OCCURRENCE OF *CAMPYLOBACTER* SPP. AMONG CATTLE IN KOTA BHARU KELANTAN

By

Mira Izzati Binti Ibrahim

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ABSTRACT

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

This study aimed to investigate the prevalence of *Campylobacter* species among cattle in Kota Bharu, Kelantan, due to its zoonotic potential, which poses a risk of transmission to humans and can cause diarrhea in both cattle and humans. A total of 31 cattle were sampled, with vaginal and rectal swabs collected from females and rectal swabs from males. Samples were transported in Cary-Blair medium and cultured on modified charcoal cefoperazone deoxycholate agar (mCCDA) agar at 42 °C for 48 hours. The colonies were isolated for DNA extraction followed by Polymerase Chain Reaction (PCR). Various risk factors, including rearing system, age, sex, and previous treatments, were assessed. Data were analyzed using Microsoft Excel, employing descriptive statistics to determine the prevalence of *Campylobacter* species and evaluate potential influencing factors. Chi-squared tests were used to compare prevalence rates among different *Campylobacter* species and their sources. The results identified two positive cases from semi-intensive farms, emphasizing the rearing system as a significant contributing factor to the presence of *Campylobacter* species. These findings highlight the need for targeted management practices to mitigate zoonotic risks.

ABSTRAK

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

Kajian ini menyiasat kekerapan spesies *Campylobacter* dalam kawanan lembu di Kota Bharu, Kelantan, kerana potensi zoonotik dan kemampuannya untuk menyebabkan cirit-birit pada manusia dan juga lembu. Sebanyak 31 ekor lembu telah disampel, di mana swab faraj dan rektum diambil dari lembu betina serta swab rektum dari lembu jantan. Sampel dihantar dengan menggunakan medium Cary-Blair, dikultur menggunakan agar mCCDA, dan koloni diasingkan untuk pengambilan DNA dan analisis PCR. Faktor risiko seperti sistem penternakan, umur, jantina, dan sejarah perubatan telah dikenalpasti. Analisis data, termasuk statistik deskriptif dan ujian chi-kuasa dua, telah dilaksanakan menggunakan Microsoft Excel untuk menentukan prevalens dan faktor-faktor yang berkaitan. Hasil kajian menunjukkan dua kes positif dari ladang semi-intensif, menekankan sistem penternakan sebagai faktor risiko yang signifikan.

CERTIFICATION

This is to certify that we have read this research paper entitled **'Occurrence Of *Campylobacter* Spp. From Cattle In Kota Bharu Kelantan'** by **Mira Izzati binti Ibrahim**, 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.

DR. MOHAMMED DAUDA GONI

DVM (University of Maiduguri), MSc (UPM), PhD (USM)
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Malaysia Kelantan
(Supervisor)

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DVM 5 Class of 2024/2025

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LIST OF ABBREVIATIONS

PCR	-	Polymerase Chain Reaction
DNA	-	Deoxyribose Nucleic Acid
mCCDA	-	modified Charcoal Cefoperazone Deoxycholate Agar
CCDA	-	Charcoal Cefoperazone Deoxycholate Agar

1.0 Introduction

Campylobacter is widely recognized as the leading bacterial cause of foodborne diarrheal disease across the globe (Silva *et al.*, 2011). The genus *Campylobacter* is highly biologically diverse, with some species causing clinical illness in animals and humans, while many others are commensals in the intestinal tract of animals (Sahin *et al.*, 2017). Among the pathogenic species, *Campylobacter jejuni* is the leading cause of bacterial foodborne gastroenteritis, accounting for 90% of human campylobacteriosis cases globally (Gillespie *et al.*, 2002). *Campylobacter coli* also contributes to these infections, albeit to a lesser extent.

Human campylobacteriosis primarily arises from consuming contaminated food, with the most common transmission being the handling and consumption of raw or undercooked poultry. Additionally, cattle often carry *C. jejuni, C. coli*, and other *Campylobacter* species, potentially transmitting these bacteria to humans via meat or milk (Wysok *et al.*, 2024). If not managed properly, this contaminated environment can lead to the transmission of *Campylobacter* to humans through dairy products, particularly unpasteurized milk (Bianchini *et al.*, 2014). Therefore, the objectives of this study were to conduct a comprehensive investigation into the occurrence of *Campylobacter* among cattle farms in Kota Bharu, Kelantan. This study aims to identify the specific species of *Campylobacter* present in these environments.

1.1 Research Problem

Campylobacteriosis, caused by the bacteria *Campylobacter*, is a zoonotic disease transmissible through direct or indirect contact with animals, notably farm animals. Given the absence of previous research on campylobacteriosis among cattle in Kelantan, the current status of *Campylobacter* presence or occurrence in Kelantan cattle remains unknown. Hence, this study was undertaken to investigate the occurrence of *Campylobacter* among cattle in Kota Bharu, Kelantan.

