SEASONAL PREVALENCE, MOLECULAR CHARACTERIZATION, ANTIMICROBIAL RESISTANCE AND FACTORS ASSOCIATED WITH THERMOPHILIC *CAMPYLOBACTER* IN CATTLE, CHICKEN AND WATER IN KAJIADO COUNTY, KENYA

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DECLARATION

I declare that this is my original thesis and has not been presented before in this university or any other university for a degree.

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DEDICATION

To my ever-loving mother Naomi Wanja; in recognition and appreciation for your constant prayers and motivation throughout my PhD journey.

Mum, you have always made sacrifices in your life, you denied yourself luxuries so that I could have a chance of stepping in school.

Today, I'm a PhD holder and I thrive in the blessings of your struggles.

Long Live Mum!

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LIST OF ABREVIATIONS AND ACRONYMS

16S rRNA = 16 subunit ribosomal ribonucleic acid

A, C, G, T = Nucleotides in DNA; Adenine, Cytosine, Guanine and Thymine

AMP = Ampicillin

AMR = Antimicrobial resistance

AMU = Antimicrobial use

AOR = Adjusted odd ratio

ASTs = Antibiotic susceptibility tests

ATCC = American Type Culture Collection

BAss = Blood agar with selective supplement

CLSI = Clinical and Laboratory Standards Institute

cmeABC = *Campylobacter* multi-drug efflux pump; comprises of 3 subunits *cmeA, cmeB, cmeC*

COR = Crude Odd Ratio

DNA = Deoxyribonucleic acid

E = Erythromycin

EDTA = Ethylenediaminetetraacetic acid

F = Forward (primer)

FAO = Food and agriculture organization

flaA = Flagellin gene subunit A

FQs = Fluoroquinolones

GEN = Gentamicin

GOK = Government of Kenya

GPS = Global positioning system

gyrA = DNA gyrase gene encoding the gyrA subunit of the gyrase enzyme

BLAST = Basic Local Alignment Search Tool

BLASTn = BLAST nucleotide database

bp = Base pairs

CDC = Centers for Disease Control and Prevention

ceuE = lipoprotein component encoding siderophore enterochelin

CI = Confidence interval

ciaB = *Campylobacter* invasion antigen B

CIP = Ciprofloxacin

hipO = hippurate hydrolysis gene

IGAD = Intergovernmental Authority on Development

ILRI = International Livestock Research Institute

KNBS = Kenya National Bureau of Statistics

LMICs = Low- and middle-income countries

MALDITOF = Matrix-assisted laser desorption/ionisation time-offlight

mCCDA = modified charcoal cefoperazone deoxycholate agar

MDR = Multidrug resistant

MEGA = Molecular evolutionary genetics analysis

MHBA = Mueller Hinton blood agar

mPCR = multiplex Polymerase Chain Reaction

NA = Nalidixic acid (NA)

NAFIS = National Farmers Information Service

NCBI = National Center for Biotechnology Information

NCs = Non-campylobacters

NJ = Neighbor-joining

OR = Odds Ratio

OTCs = other thermophilic *Campylobacter* species

R = Reverse (primer)

RNA = Ribonucleic acid

sPCR = singleplex Polymerase Chain Reaction

TBE = Tris-Borate-EDTA

TE = Tetracycline

TSB = Tryptose soya broth

UV = Ultra-violet

WHO = World Health Organization

DEFINITION OF TERMS

"Miti shamba" = A swahili words for herbal medicine

"Dawa za kienyenji" = A swahili phrase referring to traditional medicine including herbs

"***Oltinka***" = A Maasai word meaning "a common animal watering point"

"**Mchungo**" = A local name for poultry litter mixed with feed leftover, commonly used as cattle feed

ABSTRACT

Members of the genus *Campylobacter* are frequently associated with abortion, gastroenteritis and/or diarrhoea in livestock. The bacteria also present a substantial public health problem resulting in major financial constraints to the health care system and economic impacts due to lost productivity. Despite the relevance of this bacterium, there is limited epidemiological information and genotypic relatedness on their occurrence in livestock in Kenya. The objectives of this study were to: (1) determine seasonal prevalence of thermophilic *Campylobacter* species among cattle, chicken and water in Kajiado County; (2) identify risk factors associated with their occurrence in cattle herds raised on integrated small-scale farms; (3) assess antimicrobial usage in cattle and chicken production systems, susceptibility profiles and resistance genes in *Campylobacter* spp. isolated from water, cattle and chicken faecal samples; and (4) determine virulence factors and genetic relatedness of *Campylobacter* isolates from the water, cattle and chicken faecal samples in Kajiado County, Kenya.

A total of 457 samples comprising 265 cattle rectal swabs, 142 chicken cloacal swabs, and 50 cattle trough water samples were aseptically collected from 55 randomly selected smallholder farms practicing mixed farming. A pre-tested semi-structured questionnaire was used to collect data on respective farm characteristics and management practices (as potential risk factors for campylobacter colonization in cattle), antimicrobial use, disease history and animal health-seeking behavior among farm owners. Individual samples were subjected to standard techniques for isolation, biochemical tests, followed by singleplex-polymerase chain reaction (sPCR) assays for identification and confirmation of *Campylobacter* genus and species. Out of the 162 isolates that were recovered and confirmed by 16S rRNA-PCR assay, 103 speciated isolates [*Campylobacter coli* (n =29) and *C. jejuni* (n = 74)] were assayed for phenotypic antimicrobial

susceptibility profiling using Kirby–Bauer disk-diffusion method against: ampicillin (AX), tetracycline (TE), gentamicin (GEN), erythromycin (E), ciprofloxacin (CIP) and nalidixic acid (NA). Additionally, detection of antimicrobial resistance genes (tetracycline resistance ribosomal protection [*tet*(O)], beta-lactamase (bla_{OXA-61}), aminoglycoside phosphotransferase (*aph*-3-1), gyrase subunit A (*gyrA*), and multi-drug efflux pump (*cmeB*) encoding resistance to multiple antibiotics) and virulence genes [encoding for motility (*flaA*), adhesion (*cadF*), invasion (*ciaB*) and cytotoxin production (*cdtA*)] among the 103 isolates were detected by PCR and amplicon sequencing. To evaluate the genetic relatedness, 36 isolates including 20 isolates based on 16S ribosomal RNA (16S rRNA) primer specific for genus *Campylobacter*, 8 isolates based on lipoprotein (*ceuE*) primer for *C. coli* and 8 isolates based on hippurate hydrolase (*hipO*) primer for *C. jejuni* were sanger-sequenced at Inqaba laboratories (South Africa). Phylogenetic analysis was done on sequences of cattle, chicken and water isolates using molecular evolutionary genetic analysis version 11 (MEGA 11).

Overall, thermophilic *Campylobacter* prevalence was 35.4% [95% confidence interval (95% CI) = 31.0–39.8]; with *C. jejuni* dominating at 55.6% (95% CI=47.9–63.3%) over *C. coli* in all sample types. The highest thermophilic *Campylobacter* prevalence was observed in chicken at 44.4% (95% CI=36.2–52.6%), followed by cattle at 30.9% (95% CI=25.3–36.5%). Thermophilic *Campylobacter* species were isolated in both seasons; with higher prevalence [39.8% (95% CI=33.6–45.9)] recorded during cold-rainy season. There was significant ($P<0.05$) association between season and thermophilic *Campylobacter* occurrence. Farm stocking dairy breeds [adjusted odds ratio (AOR) = 12.7, 95%CI: 3.2-60] were significantly associated with *Campylobacter* carriage in cattle. There was a significant interaction between farms that kept companion animals and those that did not co-graze cattle with other ruminants; modifying the

odds of acquisition of thermophilic *Campylobacter* in cattle by 10 times (AOR: 10, 95%CI=1.2–95.9). Tetracyclines, aminoglycosides and β-lactam-based antimicrobials were the most commonly used antimicrobials; with 54.5% of the farms generally reporting using antimicrobials in chicken production systems than in cattle. Overall, antimicrobial resistance among *Campylobacter* isolates ranged between 100% for ampicillin to 11.7% for gentamicin. Multi-drug resistance (MDR) was observed in 99 of 103 (96.1%) isolates; with the AX-TE-E-CIP being the most common MDR pattern at 29.1%. All the *C. coli* isolates irrespective of source and all the chicken isolates irrespective of *Campylobacter* species displayed multi-drug resistance. The *tet*(O), gyrA and *cmeB* genes were detected at 93.2%, 61.2% and 54.4% in all the *Campylobacter* isolates, respectively. Of the virulence genes; *ciaB* and *flaA* were the most detected genes in *C. jejuni* (89.7% and 62.2%, respectively), and in *C. coli* (81.1% and 62.1%, respectively) isolates irrespective of source. Phylogenetic analysis showed that *Campylobacter* sequences of the isolates from cattle, chicken and water sources were highly related (i.e. similar lineage), suggesting existence of a complex web transmission.

