter spp*. in stray cats in Kelantan. However, Goni *et al*., 2017, E Acke, 2018 suggested that the occurrence of infections in immature cats is higher than in adult cats due to immaturity of the immune system of the young but none were detected in this case due to sample size limitation. Apart from that, significant correlations were found for body condition score (P = 0.0154) and health status (P = 0.0039). Stray cats with poor body condition or compromised health were more likely to test positive for *Campylobacter jejuni*. These findings suggest that stress, malnutrition, or underlying health issues may increase the susceptibility of stray cats to bacterial colonization since also several studies have confirmed that animals with diarrhea have a higher frequency of campylobacteriosis compared to those without diarrhea, correlating with the health status (Carbonero et al., 2012; Verma et al., 2014; Acke, 2018) of which in this study, 2 out 4 positive sample cats had crusty feces on the anal region indicating previous history of diarrhea.

The identification of antimicrobial-resistant *Campylobacter jejuni* in stray cats presents significant public health concerns, particularly regarding zoonotic transmission. Stray cats frequently inhabit environments shared with humans, providing potential pathways for the spread of resistant bacteria (Sandøe *et al*., 2019). Furthermore, the strong association between bacterial occurrence and factors such as body condition score and health status of the cat underscores the necessity of incorporating stray animal health into broader strategies in conjunction with public health.

In this study, antimicrobial susceptibility testing revealed resistance patterns among the isolates. Resistance was particularly high for Trimethoprim, Nalidixic Acid, Ceftazidime, and Compound Sulfonamide, with all isolates (100%) resistant to these antibiotics. Streptomycin exhibited moderate resistance, with 66.67% of isolates resistant and 33.33% displaying intermediate susceptibility. Ciprofloxacin, Enrofloxacin, and Ampicillin/Sulbactam showed an equal distribution of susceptibility, intermediate susceptibility, and resistance (33.33% each). Amoxicillin demonstrated 33.33% resistance and 66.67% intermediate susceptibility, with no fully susceptible isolates. These findings reflect the global concern surrounding antimicrobial resistance in *Campylobacter spp*., particularly to commonly used antibiotics in the veterinary

clinic and medical centres. The complete resistance to Trimethoprim, Nalidixic Acid, Ceftazidime, and Compound Sulfonamide indicates the limited effectiveness of these drugs for treating *Campylobacter* infections in animals. Resistance to ciprofloxacin and enrofloxacin is especially worrying, as these antibiotics are vital for treating severe bacterial infections in humans. Parallel to that, *Campylobacter spp*. have developed various mechanisms to resist antibiotics, one of it includes genetic mutations in drug target-associated genes and the utilization of efflux pumps to expel the antimicrobial reaction from bacterial cells notably, the mutations in the gyrA gene are linked to fluoroquinolone resistance (Shen *et al*., 2018) , therefore, in this study, ciprofloxacin (Cip10) and Enrofloxacin (EX5) were used to test antimicrobial susceptibility. Meanwhile, according to a study, *Campylobacter jejuni* accounts for 19.2% resistance for nalidixic acid (Mustafa Yildiz *et al*., 2023). In this study, nalidixic acid revealed 100% resistance to *Campylobacter jejuni* isolates.

Campylobacteriosis in both humans and animals are considered self-limiting (Wieczorek & Osek, 2013) and the use of antibiotics is debatable but are indeed used in severe cases in humans. Therefore, the rising resistance of *Campylobacter spp.* to fluoroquinolones group and possibly other groups are quite concerning. Besides, a comprehensive review has highlighted alarming trends in antimicrobial resistance (AMR) among *Campylobacter* isolates from both human and animal sources, with globally high resistance levels to ciprofloxacin and tetracycline, posing significant challenges to the treatment of campylobacteriosis despite ciprofloxacin as one of the common lines of antibiotic used for campylobacteriosis in humans (Barata *et al*., 2024, CDC, 2017). However, resistance patterns vary widely depending on geographic location and local antibiotic use practices; for example, studies in Asia have reported a high prevalence of multidrug-resistant strains (Barata *et al*., 2024, Kim *et al.*, 2024).

This study had several limitations, including variability in sample collection across locations and a small number of positive samples, which may limit the generalizability of the results. Future research with larger sample sizes and coverage of additional regions would offer a more comprehensive understanding of the occurrence and resistance patterns of *Campylobacter spp*. in stray cats in Kelantan.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In conclusion, *Campylobacter jejuni* was the most detected species, with *Campylobacter coli* not identified. The study also highlighted significant antimicrobial resistance, with high resistance rates to antibiotics such as Trimethoprim, Nalidixic Acid, Ceftazidime, and Compound Sulfonamide (100%), alongside varied resistance patterns to other commonly used antibiotics, including Ciprofloxacin (33.33%) and Enrofloxacin (33.33%). These results underscore the public health risks posed by antimicrobial-resistant *Campylobacter jejuni* in stray cats, which could act as reservoirs for zoonotic transmission. Additionally, factors like body condition score ($P = 0.0154$) and health status ($P = 0.0039$) were significant in detecting *C.jejuni*, suggesting that malnourished or sick stray cats are more likely to harbor *Campylobacter jejuni.* Therefore, emphasizing the need to improve the health and welfare of stray animals, particularly their nutrition and overall health, should be incorporated into public health strategies to reduce the risk of campylobacteriosis in both humans and animals especially in areas with high human-animal interaction such as Kelantan plus the impact of environmental factors and human-animal interactions on the transmission of resistant Campylobacter strains should be explored further. Additionally, Future research should involve larger, geographically diverse sample sizes to gain a more comprehensive understanding of *Campylobacter* spp. resistance patterns and identify additional potential reservoirs of infection.

APPENDICES

Figure 8 : MHA agar preparation.

Figure 9 : AST preparation.

Figure 10 : Sample streaking from Cary-Blair transport media onto mCCDA agar.

Figure 11 : PCR working boxes.

Figure 12 : Sample collection with a colleague.

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