1.2 Research Question

I. Is there an occurrence of Campylobacteriosis among cattle in Kota Bharu, Kelantan?

II. What are the *Campylobacter* species that mostly affect cattle in Kelantan?

1.3 Research Hypothesis

I. *Campylobacter spp*. has a high occurrence among cattle in Kota Bharu Kelantan

II. *Campylobacter fetus*, *Campylobacter coli* and *Campylobacter jejuni* are present among cattle in Kota Bharu, Kelantan.

1.4 Research Objectives

I. To investigate the occurrence of *Campylobacter spp*. among cattle in Kelantan.

II. To identify the species of *Campylobacter* present among cattle in Kota Bharu Kelantan.

2.0 Literature Review

2.1 Background of the *Campylobacter spp.*

Campylobacter are gram-negative, non-spore-forming, slender curved rods measuring 0.2-0.9μm in width and 0.5-5.0μm in length. They possess a capsule and are motile due to a polar flagellum located at one or both ends of the cell. When multiple bacterial cells cluster together, they can create S-shaped or gull-winged formations, which may look like spirals (Duhamel, 2022). *Campylobacter* species require a microaerobic environment with 3-10% oxygen and 3-15% CO_2. The majority of species are thermotolerant and can grow at temperatures up to 42 °C. They exhibit oxidase-positive activity and do not ferment or oxidize carbohydrates. Instead, they generate energy by oxidizing amino acids or tricarboxylic acid intermediates via the respiratory pathway (Duhamel, 2022).

The genus *Campylobacter* comprises nine species of veterinary importance, including two species that are further divided into subspecies and one species divided into three biovars. Most *Campylobacter* species are pathogenic, causing reproductive and intestinal diseases in both animals and humans. Non-pathogenic species are commensal bacteria found in the intestinal tracts of humans, domestic and wild mammals, birds, and shellfish. Species of veterinary significance include *Campylobacter fetus, Campylobacter jejuni subsp. jejuni, Campylobacter coli, Campylobacter upsaliensis, Campylobacter lari, Campylobacter hyointestinalis, Campylobacter helveticus, Campylobacter sputorum, and Campylobacter mucosalis. C. fetus and C. jejuni* are notable for causing reproductive failures in ruminants. Several species, particularly *C. jejuni and C. coli*, are significant causes of foodborne and waterborne enterocolitis in humans, and they are also minor causes of disease in animals (Duhamel, 2022).

2.2 Transmission of *Campylobacter* spp.

For reproductive diseases primarily caused by *C. fetus ssp. venerealis*, transmission occurs mainly through coitus, although it can also spread via artificial insemination using contaminated semen or equipment. In contrast, *C. fetus ssp. fetus* and *C. jejuni* infections occur through the ingestion of organisms present in food or water contaminated by the feces of carrier ruminants, or by exposure to fetal fluids and placental membranes from aborted fetuses. However, campylobacters linked to enteric diseases, such as *C. jejuni and C. coli*, are primarily transmitted through the ingestion of contaminated food or water (Duhamel, 2022).

2.3 Clinical importance of *Campylobacter* in cattle

Various *Campylobacter* subspecies have been isolated from both healthy and diseased cattle. *Campylobacter fetus subsp. fetus* is known to cause sporadic abortions in cows and epizootic abortions in sheep. *Campylobacter fetus subsp. venerealis* leads to infectious infertility in cattle, typically transmitted by carrier bulls (Skirrow 1994). *Campylobacter jejuni subsp. jejuni* and *Campylobacter hyointestinalis* have occasionally been associated with bovine abortions (Diker *et al.* 1990; Van Donkersgoed *et al.* 1990), and *Campylobacter jejuni subsp. jejuni* has also been identified in cases of bovine mastitis (Morgan *et al.* 1985). Additionally, *C. jejuni* commonly colonizes the intestines of young ruminants, the infection often leads to self-limiting diarrhea, while in older cattle, the infection is usually asymptomatic. (Duhamel, 2022).

The *C. fetus* species is classified into two subspecies, namely *C. fetus subsp. fetus* and *C. fetus subsp. venerealis*, both recognized for causing reproductive failures in ruminants. Despite their close genetic relationship, these subspecies exhibit distinct ecological and epidemiological characteristics in ruminants. *C. fetus subsp. fetus* has a wide host range, colonizes the

gastrointestinal tract, and is typically associated with horizontally transmitted abortion in sheep and cattle. On the other hand, *C. fetus subsp. venerealis* has a limited host range, primarily found in the bovine genital tract, and is the main cause of venereally transmitted infectious infertility and embryonic mortality in cattle (Sahin *et al.*, 2017).

Campylobacter fetus venerealis induces venereal disease primarily leading to infertility or early embryonic demise, occasionally culminating in abortion between 4 and 8 months of gestation. *C. fetus fetus* and *C. jejuni* are transmitted via ingestion and subsequent hematogenous dissemination to the placenta. Both varieties contribute to sporadic abortions, typically occurring in the latter half of gestation. Fetal specimens may present as fresh with partially expanded lungs or severely autolyzed. Additionally, mild fibrinous pleuritis and peritonitis might be observed, alongside bronchopneumonia. Placentitis manifests mildly, characterized by hemorrhagic cotyledons and an edematous intercotyledonary region (*The Merck Veterinary Manual*, 2016b).