In conclusion, the study provides insight into *Campylobacter* spp. in livestock and environmental reservoirs, possible transmission dynamics and the relevance for therapeutic regimens in Kajiado County. Seasonality and/or animal husbandry practices play a role in the epidemiology of the organisms in livestock and environment; therefore, understanding pathogen-specific seasonal patterns and associated risk factors is important for improving existing disease prevention and control strategies. Moreover, the existence of major virulence genes associated with the pathogenicity of *Campylobacter* spp., demonstrates that they can potentially infect humans. Consequently, this calls for implementation of one-health approach to reduce the impact of this foodborne zoonotic bacterium for the wellbeing of human and animal health.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background information

Kenya's economy is majorly agricultural one, just like many developing countries in Africa. Therefore, contribution of the livestock sector at household and national level cannot be underestimated. For instance, livestock serves as source of food, draught power, manure for their crops, source of income and dowry, especially in pastoral communities (Muma *et al*., 2014) and other small-holder farmers. At the national level, the livestock sector is a key subscriber to over 12% of Kenya's gross domestic product (IGAD, 2013). Kenya is, however, unable to exploit the sector entirely. This is occasioned by adverse weather and/or climate change, droughts and incidences of animal diseases (Bett *et al*., 2009; KNBS, 2018). Other than natural calamities; incidences of diseases are of important concern, since it is a menace to livestock health, livelihoods, food safety, and supply in agricultural-based economies.

With respect to diseases, to-date, emphasis has been on mastitis, infectious viral and bacterial diseases, helminthoses, tick-borne and other arthropod-borne diseases. There is a need to investigate the burden of other diseases to evaluate their impact on livestock production performance. One such livestock disease with scanty information is campylobacteriosis.

Thermotolerant campylobacters (cause of campylobacteriosis) are important points of reference for animal and human health research, owing to their zoonotic potential, wide variety of reservoir hosts, and environmental perseverance e.g. survival in water (Hannon *et al*., 2009). They are found in the gut of healthy and diseased wild animals and livestock, including poultry. Some of the thermotolerant *Campylobacter* species include; *C. jejuni, C. coli, C. lari* and *C. upsaliensis.* However, only *C. jejuni* and *C. coli* have been implicated as having the greatest disease impact

in human beings and livestock. In farm animals; campylobacteriosis manifests as septic abortion in cattle and ewes; mastitis in cattle; infectious hepatitis in chicken; and gastro-enteritis and/or diarrhoea in most farm and companion animals, including chicken, dogs, cats and pig (Prescott and Monroe, 1982; Gudmundson and Chirino-Trejo, 1993; Carter *et al*., 1995; Bae *et al*., 2005; Markey *et al*., 2013). Several thermotolerant *Campylobacter* species are zoonotic; and are foremost important causal agents of gastroenteritis in man (Markey *et al*., 2013).

Contaminated water plays a major role as an environmental exposure for fecal-oral mode of disease spread in both livestock and humans. Although the relative contribution of water sources to *Campylobacter* infections in livestock is unclear, due to lack of data linking water to such infections in Kenya, studies conducted elsewhere have shown an association between water source and *C. jejuni* carriage (Häkkinen and Hänninen, 2009; Bianchini *et al*., 2014).

In order to further elucidate the epidemiology of thermophilic *Campylobacter* colonization in livestock (cattle and chicken); other factors that may influence prevalence estimates need to be weighed-in as well. Such factors include herd size, breed, age, animal husbandry practices, location, season (Ellis-Iversen *et al.,* 2009b**;** Hannon *et al*., 2009; Klein *et al*., 2013; Hoque *et al*., 2021) and probably presence of infected poultry, just to mention a few.

Laboratory isolation and confirmation of *Campylobacter* by culture-based methods are tedious and laborious, owing to the organism's fastidious nature. Furthermore, distinction among species through standard biochemical tests is hard, since there are few discerning features. Consequently, molecular techniques such as polymerase chain reaction (PCR) and sequencing have been employed for identification purposes, and also for epidemiological characterization (Miller *et al*., 2010). Genotyping of strains from different sources generates epidemiological linkage data that

may be valuable in evaluating impending risk of infection, controlling disease, and establishing sources of *Campylobacter* infection (Han *et al*., 2007). Polymerase chain reaction and sequencing method have also been used to detect and confirm virulence associated markers in an organism (Barakat *et al*., 2020).

Resultant animal diseases burden has necessitated prophylactic and therapeutic usage of antimicrobial drugs to enhance growth and minimize disease incidences in livestock. Unfortunately, this has ensued in emergence and escalation of antimicrobial-resistant bacteria, such as antimicrobial-resistant *Campylobacter* strains (Chen *et al*., 2010; Abdollahpour *et al*., 2015). Antimicrobial resistance is a pressing worldwide threat to community health and food security. It poses a menace to public and livestock health by limiting the choice of antimicrobial agents available for treatment. More-over there is a likelihood of transfer of antimicrobial resistant pathogens (thermotolerant *Campylobacter* species included) to people via ingestion of contaminated foods of animal origin (Facciolà *et al*., 2017).

1.2 Objectives

1.2.1 Overall objective

To investigate seasonal occurrence, molecular characteristics, antimicrobial resistance profiles and risk factors associated with thermophilic *Campylobacter* spp. in cattle, chicken and water in Kajiado County, Kenya.

1.2.2 Specific objectives

1. To determine seasonal prevalence of thermophilic *Campylobacter* species among cattle, chicken and water in Kajiado County.
2. To identify risk factors associated with occurrence of thermophilic *Campylobacter* species in cattle herds raised on integrated small-scale farms in Kajiado County, Kenya.

3. To assess antimicrobial use, antimicrobial susceptibility profiles and presence of resistance genes in *Campylobacter* isolated from cattle, chicken and water samples in Kajiado County, Kenya.
4. To determine virulence factors and genetic relatedness of *Campylobacter* isolates from cattle, chicken and water samples.

1.3 Null hypotheses

H_1: There is no seasonal variation in prevalence of thermophilic *Campylobacter* species among cattle, chicken and water samples from Kajiado County.

H_2: There are no risk factors associated with occurrence of thermophilic *Campylobacter* species in cattle herds in integrated small-scale farms in Kajiado County.

H_3: There is no usage of antimicrobials in cattle and chicken production systems;

H_4: *Campylobacter* isolates from cattle, chicken and water samples in Kajiado County are not resistant to the selected antimicrobials tested, nor do they possess antimicrobial resistance genes.

H_5: *Campylobacter* isolates from cattle, chicken and water samples in Kajiado County do not possess virulent genes and are genetically heterogenous.

1.4 Problem statement

Foodborne pathogens cause illness in 1 in every 10 people worldwide annually (Knechtges *et al.*, 2018), with *Campylobacter* leading (WHO, 2018). *Campylobacter* infections in humans usually present as mild enteric illness persisting for 5 to 7 days (Kim *et al*., 2018). However, life-threatening infections and sometimes deaths may occur in kids, the aged, or immuno-compromised people (Same and Tamma, 2018). In some parts of Kenya, *Campylobacter*

infections in humans have been increasing (Zachariah *et al*., 2021); this may be attributed to human behaviour (more so hygiene) or ingestion of beef or poultry meat or water contaminated with *Campylobacter*.

Currently, a deductive reasoning on thermophilic *Campylobacter* prevalence among cattle, chicken and farm environment (water) in Kajiado County and Kenya as a whole, is either lacking or poorly comprehended (especially in arid and semi-arid areas); and has not been fully characterized probably due to ubiquitous and fastidious nature of the organism. Furthermore, there is little published information on prevalence, seasonality, antimicrobial resistance patterns and genotypic information of thermotolerant *Campylobacter* species from environmental sources and in food animals; as many laboratories do not routinely test for the bacteria. *Campylobacter jejuni* and *C. coli* are the most significant species; with various reservoirs including chicken and cattle being the most implicated sources of human illness. Consequently, controlling *Campylobacter* colonization in cattle and chicken would assist in minimizing incidence of *Campylobacter* infections in humans. An in-depth understanding of occurrence and genotypic characteristics of livestock-derived *Campylobacter* isolates is presently lacking in Kenya and other African states; due to the shortfall in surveillance systems (Mpalang *et al*., 2014). Additionally, the significance of diverse environments in Kenya, including water, as possible risk factors and sources for acquisition of *Campylobacter* in livestock (particularly cattle) is far less known. Globally, most studies have focused on *Campylobacter* isolation from chicken with limited attention on other reservoirs including cattle and environment (water).

Furthermore, knowledge or data on the magnitude of antimicrobials resistance (AMR) among Campylobacter isolates from cattle, chicken and environmental water samples from Kajiado County is lacking. The few existing studies on antimicrobial resistance of thermotolerant

Campylobacter organisms done elsewhere in Kenya has focused on man and chicken (Shapiro *et al*., 2001; Brooks *et al*., 2006; Nguyen *et al*., 2016). There is little to no information on antimicrobial susceptibility profiles of *Campylobacter* isolated from cattle or environment. In addition, there's lack of information on use of antimicrobials in animal husbandry practice; often without relying on empiric findings from antimicrobial susceptibility tests (ASTs) for choice of antimicrobials. This contributes to the development of resistance; a looming disaster which is, perhaps, more aggravated in developing countries including Kenya. To tame this menace, more AMR studies are needed.

Therefore, an investigation on seasonal prevalence, molecular determinants of virulence and resistance, genetic heterogeneity, antimicrobial usage and susceptibility profiles and factors associated with carriage of thermophilic *Campylobacter* in cattle, chicken and water in Kajiado County, Kenya, was carried out

1.5 Justification of the study

Although chicken, cattle and/or their products (beef, milk or chicken meat) may harbour or get contaminated with *Campylobacter* at farm or slaughter level, it is imperative to investigate *Campylobacter* prevalence in live animals at farm level. However, some studies on incidence of *Campylobacter* spp. in chicken have been reported in Kenya (Osano and Arimi, 1999; Nguyen *et al*., 2016; Mageto *et al*., 2018). Up to date information on thermophilic *Campylobacter* infection in cattle and environmental water samples is missing in Kajiado. *Campylobacter* strains from farm and wild animals and environmental sources have been associated with human gastroenteritis (Kwan *et al*., 2008); and therefore, pose a major public health concern. Consequently, establishing the status of *Campylobacter* carriage in livestock and environment will assist in identifying the type of livestock production systems and/or environments where

future studies ought to focus-on in order to reduce its prevalence. Livestock including chicken, play a significant role in *Campylobacter* infections in humans. The significance of cattle and chicken as reservoir for *Campylobacter* infections in human is related to: (1) contaminated foods (raw milk, beef and poultry meat), (2) environmental and water pollution, (3) direct transmission to humans from infected animals. Several studies have identified chicken meat and beef as the main food vehicles associated with sporadic cases of campylobacteriosis in humans (Modi *et al*., 2015; Bertasi *et al*., 2016; Barakat *et al*., 2020). Ultimately, reducing the prevalence from farm-to-slaughter and farm environment can reduce the burden of *Campylobacter* infections in humans. This is despite the significance of campylobacteriosis disease in livestock. Few studies have considered the role that environmental sources and poultry play in the epidemiology of campylobacteriosis and/or transmission in cattle. *Campylobacters* grow best at 42°C, a temperature found in intestines of chicken; as such chicken are the most important natural reservoirs. Their (chicken) roaming behavior is an important aspect in that they continuously spread faecal material laced with *Campylobacter*; leading to environmental (water or pasture) contamination. Genotyping assists in elaborating the transmission dynamics of *Campylobacter* via source ascription and characterizing genotypes associated with human infections (Eberle and Kiess, 2012).

Antimicrobial resistance (AMR) is a pressing global threat to community health, food security and development. By 2050, antimicrobial resistance (AMR) is projected to cause 10 million deaths per year, with 4.5 million deaths coming from sub-Saharan Africa, overpassing diseases such as cancer (WHO, 2016). Furthermore, without intervention, AMR is projected to decrease global GDP by as much as 5%, and many of these losses are experienced in low-income countries including Kenya (World-Bank, 2016). As such, there is need for monitoring and

surveillance of antimicrobial use in cattle and chicken production systems and presence of antimicrobial resistance in thermotolerant *Campylobacter* isolated from these animals. This will aid in timely detection of resistant strains in farm animals that may be transferred to humans. Increase in the rate at which resistant *Campylobacter* species are isolated necessitates research on alternative therapeutic agents.

Studies on molecular characterization of *Campylobacter* isolated from animals and environment are important in establishing pathogenic potential of *Campylobacter* species to humans. *Campylobacter* species possess a virulome that is necessary for adhesion, colonization, attack, and toxins production, leading to their enhanced incidence and epidemiology in contrast to other enterobacteria (Bolton, 2015; Otigbu *et al*., 2018); even though, the pathogenicity mechanisms contributing to *Campylobacter* infectivity are elusive (Nguyen *et al*., 2016). Additionally, determination of genetic relatedness using phylogenetic assays may be used as a molecular tool in studies in the surveillance of *Campylobacter*-like bacteria (Nayak *et al*., 2014); allowing discernment of the sources of infection.

Comprehending seasonal occurrence and associated factors contributing to genetic heterogeneity will assist researchers and farmers establish when and where risks for acquisition of *Campylobacter* remains high. Additionally, findings on predisposing factors will provide potential areas to guide public health players when formulating control strategies for minimizing and preventing zoonotic transmission of *Campylobacter*. Findings on antimicrobial susceptibility profiles will be crucial for effective therapy i.e. will generate information on which antimicrobials are relevant in the wake of AMR. The policies or legislations governing use of antimicrobials in livestock production systems ought to take into consideration animal-health seeking behaviour among livestock owners that cause and aggravate antimicrobial resistance.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of livestock production systems in Kenya

2.1.1 Spotlight and significance of cattle and poultry production systems

The Kenya, cattle and poultry sectors are major contributors to Kenya's food security and gross domestic product (GDP). The sectors impact positively through provision of livelihoods (source income), food (milk, meat and eggs) and/or nutrition at household level among the livestock dependent communities. However, the sectors can also have negative impacts, especially through zoonotic diseases that spill-over from animal interface to humans (FAO, 2018). Kenya is endowed with a great variety of animal genetic resources, with cattle and poultry contributing around 70 percent to the total livestock production (ILRI, unpublished), projected at $1,622 billion as of 2016. However, the sector is set to expand exponentially over the next three decades and support the country's food supply amidst a rapid growth in human population (KNBS, 2018). Poultry contributes 8 percent to agricultural value, with the country producing over 25 000 tons of poultry meat and 1.3 billion eggs per year, jointly valued at KES 28.5 billion (FAO, 2017). Per capita consumption is approximately 0.56 kg of poultry meat and 45 eggs per year (FAO, 2017). The sector is highly diverse, consisting of large number of small-holder free-range and backyard indigenous chicken farmers; a sizable number of commercialized small-holder layers and broiler farms; and limited industrial integrated layer and broiler farms. Chickens constitute about 98 percent of the total poultry raised in the country and 65 percent of Kenyan households are estimated to keep at least one bird. The three major poultry production systems in Kenya are free range, semi-intensive and intensive systems (FAO, 2017).

Livestock sector is an important supply source for milk, hides, skin, meat, eggs, feathers, blood and bone meal. Beef is the most significant source of red meat, with a contribution of around

80% of the slaughtered ruminant off-take (IGAD, 2013). However, poultry greatly contributes to white meat protein which is preferred over red meat. While healthy animals produce healthy, nutritional and disease-free food, losses occur in an event of diseases. As such, diseases are a threat to animal health, food security and livelihoods (Nabarro and Wannous, 2014), especially in agricultural-based economies.

2.1.2 Future perspective and challenges facing livestock production systems

Global livestock production has drastically changed in the last decade, from subsistence farming to intensive system. In Kenya, the cattle and poultry sectors are projected to rapidly expand and revolutionize the economy in the coming years, owing to the increasing demand for animal-source protein and growing population. However, the long-term future of Kenyan livestock, and of the cattle and poultry sectors in particular, is at threat of significant production losses due to emergence and spread of multi-drug-resistant disease-causing organisms (Kiambi *et al*., 2021). Diseases are the major causes of declining livestock productivity. This has necessitated to re-orientation of production systems to conform with this reality; adapting practices that will counter the increasing disease outbreaks incidences (some being emerging and re-emerging) and ensure safety of livestock by-products (meat, eggs, and milk, among others).

Additionally, over the years, livestock keepers, particularly pastoralists, have had to encounter a number of livelihoods threatening-challenges including; changes in climate, land tenancy and use, population increase, rapid urbanization, globalization, intensification and communal conflicts (Said *et al*., 2019). Livestock sector (including cattle and poultry), like all other agricultural sub-sectors are affected by the adverse effects of climate change that are being experienced globally. Consequently, majority of farmers are slowly adopting diversified or integrated farming; where they raise manageable herds of cattle and other livestock species

including poultry, alongside numerous farm produce (i.e. integrated crop–livestock farming). In addition, farms stocking several species of animals may have different biosecurity barriers in place than farms that only keep one type of animal species (Ellis-Iversen *et al*., 2009a). It is also a common practice in some farms to apply raw manure on grazing fields or on fodder crops such as napier grass to improve soil fertility. Application of raw manure can introduce faecal entero-pathogens (including *Campylobacter* spp.) thereby contaminating pasture.

Most countries are advocating for diversification of farming systems as a substitute for intensive agriculture, affirming readiness to change to ecofriendly agriculture, in agreement with the United Nations Sustainable Development Goal number 12 (Patterson *et al*., 2022). Even with diversification, each farm is a unique combination of numerous environmental characteristics that could set baseline conditions for the presence of pathogenic bacteria (Strawn *et al*., 2013).

This study aims at validating and improving strategies for management and adaptation measures that can be used specifically in the livestock sector to minimize the impacts of climate change associated with campylobacteriosis. The results will provide a basis for development of models for prediction of weather-related disease occurrences (Patterson *et al*., 2022).

2.1.2.1 Climate change and livestock diseases interrelationship

The adverse magnitude of livestock diseases is high in third world countries (including Kenya); with the diseases believed to kill 20% of ruminants and over 50% of poultry, causing economic losses of roughly USD 300 billion each year (Grace *et al*., 2015). Climate change can aggravate disease outbreaks in livestock and, 58% of the 65 diseases of significance to poor livestock keepers being sensitive to changes in climate (Grace *et al*., 2015). Climate sensitive diseases have great economic impacts and remain the most important diseases among the vulnerable

livestock farmers; as presented in Table 2.1. From the table, campylobacteriosis is one of the top climate-sensitive livestock diseases, in addition to being a human health burden.

Table 2.1: Important bacterial diseases of livestock among the poor and vulnerable livestock farmers

Livestock disease	Importance index	Zoonotic potential	Region				Farming system		
			WA	ECSA	SA	SEA	Pastoral	Agro pastoral	Peri-urban
Salmonellosis	13	++	++	++	++	++	++	++	++
Campylobacteriosis	13	++	++	++	++	++	++	++	++
Leptospirosis	12	++	++	++	++	++	++	++	++
Botulism	12	++	++	++	++	++	++	++	++
Listeriosis	11	++	++	++	++	++	++	++	++
Colibacillosis	10	++	++	++	++	++	++	++	++
Anthrax	9	++	++	±	++	++	±	++	++
Percent of disease present		39	66	63		63	50	68	71

Adopted from Grace *et al*. (2015). **Key**: ++= an important problem; ± = a minor problem; WA = West Africa; ECSA = Eastern, Central and Southern Africa; SA = South Asia, SEA = South-East Asia

While most studies have focused on effects of climate change on arthropod-borne diseases, such as Rift Valley fever, tick-borne diseases and trypanosomosis among others; little attention has been given to non-vector-borne diseases, e.g. *Campylobacter* infection. Thus, there is paucity of published data on the likely impact of non-vector-borne diseases under varying climate settings. Another knowledge gap exists owing to the complexity of disease transmission dynamics that is, there are multiple pathways, direct and indirect, by which climate can influence pathogen/disease (Grace *et al*., 2015). While climate change obviously contributes to variation in disease occurrence, spread and abundance, deviations in management practices, livestock population and technology could preponderate these.