2.4 Health effect of Campylobacteriosis in Humans

Campylobacter fetus subsp. fetus rarely leads to human infections, unlike *C. fetus subsp. venerealis*. Individuals with exposure to cattle and sheep are at higher risk of infection, including veterinarians, farmers, packinghouse workers, and others involved in cattle and sheep handling. Possible infections include bacteremia, septic arthritis, endocarditis, septic abortions, peritonitis, salpingitis, meningitis, and thrombophlebitis (Carter, 1986).

Campylobacter jejuni is the most commonly isolated pathogen found in stool samples associated with gastroenteritis in humans. The organism originates from various sources, including milk, poultry carcasses, feces of both animal and human carriers, as well as food and water contaminated by feces. Additionally, it can be found in the feces of dogs and cats, with or without diarrhea. The

typical duration of illness ranges from 1 to 7 days and presents with symptoms such as fever, abdominal pain, nausea, vomiting, and watery diarrhea, sometimes with blood in the stool. Most infections are asymptomatic, but disseminated infections may occur in individuals with weakened immune systems (Carter, 1986).

This is also supported by another source, which notes that *Campylobacter jejuni and C. coli* are commonly found in large numbers as commensals in the intestinal tracts of companion and food animals. Humans typically contract infections from consuming undercooked meat, particularly poultry, which is contaminated predominantly by *C. jejuni (*95%) and to a lesser extent by *C. coli (*5%). Following an incubation period of 24 to 72 hours, individuals experience severe diarrhea, often accompanied by blood and leukocytes in the feces, as well as fever, vomiting, and abdominal pain. The illness is generally self-limiting (Songer & Post, 2004).

2.5 Diagnosis of *Campylobacter* infection

Diagnosing campylobacteriosis is relatively simple but requires bacteriological culture. For reproductive tract infections, samples such as smegma, preputial scrapings, vaginal fluid, or fetal stomach contents should be collected following standard bacteriological protocols. These samples should then be transported in enrichment media like Clark's medium or thioglycollate broth to enhance the survival rate of *Campylobacter spp.* (Clark, 1971; Hum *et al.*, 1994) (Songer & Post, 2004). However, more recently, several selective broths, such as Bolton broth (BB), *Campylobacter* enrichment broth (CEB), and Preston broth (PB), have also been utilized as enrichment media (Silva *et al.*, 2011).

Various selective agars have been developed and evaluated for their effectiveness in isolating *campylobacters*. Preston, charcoal cefoperazone deoxycholate (CCDA), and Butzler agars have

all demonstrated equal efficacy (Zanetti *et al.*, 1996). Enteric *campylobacters* are best isolated from intestinal samples using selective media containing antimicrobial drugs, such as Campy-CVA medium (cefoperazone, vancomycin, and amphotericin B) or Skirrow medium. *C. jejuni, C. coli, and C. lari* are best isolated using cephalothin-containing medium such as Butzler's and Campy-BAP media (Duhamel, 2022). Nevertheless, the preferred method involves using CCDA and incubating at 42°C instead of 37°C, as this approach facilitates the isolation of a greater number of *Campylobacter* strains (Zanetti *et al*., 1996). However, the most reliable confirmation methods are those based on the polymerase chain reaction (PCR), as phenotypic reactions are often atypical and challenging to interpret (Silva *et al*., 2011).

2.6 Treatment of *Campylobacter* spp. Infection

Campylobacter is frequently exposed to antibiotics administered to food-producing animals, companion animals, and humans. This organism is remarkably adaptable to antibiotic pressure, having developed numerous resistance mechanisms (Wieczorek & Osek, 2013). In regions with a high prevalence of fluoroquinolone resistance, macrolide antibiotics like azithromycin are recommended as the first line of treatment for campylobacteriosis (Tribble, 2017). Furthermore, carbapenems have been effective in treating Campylobacter-related bacteremia and sepsis, and are proposed as an alternative treatment option for systemic infections caused by *Campylobacter*.

However, according to Peter (2020), cows are typically not treated for genital campylobacteriosis due to practical reasons. Conversely, in bulls, the infection can be eradicated with 1-2 treatments of streptomycin at a dosage of 20 mg/kg administered subcutaneously, along with the application of 5 g of streptomycin in an oil-based suspension to the penis for three consecutive days. Culling infected cattle would be the easiest way of controlling the infection.

3.0 Methodology

3.1 Ethical Consideration

This study obtained ethical approval from the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Malaysia Kelantan under approval code UMK/FPV/ACUE/FYP/010/2024.

3.2 Study Area & Design

A cross-sectional study was conducted in Kelantan, a state located on the East Coast of Peninsular Malaysia, specifically in the Kota Bharu district.

3.3 Study Population

The study focused on cattle from farms in Kota Bharu, Kelantan. Using calculator.net, the sample size was determined with a 95% confidence level, a 5% margin of error, a 50% population proportion, and a population size of 92,566 (DVS, 2019). The calculated sample size was 383, but due to practical constraints, it was reduced to only 31 cattle.