2.2 Overview of genus *Campylobacter* with emphasis on the enteric thermotolerant group

2.2.1 Current taxonomy of *Campylobacter* species

The word *Campylobacter* originates from ancient Greek meaning curved rod referring to its typical shape (Tresse *et al*., 2017). To date, the genus *Campylobacter* falls under the phylum Proteobacteria, class Epsilonproteobacteria, order Campylobacterales, and the family Campylobacteraceae (Ammar *et al*., 2021). The genus *Campylobacter* comprises 34 formally published species (Parte, 2018) and 13 subspecies (Chlebicz and Śliżewska, 2018); with *C. upsaliensis*, *C. coli*, *C. rectus*, *C. lari*, *C. jejuni*, *C. concisus*, *C. mucosalis*, *C. sputorum*, *C. laridis*, *C. helveticus*, *C. fetus*, *C. hyointestinalis*, *C. insulaenigrae*, and *C. ureolyticus* being the most implicated pathogens (Heredia and García, 2018) in both human and veterinary medicine. *Campylobacter jejuni* comprises two subspecies, namely *C. jejuni* subspecies *jejuni* and *C. jejuni* subspecies *doylei*. Subspecies *jejuni* is infective; whereas the role of subspecies *doylei* in animals and humans is unclear (Markey *et al*., 2013). *Campylobacter fetus* subspecies *fetus* and *C. fetus* subspecies *venerealis* represent the mesophilic campylobacters and significant genital pathogens of veterinary importance (Markey *et al*., 2013).

Campylobacter species are grouped into five distinct groups, namely, the *C. fetus* group, C. *jejuni* group (thermotolerant), *C. lari* group, *C. concisus* group, and *C. ureolyticus* group. *Campylobacter jejuni*, *C. upsaliensis*, *C. coli*, *C. helveticus* and *C. hepaticus*, among others, form a genetically homogenous grouping and are identified as the thermophilic campylobacters (Costa and Iraola, 2019) as presented in Figure 2.1.

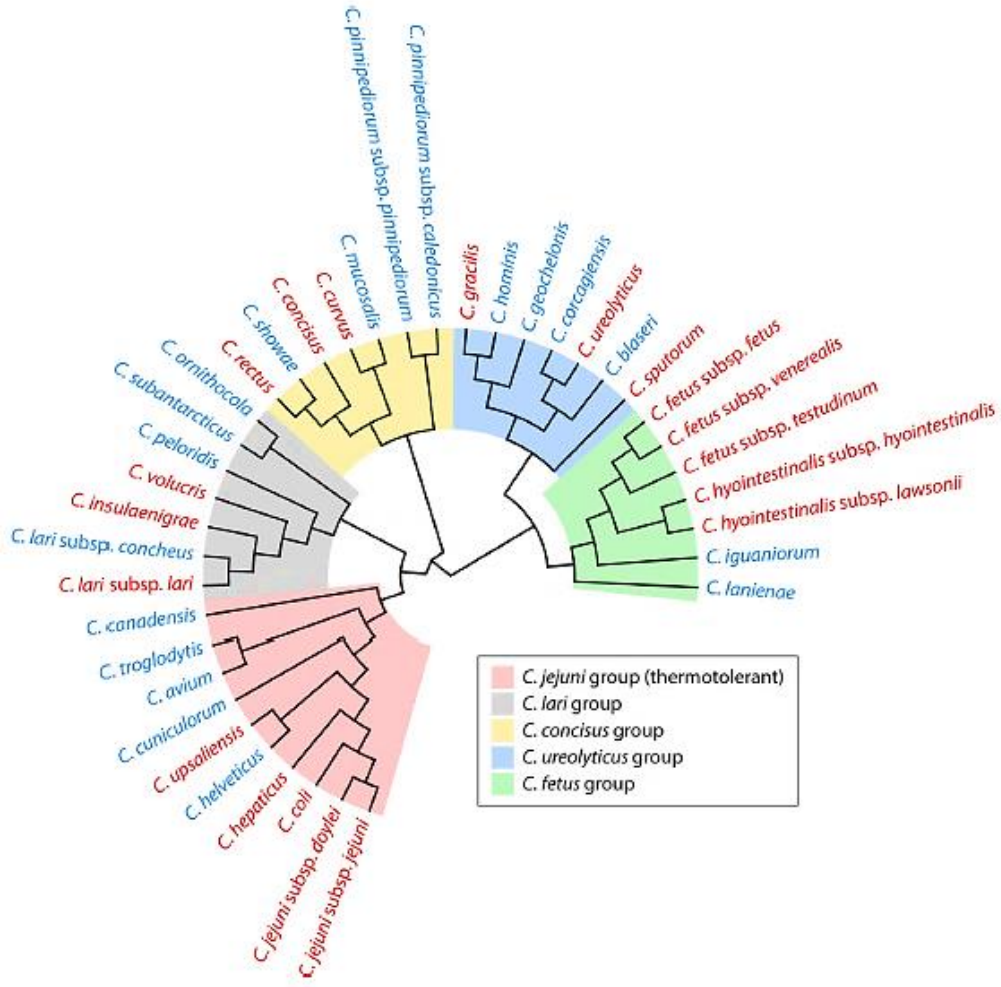


Figure 2.1: Phyletic relationship among members of *Campylobacter* based on the most clinically relevant species within each group (Costa and Iraola, 2019). The red font tip labels indicate species reported to cause infections in man and/or other animals while the blue font tip labels indicate species not reported to cause infections

Thermotolerant species thrive optimally at 42 ℃. Additionally, thermotolerant campylobacters can grow sub-optimally at 37 ℃ but not at 25 ℃. The organisms, particularly *C. jejuni* and *C. coli* are of most clinical relevance and also most frequently encountered pathogens in livestock (Markey *et al*., 2013) and humans (Facciolà *et al*., 2017). Other species *C. concisus*, *C. lari*, *C. upsaliensis* and *C. ureolyticus* are described as emerging *Campylobacter* species and have been underrated as causal agents of human gastroenteritis because of biases in the existing

identification methods (Costa and Iraola, 2019; Soto-Beltrán *et al*., 2022). Consequently, this study explored the "known" enteric thermophilic campylobacters, with inclination towards *C. jejuni* and *C. coli*.

2.2.2 Natural habitat and spatial distribution of *Campylobacter* species

Thermotolerant *Campylobacter* organisms are extensively spread in nature. The organisms are ubiquitous on the oral cavity mucosa, in the gut of both wild and farmed animals, as well as birds (Markey *et al*., 2013) and in the environment (soil or water contaminated with animal faeces). While most farm animals are potential reservoirs for the organisms, it is the poultry source that carries the most risk burden (Guévremont *et al*., 2014). This is so considering that these organisms grow best at temperature of 42°C found naturally in avian intestines. However; the probable significance of chicken as a reservoir of *Campylobacter* organisms aiding in transmission of disease to cattle has not been fully investigated.

2.2.3 Transmission and sources of thermophilic campylobacters in livestock

Most transmission of campylobacters in livestock occurs from the environment (faecal-oral route via consumption of contaminated pasture and water), as well as through horizontal transmission between flocks/herds (via touching faeces from diseased animals). It is likely that complex transmission pathways exist among domestic animals (cattle and chicken) and wild animals and their immediate environment including pasture, soil and water (Figure 2.2). However, once thermophilic campylobacters colonize a herd/flock; the spread is rapid, making disease elimination difficult (Sibanda *et al*., 2018).