3.4 Sampling Method

A convenient sampling method was employed to gather rectal swabs and vaginal swabs for females, and only rectal swabs for males, from cattle in the Kelantan area following consent from the owners.

3.5 Sampling Procedure

Samples were collected upon the approval of the Animal Care and Use Committee (ACUC) of University Malaysia Kelantan. A total of 31 cattle were selected for the study. Each underwent a physical examination, during which all identifying details and any abnormalities among the cattle were documented.

Cattle were restrained in a crush, and rectal and vaginal swabs were collected aseptically from both healthy and sick animals (n=31) using sterile cotton swabs and disposable gloves. These samples came from farms located around Kota Bharu District. After collection, all samples were labeled accordingly before being transported to the lab in a cooler box. They were stored in a Cary-Blair enrichment medium and maintained at 4°C.

Furthermore, before sampling, a thorough set of data was collected, including the subjects' age (classified as young or adult), sex (male or female), medical and vaccination history, and management practice type (intense, extensive, or semi-intensive).

3.6 Bacterial Culture and Isolation

Rectal and vaginal swabs were streaked directly onto modified charcoal cefoperazone deoxycholate agar (mCCDA, Thermo Scientific Microbiology Sdn. Bhd, Malaysia) for isolation. The plates were incubated for 48 hours at 42ºC under microaerophilic conditions, achieved by using BD CampyPak™ gas packs (Becton, Dickinson and Company) in an anaerobic jar. Two types of colonies were identified from the sample: yellowish mucoid colonies and small gray colonies on the media.

3.7 DNA Extraction

DNA was extracted from the eight samples that exhibited shiny, glistening colonies with a whitish to grayish appearance using the boiling method. Each Eppendorf tube was first labelled, and then a single colony was placed into a microcentrifuge tube containing 1 mL of sterile sodium chloride (NaCl) solution to remove any residual media. The tube was centrifuged at 12,000 rpm for 5 minutes, allowing the bacterial cells to form a pellet at the bottom. The supernatant was carefully removed, and 200 µL of sterile distilled water was added to resuspend the pellet. The sample was briefly vortexed for uniform mixing and then heated in a 100°C water bath for 10 minutes to lyse the cells and release the DNA. Following this, the tube was immediately cooled on ice for 5 minutes to keep the DNA from degradation. The sample was then centrifuged again at 12,000 rpm for 5 minutes to separate the cell debris. Finally, the supernatant containing the extracted DNA was gently transferred to a new microcentrifuge tube, ensuring the pellet remained undisturbed. This method was also used by Dashti *et al.* (2009).

3.8 Species Identification Using Polymerase Chain Reaction

Before gene amplification, the reagents were prepared. The amplification was carried out in a total reaction volume of 25 µL, which included 5.0 µL of extracted DNA sample, 1.0 µL of forward primer, 1.0 µL of reverse primer, 12.5 µL of Thermo Scientific™ DreamTaq™ PCR Master Mix (2X), and 5.5 µL of nuclease-free water. The primers employed in this process are detailed in Table 1.

Table 1: Primer sequences used for the multiplex PCR assay and the predicted sizes of PCR products (Aung *et al.,* 2015)

Species	Size (bp)	Target Gene	Primer	Sequence
C. jejuni	735	hip gene	HIP400F	5′-GAA GAG GGT TTG GGT GGT G-3′
			HIP1134R	5′-AGC TAG CTT CGC ATA ATA ACT TG-3′
C. coli	894	ceuE gene	F	5′-ATG AAA AAA TAT TTA GTT TTT GCA-3′
			R	5′-ATT TTA TTA TTT GTA GCA GCG-3′
C. fetus	359	cstA	MG3F	5′-GGTAGCCGCAGCTGCTAAGAT-3′
			CF359R	5′-AGCCAGTAACGCATATTATAGTAG-3′

Following that, the amplification process was carried out using a Thermocycler (Bio-Rad T100™ Thermal Cycler) with the following cycling conditions: an initial denaturation at 95°C for 15 minutes, followed by 25 cycles of 95°C for 30 seconds for denaturation, 58°C for 1.5 minutes for annealing, 72°C for 1 minute for extension, and a final extension at 72°C for 7 minutes.

The PCR-amplified products were subjected to gel electrophoresis using a 1.5% (w/v) agarose gel prepared with 80 mL of Tris-EDTA-Sodium Chloride (TES) buffer and a DNA ladder. Electrophoresis was conducted at 100 V for 45 minutes. Afterwards, the DNA fragments were visualized using a UV illuminator (UVP GelMax 125 Imager, USA). The target DNA was then compared and verified against reference DNA ladder markers to determine their sizes accurately.

3.9 Statistical Analysis

The data was analysed using Microsoft Excel. Descriptive statistics were used to determine the prevalence of *Campylobacter* species and characteristics such as age, gender, management, and previous treatments that may influence their presence. The chi-square test was performed to determine the association between the prevalence of *Campylobacter* species from various sources and associated risk factors. The level of significance was set at 0.05.

4.0 Result

4.1 Bacteria culture

After 48 hours of incubation at 42°C in a microaerophilic environment, 8 samples exhibited shiny, glistening colonies with a whitish to grayish appearance, which, according to Wanja *et al*. (2022), were considered positive for *Campylobacter spp.*

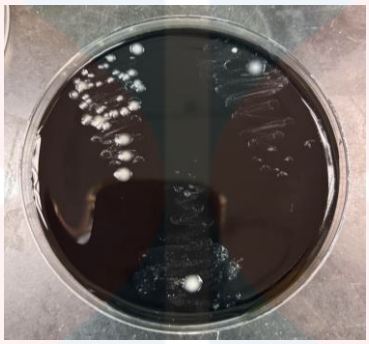

Figure 1: Suspected *Campylobacter* colonies on mCCDA plate

4.2 Species Identification Using Polymerase Chain Reaction

PCR was performed to identify the presence of the target species in the samples. After amplification, the results were analyzed to determine the presence of the species of interest (Table 2). Out of the eight samples tested, two samples were identified as positive for *Campylobacter jejuni*. Figure 2 showed positive *Campylobacter jejuni* on gel electrophoresis.

Table 2: Summary and information of samples that tested positive for *C. jejuni*

Variables	Sample 1202	Sample 1216
Age	Adult	Adult
Sex	Female	Female
Management	Semi-intensive	Semi-intensive
Presence of other species	Yes	Yes
Previous Treatment	No	No

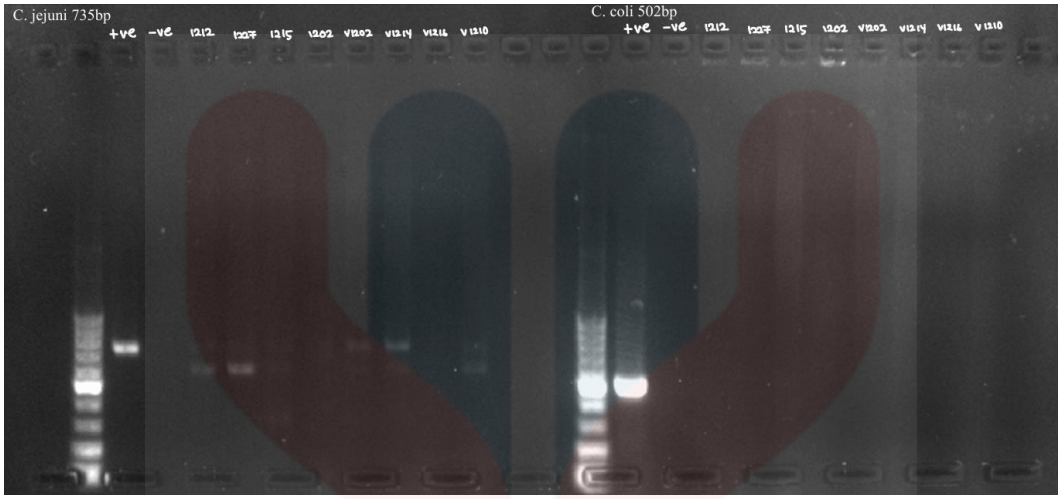

Figure 2: PCR amplification of *Campylobacter jejuni* and *Campylobacter coli* isolated from cattle.

5.0 Occurrence of *Campylobacter* and associated risk factors

In this study, a prevalence rate of 6.45% was observed, with *Campylobacter jejuni* being detected in 2 out of 31 cattle swab samples. Statistical analysis revealed no significant association between the risk factors of age, sex, rearing system, and previous treatment, with p-values of 0.5735, 0.6322, 0.1310, and 0.7793, respectively (Table 3). Even though no statistical significance was reported, the findings remain biologically significant.

Table 3: Prevalence and p-values calculated using Microsoft Excel

Variables		n	Prevalence	P-Value
Age	**Young**	4	0	0.5735
	Adult	27	7.407	
Sex	**Male**	3	0	0.6322
	Female	28	7.142	
Rearing system	**Intensive**	16	0	0.1310
	Semi-intensive	15	13.333	
Previous Treatment	**Yes**	1	0	0.7793
	No	30	6.666	

6.0 Discussion

From this study, out of the 31 cattle sampled, the two that were confirmed positive originated from the same farm, where multiple animals were kept in close proximity, creating a higher risk for disease transmission. The workers on this farm not only worked but also socialized with each other on a daily basis, which could further facilitate the spread of infections. With that being said, controlling *Campylobacter* on farms is complex, as the pathogen can persist in a wide range of environments and hosts commonly found on farms (Lynch *et al.*, 2011). Infectious diseases in livestock often can be transmitted through fomites, which are objects capable of carrying infectious agents. Personnel may unknowingly carry pathogens on their clothes, equipment, or vehicles, thereby transmitting the infection to susceptible animals (Rossi *et al*., 2017).