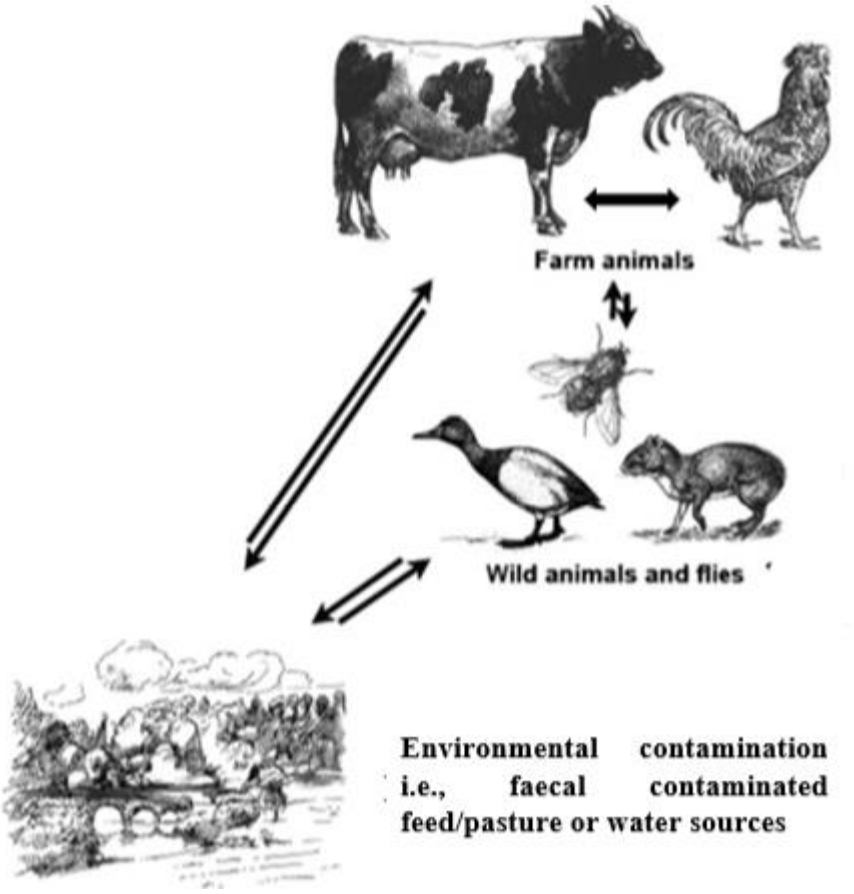


Figure 2.2: Possible transmission dynamics for *Campylobacter* in livestock; modified from Bronowski *et al*. (2014)

Thermophilic campylobacters have been isolated in a wide variety of animal reservoirs in Kenya. Most studies on thermophilic campylobacters infections in livestock in Kenya have reported live chicken and contaminated chicken meat as major reservoirs (Chepkwony, 2016; Nguyen *et al*., 2016; Carron *et al*., 2018; Mageto *et al*., 2018; Abubakar *et al*., 2019; Kariuki *et al*., 2020). However, colonization in other livestock, such as pigs, ducks, goats, cattle and sheep has also been described (Turkson *et al*., 1988; Osano and Arimi, 1999; Chepkwony, 2016). Nevertheless, Chai *et al*. (1990) and Ngotho *et al*. (2006) have also reported outbreaks of *Campylobacter* infections in colonies of captured vervet monkeys.

Thermophilic campylobacters have also been detected in wild birds, raw milk and associated milk products and environmental sources such as surface water, soil/manure and feed samples (Markey *et al*., 2013; Merialdi *et al*., 2015; Del-Collo *et al*., 2017; Uddin *et al*., 2021). Occurrence of campylobacters in environmental sources is mainly linked to faecal contamination. Additionally, the microorganisms can also be carried into farms via fomites such as gumboots, clothing and farm equipment such as contaminated feed trough (Sibanda *et al*., 2018). However, their survival outside the host body and/or in the environments is generally thought to be poor; with some studies suggesting that the ability to survive may vary with strain (Mustafa, 2016; Mulder *et al*., 2020). The role of surface-water as a vehicle of transmission for *Campylobacter* among domestic animals including cattle need further investigation.

2.2.4 Culture-dependent diagnosis

2.2.4.1 Laboratory isolation, culture conditions and colony morphology

Laboratory isolation based on conventional culture method is considered the gold standard for campylobacteriosis diagnosis; nevertheless, it is laborious and arduous owing to the fastidious growth requirements of the bacterium (Li *et al*., 2014). The organisms are mostly isolated from fecal specimens (cloacal or rectal swabs) and from environmental swabs using selective media such as Skirrow, Blaser's Campy-BAP medium, Karmali, Preston, charcoal-cefoperazole-deoxycholate agar and/or modified charcoal cefoperazone deoxycholate agar (Martin *et al*., 2002; Markey *et al*., 2013). A selective supplement containing combination of either of the following antimicrobials: vancomycin, cephalothin, amphotericin B, trimethoprim lactate, rifampicin, colistin, polymyxin B or cycloheximide sulphate is usually incorporated to inhibit non-campylobacter organisms (Markey *et al*., 2013).

Campylobacters are obligate anaerobes or micro-aerobes (grow at 5-10% oxygen and 5-10% carbon (iv) oxide conditions); however, some species, including *C. concisus*, *C. curvus*, *C. gracilis*, *C. mucosalis*, *C. rectus*, C. *showae* and *C. hyointestinalis* require 3-7% hydrogen-enriched conditions for them to grow. It is for this reason that most laboratories do not routinely test for this bacterium. As such, inoculated plates ought to be incubated under the given atmospheric conditions described above (micro-aerobically at 42°C, 37°C is suboptimal) for two to three days. *Campylobacter* colonies appear as grey/white or creamy grey in colour and moist on modified charcoal cefoperazone deoxycholate agar (mCCDA). The colonies may appear as a layer of growth over the surface of the agar. The colonies are translucent, non-hemolytic and spreading on blood agar (Markey *et al*., 2013).

2.2.4.2 Cell morphology

Campylobacters normally occur as small (0.2–0.8 μm × 0.3–5 μm), Gram-negative, motile, curved rods; but occasionally many cells are involved, joined to form long (8 μm) spiral chains. They exhibit a typical spiraling motility. However, under unfavourable culture conditions, the curved rods degenerate to coccoid forms (MSD Veterinary Manual, 2022). Like other bacteria, the coccoid forms were first believed to be a viable but non-culturable (VBNC) form with ability to survive in unfavourable circumstances. Nonetheless, subsequent research on a closely related bacterium *Helicobacter pylori* hinted that the coccoid forms are non-viable and degenerative forms (Ikeda and Karlyshev, 2012). The issue on whether the coccoid forms are viable and infective is exceedingly contentious (Ikeda and Karlyshev, 2012). However, Frirdich *et al*. (2019) demonstrated that evolution to coccoid forms resulted in variations in pathogenicity and/or virulence. Frirdich *et al*. (2019) further reported that coccoid forms of *C. jejuni* were non-motile-non-infectious, with minimal invasion of and adhesion to epithelial

cells, inability to stimulate neutrophil chemo-attractant or interleukin-8 (IL-8). Thus, formation of coccoid forms presents an important survival mechanism (Frirdich *et al*., 2019).

2.2.4.3 Phenotypic and biochemical characteristics

Campylobacter organisms may be diagnosed by conventional culture dependent biochemical tests and molecular assay. Although conventional techniques are useful, they have some shortfalls due to; fastidious disposition of the organism, requiring specific culture conditions. Furthermore, lack of many discerning biochemical tests is the other problem encountered in laboratory identification of *Campylobacter* species. Thermophilic *Campylobacter* species do not metabolize sugars aerobically or anaerobically (they are asaccharolytic). *Campylobacter jejuni* is differentiated on its ability to hydrolyze hippurate, and *C. upsaliensis* has negative or weak catalase production and is differentiated from other campylobacters because of its sensitivity to nalidixic acid. *Campylobacter helveticus* is also catalase negative but can be difficult to differentiate biochemically from *C upsaliensis* relying on distinctive colony morphologies (Markey *et al*., 2013; Bojanić, 2016).

Hydrolysis of hippurate salt is the only biochemical test for differentiation of *C. jejuni* from *C. coli* (Skirrow and Benjamin, 1980). However, variable hippurate activity hippuricase *C. jejuni* has been reported (Totten *et al*., 1987). Owing to the variable results, culture-based diagnosis of *Campylobacter* ought to be supported by molecular tools such as polymerase chain reaction (PCR).

2.2.5 Molecular diagnostic assays and genotyping

Owing to the shortfalls in conventional culture-based methods; there has been a rapid move towards molecular methods for both surveillance and routine analysis. Nevertheless,

campylobacters are genetically diverse organisms with frequent genetic recombination processes (Ragimbeau *et al*., 2008). Consequently, molecular-typing techniques are extensively utilized to characterize several strains. Numerous genotyping methods have been established for epidemiological studies of *Campylobacter* infections, to distinguish between strains below the level of the species (Wassenaar and Newell, 2000). Commonly used molecular typing methods include multilocus sequence typing (MLST), flagellin typing, PCR (conventional amplification), pulsed-field gel electrophoresis (PFGE), amplified fragment-length polymorphism (AFLP), and ribotyping (Eberle and Kiess, 2012).

The advantage of using PCR is its cost effectiveness and simplicity, and PCR-based assays provide an accurate confirmation for the discrimination of those isolates that are indistinguishable by biochemical assays. In *Campylobacter* organisms, several copies of ribosomal RNA (rRNA) gene loci, namely 5S, 16S, and 23S rRNA, are occupied in different chromosomal locations (Wassenaar and Newell, 2000); of these, the 16S rRNA gene is highly specific, ubiquitous and extremely conserved region within the *Campylobacter* genomic DNA and has been widely used for genus identification (Patton *et al*., 1991). However, this tool has limitations due to the low polymorphism of 16S rRNA gene sequence data and high intraspecies diversity in some cases (Korczak *et al*., 2006). Consequently, other molecular tools are required to elucidate classification of genus *Campylobacter* and, at the same moment, to enable the genetic characterization of bacteria belonging to this genus (Korczak *et al*., 2006).

Apart from 16S rRNA PCR assay, other PCR assays targeting genus-specific or species-specific including *ceuE*, *lpxA*, *hipO*, *mapA*, and *ask*, have been developed in the recent past to facilitate the accurate identification of *Campylobacter* strains, especially *C. jejuni* and *C. coli* (Dennis *et al*., 1999; Han *et al*., 2016; Chowdhury *et al*., 2019). Subsequently, the hippurate hydrolysis

(*hipO*) gene-based polymerase chain reaction (PCR) assay is used to differentiate *C. jejuni* from the other campylobacters (Al-Amri *et al*., 2007); whereas a lipoprotein encrypting siderophore transport gene (designated as *ceuE*) has recently been used in identification of *C. coli*. polymerase chain reaction (PCR) assays of *ceuE* and *hipO* genes have greatly supported the confirmation of *C. coli* and *C. jejuni*, respectively, with reasonable specificity (Han *et al*., 2017); where regions appear conserved. More recently, assays targeting other genes have been developed to increase specificity and sensitivity, as well as for virulence characterization (Bang *et al*., 2003).