Therefore, farms with confirmed *Campylobacter* infections, particularly diversified small-scale farms (DSSFs) that reared multiple animal species such as goats, deer, and poultry on the same land, face additional challenges. Personnel on these farms often care for multiple species, increasing the risk of cross-contamination. Several herd-level factors, such as rearing systems (intensive vs semi-intensive), stocking density, access to pasture, manure management, and the presence of wildlife, may contribute to *Campylobacter* prevalence. Additionally, smaller farms often do not segregate animals by age, further heightening the risk of transmission (Pires *et al.*, 2019). Similarly to affected farm which did not segregate the animal by age and sex which exacerbate the potential risk of having diseases. This environment likely increased the susceptibility of cattle to get *Campylobacter* infection, highlighting the importance of considering biological relevance alongside statistical outcomes. Despite the lack of statistical significance, the findings remain biologically significant.

Cross-contamination is a significant concern because *Campylobacter* can survive in diverse environments, including fecal pads on pastures, stored or composted manure, and soil. Cattle that defecate on soil or pasture may transmit the pathogen to other cattle while grazing. Furthermore, *Campylobacter* has been found in air, soil, water, dust, and various abiotic surfaces, demonstrating its environmental resilience (Bull *et al.,* 2006; Ellis-Iversen *et al*., 2009).

Effective biosecurity measures can significantly reduce *Campylobacter* prevalence. Gibbens *et al.* (2001) showed that well-implemented disinfection protocols could decrease *Campylobacter* prevalence from 80% to less than 40%. Measures such as installing hygienic barriers between internal and external farm environments, controlling personnel access, enforcing strict hygiene practices (e.g., handwashing and sanitizing), and requiring boots and overalls to be changed before entry have been proven effective (Silva *et al*., 2011).

However, these practices were absent on a farm with confirmed *Campylobacter* infection. The cattle on this farm were kept in a compound enclosed by wire mesh fencing with soil bedding. They were confined to the compound at night but allowed to graze freely outside during the day. There were no foot dips for personnel entering the farm, increasing the likelihood of contamination. Additional risk factors included poor hygiene, the presence of other farm animals, rodents, insects, and seasonal changes, all of which are known to exacerbate *Campylobacter* colonization (Russa *et al*., 2005).

In contrast, another farm tested negative for *Campylobacter*. This farm practiced intensive rearing, where cattle were housed in individual pens with cement flooring, minimizing contact with soil and feces. Feed was provided through a cut-and-carry method, significantly reducing the risk of

cross-contamination among animals. This comparison highlights the importance of robust biosecurity and management practices in preventing *Campylobacter* transmission.

7.0 Conclusion and Recommendation

In conclusion, this study investigated the prevalence of *Campylobacter* species among cattle in Kota Bharu, Kelantan, and identified a prevalence rate of 6.45%, with *Campylobacter jejuni* detected in two out of 31 cattle samples. The findings highlight that while risk factors such as age, sex, rearing system, and previous treatment showed no statistical significance ($p > 0.05$), their biological relevance remains significant. The positive cases originated from a semi-intensive farm with diverse animal species and limited biosecurity measures, underscoring the role of farm management practices in influencing disease prevalence.

Although the statistical analysis did not reveal significant associations, the biological context such as the impact of mixed-species farming, close proximity of animals, and inadequate hygiene emphasizes the potential risks. These findings align with previous studies suggesting that factors like rearing systems, manure management, and the presence of wildlife can create environments conducive to *Campylobacter* transmission. This underscores the importance of addressing biological factors in disease management strategies, even when statistical significance is not evident.

To mitigate the prevalence of *Campylobacter* and its zoonotic risks, farms should enhance biosecurity measures, improve hygiene standards, and segregate animals by species, age, and sex to minimize cross-contamination. Routine surveillance, worker education on proper animal handling, and environmental management, such as maintaining clean housing conditions and reducing exposure to contaminated resources, are essential. Future research with larger sample

sizes and molecular epidemiology is recommended to better understand transmission dynamics and refine prevention strategies, ensuring improved animal and public health outcomes.

9.0 Appendices

Figure 4: mCCDA agar incubated in anerobic jar

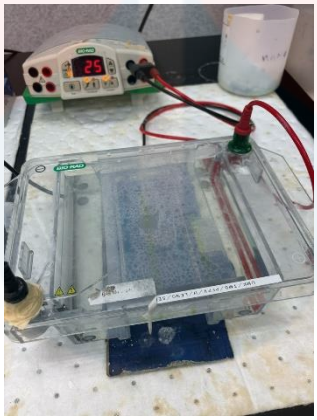

Figure 5: Gel electrophoresis

Figure 6: Cattle during sampling

10.0 References

Carter, G. R. (1986). *Essentials of veterinary bacteriology and mycology*. http://fipak.areeo.ac.ir/site/catalogue/18329669

Songer, J. G., & Post, K. W. (2004). *Veterinary Microbiology: Bacterial and fungal agents of animal disease*. http://ci.nii.ac.jp/ncid/BA84202726

Sahin, O., Yaeger, M., Wu, Z., & Zhang, Q. (2017). Campylobacter-Associated diseases in animals. *Annual Review of Animal Biosciences*, *5*(1), 21–42. https://doi.org/10.1146/annurev-animal-022516-022826