2.2.6 Genotyping and phylogenetic analysis

Since thermophilic *Campylobacter* species are isolated from a wide range of farm animals (chicken, cattle, shoats, pigs) and wild birds, and environmental samples (feeds, water and soil) it is nearly impossible to ascertain the sources and routes of infection using conventional and biochemical tests. Consequently, use of molecular methods in establishing phyletic relationships among hosts have appealed much attention and have an important role to play especially in reorganization and classification of *Campylobacter* (Woese, 1987). Most importantly, phylogenetic assays have come handy in tracing the sources of clinical field isolates by exploiting differences in the genotypic characteristics and frequency of thermophilic *Campylobacter* strains harboured in diverse host animals and environments (Dearlove *et al*., 2016). Besides, sequencing of isolates from discrete sources provides epidemiological linkage data that may be utile in evaluating risk of future infection and controlling disease (Han *et al*., 2007).

Molecular tools including phylogenetic analysis have been used in identification of genes within conserved regions in a given *Campylobacter* lineage and also in detection of those that are

phyletically distributed at species level. Furthermore, phylogenetic characterization of sequences obtained have been used to trace sources of campylobacteriosis by taking advantage of differences in the genotypes and incidence of *Campylobacter* species that thrive in various animals and habitats (Dearlove *et al*., 2016). For instance, Sanad *et al.* (2013**)** demonstrated that starlings carry *C. jejuni* strains that were genotypically similar to those in cattle; proving the usefulness for this purpose. More-over, with the advent of high-throughput sequencing technique; complex and diverse microbiota can be sequenced to unparalleled complexity with more precise reporting (Ocejo *et al*., 2019b). One such sequencing technology described in the current work is targeted and/or amplicon sequencing. The technique entails amplification of conserved regions such as 16S ribosomal RNA (rRNA) genes in order to profile and analyze bacterial community (Ocejo *et al*., 2019b). Genomic DNA sequencing and analysis of genes targeting 16S ribosomal RNA (16S rRNA) has intensely improved the classification and documentation of many bacterial genera.

Amplified sequences of 16S rRNA genes has been used to establish phylogeny relationships within the genus *Campylobacter* (Korczak *et al*., 2006; Al-Nasrawi, 2016; Chukwu *et al*., 2019; Hassler *et al*., 2022;). Furthermore, sequencing of 16S rRNA amplicon is important in genus identification and speciation of bacteria (Hansson *et al*., 2008). Additionally, certain regions of the 16S rRNA gene are extremely conserved, and that any alterations within the sequence are so likely to be precise measure of evolution, making a useful molecular marker for the study of phylogenetic linkages (Janda and Abbott, 2007). However, the 16S rRNA sequencing technique has limitations due to high similarity (low polymorphism) found among members of the *Campylobacter* genus making it hard to distinguish between species including *C. coli* and *C. jejuni* based on 16S rRNA gene (Harrington and On, 1999; On, 2001). Consequently, extra

genetic tools are needed for *Campylobacter* genus identification while still confirming species identity.

Lack of genotypic information on *Campylobacter* strains emanating from chicken, cattle and environment (water) in Kajiado County, and Kenya at large, spotlights the necessity to monitor their genotypes and potential relationships. *Campylobacter coli* and *C. jejuni* are closely related by genetic make-up and phylogeny (Dedieu *et al*., 2004) further limiting 16S rRNA amplicon sequencing technique. Consequently, phyletic relationship and homogeneity among *C. coli* and *C. jejuni* strains in this study was inferred using amplicon amplification and Sanger dideoxy approach for the 16S rRNA gene (genus *Campylobacter* specific), *ceuE* gene (*C. coli*) and *hipO* (specific for *C. jejuni*).

2.2.7 Pathophysiology of *Campylobacter* species

Campylobacter jejuni and *C. coli* can infect ruminants, poultry, pets, wild animals, pigs and non-human primates causing enteritis. The organisms are also commonly associated with gastroenteritis and systemic illness in humans. As such, campylobacters are important targets for animal and human health research because of their zoonotic potential, wide variety of reservoir hosts, and environmental persistence and survival especially in water (Hannon *et al*., 2009).

2.2.7.1 *Campylobacter* species infections in chicken and other avian species

Natural reservoirs for *Campylobacter* include chicken and other domestic poultry (including turkey, ducks, and geese) (Conrad *et al*., 2018). Poultry remains the most important reservoir owing to: (1) the high body temperature of chicken of 42°C, which makes their gut ideal niche for optimum growth of campylobacters, (2) the free-roaming nature of chicken, more so under free-range system; this may spread *Campylobacter* through fecal contamination of the

environment, pasture, and surface water. More-over, studies have demonstrated that poultry and wild birds are the most important contributors to surface water contamination with campylobacters, followed by ruminants and swine (Mughini-Gras *et al*., 2016).

Thermophilic campylobacters normally induce minimal or no pathologies in avian species (Ellerbroek *et al*., 2010). However, poultry, including intensively reared chicken, tend to have higher colonization and carriage rates of *Campylobacter jejuni* than other animals. However, the bacterium may colonize the palatine lymphoid tissues and the crops in broilers, with higher colonization in intensive systems (MSD Veterinary Manual, 2022), despite appearing healthy.

Campylobacter jejuni appears to be normal flora/commensals in the gut of most domestic and wild birds; its role in vibrionic hepatitis, a spotty liver disease characterized by presence of greyish-white focal lesions, is not clear (Markey *et al*., 2013). Despite the organism being associated with vibrionic hepatitis, the causal role is insufficient since the same bacterium for the disease can be found in the livers of apparently healthy birds (Jennings *et al*., 2011). A few studies have reported possible negative health implications in chickens caused by *C. jejuni* colonization of the intestines, therefore this pathogen is considered to have a near-commensal relationship with poultry (Thibodeau *et al*., 2015).

Campylobacter jejuni and occasionally *C. coli* causes acute enteritis with rapid onset of diarrhea and death, in poults and newly hatched chicks. Avian species have been incriminated to have a major role in the epidemiology of *Campylobacter* species for humans and other farm animals; even though other sources (environmental) and/or risk factors may be important (Mughini-Gras *et al*., 2012).

2.2.7.2 *Campylobacter* infections in cattle

Apparently healthy cattle can harbour one or more *Campylobacter* species including; *C. jejuni*, *C. lari*, *C. coli*, *C. hyointestinalis* and *C. fetus* (Salihu *et al*., 2009), alluding a likely commensal role for the bacterium. In a number of studies, cattle have been found to harbor *Campylobacter* in the gallbladder, spleen, lymph nodes, large and small intestines, and liver (Enokimoto *et al*., 2007; Mohapatra *et al*., 2020). The carriage of thermophilic *Campylobacter* spp. is quite variable; and ranges from 4% to 89.4% according to a number of studies (Harvey *et al*., 2004; Stanley *et al*., 1998; Häkkinen *et al*., 2007; Kwan *et al*., 2008; Fernandez and Hitschfeld, 2009; Châtre *et al*., 2010). In studies that compared *C. jejuni* carriage in healthy cattle and in cattle with diarrhea, the frequency of *Campylobacter* spp was not notably different, and the role of *Campylobacter* spp in enteric disease of ruminants remains inconclusive (Klein *et al*., 2013). Subsequently, both apparently healthy and *Campylobacter*-infected cattle may excrete the organisms in faeces; thereby predisposing other farm animals or humans to infections. Cattle-derived isolates can infect poultry, painting a picture that cattle could be a source of infections to chicken (Ziprin *et al*., 2003), and *vice versa*.

Campylobacters have been reported as primary or secondary causes of clinical gastroenteritis and/or diarrhoea in calves (Acha *et al*., 2004; Klein *et al*., 2013), septicaemic abortion in ewes and cows (Bae *et al*., 2005) and bovine mastitis (Gudmundson and Chirino-Trejo, 1993; Modi *et al*., 2015). The enteric disease signs may be more severe in young stock (calves); characterized by thick, mucoid diarrhea with occasional streaks of blood, either with or without a fever.

2.2.7.3 *Campylobacter* infections in other farm and companion animals

The organisms have been reported to cause pathological conditions in other farm animals including: *C. jejuni* subsp *jejuni* (enteritis and abortion in cattle and shoats), *C. coli* and *C.*

mucosalis (enteritis in pigs); *C. upsaliensis* and *C. helveticus* (enteritis in dogs and cats), *C. hyointestinalis* subsp *hyointestinalis* (enteritis in cattle and pig); *C. sputorum* (ovine abortions) as described in MSD Veterinary Manual (2022). Moreover, campylobacters have been recovered from apparently healthy farm and companion animals including shoats, pigs, dogs and cats; suggesting that these animals could act as reservoirs/sources of infection for cattle and chicken, and *vice versa* (Mpalang *et al*., 2014; Conrad *et al*., 2018; Chala *et al*., 2021).

2.2.7.4 Public health significance of *Campylobacter* species and their associated risk factors in humans

Campylobacteriosis, a bacterial zoonosis, has been documented as the major cause foodborne infections, including gastroenteritis, in humans globally (Acheson and Allos, 2001; Gupta *et al*., 2017). *Campylobacter coli*, *C. curvus*, *C. helveticus*, *C. hyointestinalis*, *C. jejuni*, *C. mucosalis*, *C. insulaenigrae*, *C. lari*, *C. rectus*, *C. hominis*, *C. showae*, *C. sputorum*, *C. concisus* and *C. ureolyticus* are associated with human infections (Igwaran and Okoh, 2019; Gupta *et al*., 2017). However, *C. coli* and *C. jejuni* are the most often recovered species of major significance in causing human disease (Friedman *et al*., 2000). *Campylobacter jejuni* has been implicated in 90% of human infection cases worldwide (Gillespie *et al*., 2002). In USA, *C. jejuni* and *C. coli* accounted for 98% of cases reported in humans in 2015-2017 (CDC, 2019); with the WHO projecting that *Campylobacter* causes 37,600 fatalities/year globally (WHO, 2015). This burden is even higher than that caused by salmonellosis (Grace *et al*., 2015).

The survival and infection mechanisms of *Campylobacter* are not well known; however, upon establishing itself in intestines, it occasionally causes symptomatic or asymptomatic infection (Igwaran and Okoh, 2019). *Campylobacter* majorly causes gastrointestinal infections; however, infections can also occur outside the gut. It is a number one cause of bacterial gastrointestinal

infections (WHO, 2018), which generally manifest as: watery or sticky diarrhea, fever, nausea, vomiting, abdominal pain, headache and muscle pain (Mezher *et al*., 2016). As such, clinical syndromes associated with *Campylobacter* are sometimes difficult to distinguish from salmonellosis and/or shigellosis (Hansson *et al*., 2018). It is also the main and frequent cause of traveler's diarrhea (Bullman *et al*., 2011) and diarrhea in children under 5 years (Liu *et al*., 2016). *Campylobacter* illness in human manifests as episodes of gastroenteritis accompanied by abdominal pains, biliousness, unsettled stomach, pyrexia, and watery diarrhea and/or dysentery (Bolton, 2015). Other than there being severe gastroenteritis and/or diarrhoea; serious post-infection complications such as: reactive arthritis, osteomyelitis, endocarditis, fatal neonatal sepsis and meningitis (Acheson and Allos, 2001); peripheral neuropathies e.g. Miller Fischer Syndrome and/or Guillain-Barré syndrome (a life-threatening demyelinating polyneuropathy) and spastic colon have been reported in infants and in patients with lowered immunity (Kuwabara and Yuki, 2013; Loshaj-Shala *et al*., 2015; Gupta *et al*., 2017).

Most human infections are sporadic and they tend to have no known source; however, studies have incriminated poultry (especially chicken) and poultry products (meat) for the increasing cases of campylobacteriosis worldwide. It is, however, surprising that a rising trend in human *Campylobacter* infection has been reported in Scandinavian countries, where thermophilic *Campylobacter* carriage in poultry flocks is low (Häkkinen and Hänninen, 2009). This therefore suggests that there are other likely sources of this bacterium. Nevertheless ruminants (particularly cattle), have been documented to be the second leading sources of *C. jejuni* infections in humans (Stanley and Jones, 2003; Kwan *et al*., 2008; Mughini-Gras *et al*., 2012; Thépault *et al*., 2017). Infected chicken and cattle may shed the bacterium in faeces, thus contaminating the environment in which humans share. Consequently, if contamination in the

said environment is not controlled appropriately, the excreted *Campylobacter* may find their way into humans Additionally, outbreaks have been traced to ingestion of contaminated animal protein (beef, pork or chicken meat), water or unpasteurized milk (Frost, 2001; Hänninen *et al*., 2003).

Fecal shedding of *Campylobacter* in cattle could result in direct contamination of milk (Mughini-Gras *et al*., 2012), or as a consequence of mastitis (Modi *et al*., 2015; Bertasi *et al*., 2016). It is thus likely to increase concerns for human health. Zoonotic contagion of campylobacters from cows occurs through consumption of unpasteurized milk; and dairy products have been documented as being the second most frequent sources of the infection in humans (Barakat *et al*., 2020). A probable role of cattle in epidemiology of human campylobacteriosis is asserted by a number of source-attribution studies, where up to 36% of human *C. jejuni* cases are ascribed to bovine-derived *C. jejuni* genotypes (Wilson *et al*., 2008; Mullner *et al*., 2009; Sheppard *et al*., 2009). Furthermore, bovine-associated campylobacters also pose an extra indirect human health hazard (Vandeplas *et al*., 2008). For instance, slurry run-off from cattle facilities and farming may contaminate surface water (river) and ground water (shallow unprotected boreholes) (Sanad *et al*., 2011).

Wild birds, such as crows and starlings, have been particularly reported to be involved in spread of *Campylobacter* organisms to humans and other livestock (Sanad *et al*., 2013). Other predisposing factors include: close interaction (contact) with companion and domestic animals, unhygienic living and/or poor sanitary standards (Gupta *et al*., 2017). However, a number of researches have postulated strong connection between campylobacteriosis in humans and palming and ingestion of raw or poorly cooked contaminated chicken meat (Gruntar *et al*., 2010). In addition, cross-contamination of fast foods during preparation, besides coming into

contact with faeces from sick humans and companion animals have also been reported as risk factors (Kaakoush *et al*., 2015; Kashoma *et al*., 2015).

2.2.8 Mechanisms of pathogenicity and/or virulence factors in *Campylobacter* species

Campylobacter infections in humans and livestock present a variable degree of virulence, ranging from: asymptomatic carriage in poultry, gastroenteritis and/or watery diarrhoea in man, to mastitis, enteritis and abortion in cattle. The pathogenesis of *Campylobacter* disease is multifaceted and still not well comprehended (Wieczorek *et al*., 2019). Exploring the molecular basis of the virulence-markers linked with *Campylobacter* pathogenicity is essential in controlling diseases and/or clinical manifestations associated with this bacterium (Fonseca *et al*., 2014). However, the molecular virulotyping of *Campylobacter* has been extensively studied elsewhere (Wysok *et al*., 2020). Consequently, some studies have explored the potential virulence and/or survival factors necessary for the pathogenicity of *Campylobacter* (Dasti *et al*., 2010). This include; response to stress, flagella-facilitated motility, adhesion and binding, invasion and adherence to epithelial cells of the intestines, chemotaxis, ability to produce toxins and ability to overcome host defense cells are now known to be involved in pathological process and/or during disease development in *Campylobacter* (Dasti *et al*., 2010; Khoshbakht *et al*., 2013; Wysok *et al*., 2020). However, it is not clear whether specific disease syndromes correlate with a particular virulence-encoding gene. All the same, virulence attributes are believed to contribute to the organism's pathogenicity and provide the aptitude to adhere to receptor cells on the host in the process of disease development: hence aid in modulating the clinical manifestations (Rawat *et al*., 2018).

2.2.8.1 Flagella-facilitated motility

Motility helps *Campylobacter* organisms to survive under different chemotactic conditions in the gastrointestinal tract (Igwaran and Okoh, 2019). Flagella-facilitated motility encompasses a chemosensory system that directs flagella movement relying on the environmental situations in which the *Campylobacter* bacteria exist (Igwaran and Okoh, 2019). Both chemotaxis (ability to sense and move in direction of suitable conditions) and flagellin are the most significant virulence factors that assists *Campylobacter* to colonization/establish itself and also help in invasion of the host cell (van-Vliet and Ketley, 2001; Igwaran and Okoh, 2019). The *Campylobacter* flagellum is not merely for motility; it is composed of extracellular filamentous structures and a hook-basal body. The extracellular filamentous structures consist of multiple monomers of protein including flagellin protein FlaA, a major protein encoding *flaA* gene; and FlaB, a minor flagellin protein encoding *flaB* gene (Lertsethtakarn *et al*., 2011). The hook-basal body is constituted of different proteins such as FliO, FlhA, FliG, FlhB, FliP, FliF, FliQ, FliR, FliY, FliM and FliN, FlgE, FlgH, FlgI and FliK (Carrillo *et al*., 2004; Bolton, 2015). The *flaA* gene appears to be important for bacterial invasion and establishment into the host epithelial cells and for attachment to the host gut (Jain *et al*., 2008). Furthermore, *flaA* gene is also involved in motility, colonization, autoagglutination, and biofilm formation; thus, contributes to infection process in *Campylobacter* (Guerry, 2007).

2.2.8.2 Invasion into the host cells

Campylobacters attack host cells through the flagella which also act as an export apparatus in the release of non-flagella proteins during invasion (Poly and Guerry, 2008). Several virulence genes are involved in *Campylobacter* invasion mechanism such as flagellin C (*flaC*) and invasion antigens (*cia*) genes. These gene products are carried into the cytoplasm of the host cell with the

help of flagella secretory system which is important for bacterial invasion and colonization (Konkel *et al*., 2004). The *Campylobacter* invasion antigen B (*ciaB*) is an important heat shock protein gene product (Jakee *et al*., 2015), necessary for the invasion of epithelial cells and colonization of avian intestines (Biswas *et al*., 2011). In addition, *Campylobacter* invasion antigens (*cia*) and *Campylobacter* invasive antigen B (*ciaB*) are vital virulence factors which aid in *Campylobacter*'s invasion of epithelial cells and subsequent attachment to the host gut (Casabonne *et al*., 2016). Other significant virulence genes and/or proteins secreted by *Campylobacter* include: 73-kDa adhesion protein; invasion antigen C protein aiding in invasion of human embryonic intestinal epithelial (INT-407) cells; invasion associated protein gene (*iamA*); periplasmic (HtrA) protein responsible for complete attachment to host epithelial cells; *CiaI*, an intracellular survival gene (Bolton, 2015); *pldA* and *hcp* invasion genes (Iglesias-Torrens *et al*., 2018).