Acheson, D., & Allos, B. M. (2001). *Campylobacter jejuni* Infections: Update on Emerging Issues and Trends. *Clinical Infectious Diseases/Clinical Infectious Diseases (Online. University of Chicago. Press)*, *32*(8), 1201–1206. https://doi.org/10.1086/319760

Gillespie, I. A., O'Brien, S. J., Frost, J. A., Adak, G. K., Horby, P., Swan, A. V., Painter, M. J., & Neal, K. R. (2002). A Case-Case Comparison of *Campylobacter Coli* and *Campylobacter jejuni* Infection: A tool for Generating Hypotheses. *Emerging Infectious Diseases*, *8*(9), 937–942. https://doi.org/10.3201/eid0809.010817

J, F. F., A, O. P., C, B. F., R, P. M., K, L. M., & A, P. L. (2006). Evaluation of direct fluorescent antibody test for the diagnosis of bovine genital campylobacteriosis. *PubMed*, *44*(3–4), 118–123. https://pubmed.ncbi.nlm.nih.gov/17063594

Hum, S., Quinn, C., & Kennedy, D. (1994). Diagnosis of bovine venereal campylobacteriosis by ELISA. *Australian Veterinary Journal*, *71*(5), 140–143. https://doi.org/10.1111/j.1751-0813.1994.tb03367.x

Clark, B. L. (1971). REVIEW OF BOVINE VIBRIOSIS. *Australian Veterinary Journal*, *47*(3), 103–107. https://doi.org/10.1111/j.1751-0813.1971.tb14749.x

Hum, S., Brunner, J., McINNES, A., Mendoza, G., & Stephens, J. (1994). Evaluation of cultural methods and selective media for the isolation of *Campylobacter fetus* subsp *venerealis* from cattle. *Australian Veterinary Journal*, *71*(6), 184–186. https://doi.org/10.1111/j.1751-0813.1994.tb03385.x

McGoldrick, A., Chanter, J., Gale, S., Parr, J., Toszeghy, M., & Line, K. (2013). Real Time PCR to detect and differentiate *Campylobacter fetus* subspecies fetus and *Campylobacter fetus* subspecies venerealis. *Journal of Microbiological Methods*, *94*(3), 199–204. https://doi.org/10.1016/j.mimet.2013.06.014

Wieczorek, K., & Osek, J. (2013). Antimicrobial resistance mechanisms among *Campylobacter*. *BioMed Research International*, *2013*, 1–12. https://doi.org/10.1155/2013/340605

Tribble, D. R. (2017). Resistant pathogens as causes of traveller's diarrhea globally and impact(s) on treatment failure and recommendations. *Journal of Travel Medicine*, *24*(suppl_1), S6–S12. https://doi.org/10.1093/jtm/taw090

Peter, D. (2020, September 8). *Bovine genital campylobacteriosis*. MSD Veterinary Manual. https://www.msdvetmanual.com/reproductive-system/bovine-genital-campylobacteriosis/bovine-genital-campylobacteriosis

Aiello, S.E. and Mose, M.A. (2016). The Merck Veterinary Manual (11th Edition).Merck & Co. Wiley Publication.

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). Campylobacter spp. as a Foodborne Pathogen: A Review. *Frontiers in Microbiology*, *2*. https://doi.org/10.3389/fmicb.2011.00200

Zanetti, F., Varoli, O., Stampi, S., & De Luca, G. (1996). Prevalence of thermophilic Campylobacter and Arcobacter butzleri in food of animal origin. *International Journal of Food Microbiology*, *33*(2–3), 315–321. https://doi.org/10.1016/0168-1605(96)01166-x

Skirrow, M. (1994). Diseases due to Campylobacter, Helicobacter and related bacteria. *Journal of Comparative Pathology*, *111*(2), 113–149. https://doi.org/10.1016/s0021-9975(05)80046-5

Diker, K. S., Diker, S., & Özlem, M. B. (1990). Bovine Diarrhea Associated with Campylobacter hyointestinalis. *Journal of Veterinary Medicine. Series B*, *37*(1–10), 158–162. https://doi.org/10.1111/j.1439-0450.1990.tb01040.x

Morgan, G., Chadwick, P., Lander, K., & Gill, K. (1985). Campylobacter jejuni mastitis in a cow: a zoonosis-related incident. *Veterinary Record/the Veterinary Record*, *116*(4), 111. https://doi.org/10.1136/vr.116.4.111

Atabay, N., & Corry, N. (1998). The isolation and prevalence of campylobacters from dairy cattle using a variety of methods. *Journal of Applied Microbiology*, *84*(5), 733–740. https://doi.org/10.1046/j.1365-2672.1998.00402.

Nobi, M. A., Haque, A. K. M. Z., Islam, M. R., Islam, S. S., Arif, M., Sikder, M. H., & Kabir, S. M. L. (2024). Detection of Campylobacter spp. in farmed sheep in Mymensingh division of Bangladesh: Prevalence, risk factors, and antimicrobial susceptibility status. *Veterinary World/Veterinary World*, 245–254. https://doi.org/10.14202/vetworld.2024.245-254

Iraola, G., Pérez, R., Betancor, L., Marandino, A., Morsella, C., Méndez, A., Paolicchi, F., Piccirillo, A., Tomás, G., Velilla, A., & Calleros, L. (2016). A novel real-time PCR assay

for quantitative detection of Campylobacter fetus based on ribosomal sequences. *BMC Veterinary Research*, *12*(1). https://doi.org/10.1186/s12917-016-0913-3

Aung, W. W., Saleha, A. A., Zunita, Z., Murugaiyah, M., Aliyu, A. B., Goni, D. M., & Mohamed, A. M. (2015). Occurrence of Campylobacter in dairy and beef cattle and their farm environment in Malaysia. *Pakistan Veterinary Journal*, *35*(4), 470–473. https://www.cabdirect.org/cabdirect/abstract/20153394247

D. Scott McVey, Kennedy, M., Chengappa, M. M., & Wilkes, R. (2022). Veterinary Microbiology. John Wiley & Sons.

Van Donkersgoed J, Janzen ED, Chirino-Trejo M, Berry C, Clark EG, Haines DM. Campylobacter jejuni abortions in two beef cattle herds in Saskatchewan. Can Vet J. 1990 May;31(5):373-7. PMID: 17423586; PMCID: PMC1480707.

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). Campylobacter spp. as a Foodborne Pathogen: A Review. Frontiers in Microbiology, 2(200). https://doi.org/10.3389/fmicb.2011.00200

Wysok, B., Rudowska, M., & Wiszniewska-Łaszczych, A. (2024). The transmission of Campylobacter strains in dairy herds in different housing systems. Pathogens, 13(4), 317. https://doi.org/10.3390/pathogens13040317

Wanja, D. W., Mbuthia, P. G., Aboge, G. O., & Bebora, L. C. (2022). Seasonal Prevalence and Molecular Identification of Thermophilic Campylobacter from Chicken, Cattle, and Respective Drinking Water in Kajiado County, Kenya. *International Journal of Microbiology*, *2022*, 1–11. https://doi.org/10.1155/2022/1526641

Rossi, G., Smith, R. L., Pongolini, S., & Bolzoni, L. (2017). Modelling farm-to-farm disease transmission through personnel movements: from visits to contacts, and back. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-02567-6

Pires, A. F. A., Patterson, L., Kukielka, E. A., Aminabadi, P., Navarro-Gonzalez, N., & Jay-Russell, M. T. (2019). Prevalence and risk factors associated withCampylobacterspp. andSalmonella entericain livestock raised on diversified small-scale farms in California. Epidemiology and Infection, 147. https://doi.org/10.1017/s095026881900205x

Lynch, Ó. A., Cagney, C., McDowell, D. A., & Duffy, G. (2011). Occurrence of fastidious Campylobacter spp. in fresh meat and poultry using an adapted cultural protocol. International Journal of Food Microbiology, 150(2–3), 171–177. https://doi.org/10.1016/j.ijfoodmicro.2011.07.037

Gibbens, J., Pascoe, S., Evans, S., Davies, R., & Sayers, A. (2001). A trial of biosecurity as a means to control Campylobacter infection of broiler chickens. Preventive Veterinary Medicine, 48(2), 85–99. https://doi.org/10.1016/s0167-5877(00)00189-6

Silva, J., Leite, D., Fernandes, M., Mena, C., Gibbs, P. A., & Teixeira, P. (2011). Campylobacter spp. as a Foodborne Pathogen: A Review. *Frontiers in Microbiology*, *2*. https://doi.org/10.3389/fmicb.2011.00200

Russa, A., Bouma, A., Vernooij, J., Jacobs-Reitsma, W., & Stegeman, J. (2005). No association between partial depopulation and Campylobacter spp. colonization of Dutch broiler flocks. *Letters in Applied Microbiology*, *41*(3), 280–285. https://doi.org/10.1111/j.1472-765x.2005.01751.x

Dashti, A. A., Jadaon, M. M., Abdulsamad, A. M., & Dashti, H. M. (2009). Heat Treatment of Bacteria: A Simple Method of DNA Extraction for Molecular Techniques. *KUWAIT MEDICAL JOURNAL*. http://applications.emro.who.int/imemrf/kmj_2009_41_2_117.pdf

Bianchini, V., Borella, L., Benedetti, V., Parisi, A., Miccolupo, A., Santoro, E., Recordati, C., & Luini, M. (2014). Prevalence in Bulk Tank Milk and Epidemiology of Campylobacter jejuni in Dairy Herds in Northern Italy. Applied and Environmental Microbiology, 80(6), 1832–1837. https://doi.org/10.1128/aem.03784-13

Bull, S. A., Allen, V. M., Domingue, G., Jørgensen, F., Frost, J. A., Ure, R., Whyte, R., Tinker, D., Corry, J. E. L., Gillard-King, J., & Humphrey, T. J. (2006). Sources of Campylobacter spp. Colonizing Housed Broiler Flocks during Rearing. Applied and Environmental Microbiology, 72(1), 645–652. https://doi.org/10.1128/aem.72.1.645-652.2006