2.2.8.3 Adhesion to epithelial cells

Campylobacter's attachment to the host epithelial cells lining gastrointestinal tract is facilitated by bacterial adhesins and a prerequisite for its establishment (Jin *et al*., 2001). Virulent genes aiding *Campylobacter*'s adherence ability include: outer membrane fibronectin binding proteins (including *Campylobacter* adhesion to fibronectin (CadF) and fibronectin-like protein A (FlpA) for); *Campylobacter* adhesion protein A (CapA), phospholipase A (PldA), surface lipoproteins (JlpA), periplasmic binding protein (Peb1A) and Type IV secretory system (virB11) gene (Bolton, 2015). Effective invasion and establishment into host epithelial cells depends on adhesion to host fibronectin. The CadF protein mediates binding of *Campylobacter* to the extracellular matrix of host intestinal cells during disease development process (Khoshbakht *et al*., 2013). Additionally, *Campylobacter* adhering to fibronectin F (CadF) protein permits the

bacteria to attach to fibronectin which foster bacterium-host cell communications and colonization (Konkel *et al*., 2010).

2.2.8.4 Ability to produce toxins

Campylobacter organisms produce diverse toxins including enterotoxins and cytotoxins. The relevance of proteinaceous exotoxins in pathological process is debatable. However, enterotoxins are believed to increase concentration of cyclic adenosine monophosphate inside the cells, and the subsequent ion flow variations result in secretion of excess fluid, which manifests as watery diarrhea (Wysok *et al*., 2022). In contrast, cytotoxins are linked with the lysis of epithelial cells causing enteritis and hemorrhagic reactions (Wysok *et al*., 2022). They have been well characterized, and it was found that cytolethal distending toxin (CDT) is a triple toxin consisting of three subunits encoded by the *cdtA, cdtB* and *cdtC* genes (Igwaran and Okoh, 2019; Wysok *et al*., 2022). These CDT genes (*cdtA, cdtB* and *cdtC*) are all responsible for the expression of cytotoxicity and destruction of intestinal absorptive cells in the host (Asakura *et al*., 2008; Carvalho *et al*., 2013). The *cdtA* and *cdtC* gene products are necessary for toxin to bind and be incorporated into the host cell while *cdtB* toxin is responsible for toxicity (Igwaran and Okoh, 2019). Cytolethal distending toxins cause diarrhea by interfering with cell division in the intestinal crypts of small intestines in both humans and animals (Carvalho *et al*., 2013).

2.2.9 Effects of seasonality and climatic factors on incidence of *Campylobacter* species

Faecal material emanating from wild and farm animals or dung-manured grazing fields are important sources of thermophilic campylobacters to which livestock may get exposed through consumption of contaminated water or pastures. The organisms initially multiply within the intestines of warm-blooded animals. Therefore, it is the post-excretion period that defines the ecology of campylobacters.

Occurrence of thermophilic campylobacters in chicken has shown a seasonal trend. In temperate countries, particularly Europe and North America, several studies have reported seasonal trends/effect of thermophilic *Campylobacter* colonization/carriage in cattle, chickens and humans; where peak shedding occur during summer or winter (Stanley *et al*., 1998; Bouwknegt *et al*., 2004; Hansson *et al*., 2004; Hofshagen and Kruse, 2005; Häkkinen and Hänninen, 2009; Taylor *et al*., 2013; Friedrich *et al*., 2016). However, seasonality of thermophilic campylobacters has not been reported in tropical low and middle-income countries, perhaps due to lack of study in these settings (Carron *et al*., 2018). Similarly, seasonality effect on occurrence of thermophilic campylobacters in Kenya has not been described previously, though a survey by Shimotori *et al*. (1986) reported varying thermophilic *Campylobacters* colonization in children at 17%, 5.4% and 12.2% in July, September and November respectively. Shimotori *et al*. (1986) findings were more or less similar, notwithstanding being carried out in different seasons. Furthermore, the results did not infer any conclusion *vis-à-vis* seasonal pattern. In another study done in Central Africa in Democratic Republic of the Congo by Mpalang *et al*. (2014), it was observed that a prevalence of thermophilic campylobacters among goats and retailed goat meat in different seasons were 16.7% (rainy) and 20% (dry). Nwankwo *et al*. (2018) reported prevalence of thermophilic campylobacters in three different seasons at 30% (wet season), 31% (cold-dry season) and 25% (hot-dry) in free-range chickens in Sokoto, Nigeria. It is however, notable that the authors found no significant difference across the seasons.

Despite this glaring evidence of seasonality of thermophilic campylobacters particularly in western countries, the reasons behind it are not fully understood (Smith *et al*., 2019). However, the seasonal peaks may coincide with levels in either fecal shedding in livestock or exposure to a common contamination source like pasture and water. Interestingly, upon environmental

contamination, the bacterium may not survive long enough to colonize grazing cattle, apart from in water, where lengthy persistence of *Campylobacter* has been documented (Cools *et al*., 2003). As such, an in-depth understanding of seasonal trends in occurrence of thermotolerant campylobacters would assist in establishing seasonal variables (weather) and risks involved in acquisition and development of clinical disease. The spread of animal diseases, and severity of disease incidence eruptions is frequently intimately associated to climate.

Climate factors may influence campylobacter infection through a number of paths, direct, and mostly indirect. For instance, seasonality trend may reflect linkage in either environmental (weather) factors (sunlight intensity, humidity and ambient temperature) or consumption of fecal contaminated pasture and water following excretion from infected animal reservoirs. On the other hand, rainfall effects may result in unintended and unnoticeable breaches of biosecurity measures, thereby increasing the entry routes by *Campylobacter* in a herd/flock (Sibanda *et al*. 2018). The resultant wetness/dampness protects the bacteria from desiccations thus may increase survival and persistence of *Campylobacter* in the environment. However, an increase in pathogen reservoirs, changes in human behavior and climate can influence the shedding and transmission of the pathogen.

2.2.10 Risk factors associated with *Campylobacter* colonization in cattle and chicken in farms

There is paucity of data on factors predisposing farm animals to *Campylobacter* colonization in Kenya. Studies conducted on risk factors in other countries have affirmed that different farm characteristics and management practices including; age of animals, source of water supply to the farm, herd size/overstocking, diarrhoeic animals, presence of other farm animals in close vicinity, no/minimal biosecurity measures, season, type of feed, and animals roaming outside the

farm can affect *Campylobacter* load or distribution (Ellis-Iversen *et al.,* 2009b**;** Hannon *et al*., 2009; Klein *et al*., 2013; Hoque *et al*., 2021).

For instance; feeding cattle on hay, indoor housing (close confinement), cleaning/disinfection of premises, existence of or co-grazing with other animals in the same farm and farm water source/supply have been reported to influence *Campylobacter* carriage in cattle herds (Ellis-Iversen *et al*., 2009b; Guévremont *et al*., 2014). In addition, ingestion of contaminated water, use of antimicrobials in production (Refregier-Petton *et al*., 2001; Ansari-Lari *et al*., 2011), unhygienic conditions (Mageto *et al*., 2018), old age of the flock, housing facilities that have been in use for a long period (Bouwknegt *et al*., 2004), access of housing facilities by wild animals including rodents and birds have been associated with *Campylobacter* carriage in poultry including chickens (Casalino *et al*., 2022).

Climatic variables such as temperature and rainfall could affect endurance and replication of *Campylobacter* organisms on foods or in environmental sources and, therefore, form part of risk factors (Weisent *et al*., 2014). Assessing farm-level predisposing factors associated with *Campylobacter* carriage in individual animal and/or herd/flock is essential in formulating workable control strategies.

It is worth noting that the significance of *Campylobacter* prevalence in livestock (cattle or chicken) relates not only to likelihood of contamination of milk and meat at slaughter, but also environmental (water or soil) contamination through discarding of slurry wastewaters. A reduction in occurrence of positive herds/flocks will substantially reduce the disease incidence in humans. This therefore necessitates need to document on-farm risk factors associated with transmission/occurrence of thermophilic *Campylobacter* organisms in herds/flocks and/or environment so as to frame suitable and operative control programs in the low-resource settings.

2.2.11 Control of *Campylobacter* organism in farm settings

Owing to the myriad probable sources of entry of *Campylobacter* into farm setting (wide range of environments and reservoirs); a complex control and prevention strategy in all aspects of livestock husbandry has to be undertaken in order to minimize risk of contracting this pathogen. Well-designed and planned biosecurity strategies such as hygiene and vaccination at farm level have been proven to be an ultimate method to countering herd/flock colonization with *Campylobacter* (Georgiev *et al*., 2017). Findings by Gibbens *et al*. (2001) have shown that thorough cleaning and disinfection could reduce *Campylobacter* prevalence from 80% to less than 40% in a flock of chicken. Indeed, installation of hygienic barriers between internal and external environments, regulation of visitors, stringent sanitary regulations and changing boots and overalls prior to entry into a farm have been evidenced to be effective (Silva *et al*., 2011).

2.3 Antimicrobial susceptibility testing, antimicrobial use in livestock, mechanisms, drivers and impacts of antimicrobial resistance (AMR)

2.3.1 Antimicrobial susceptibility testing of *Campylobacter* strains

2.3.1.1 Phenotypic antimicrobial susceptibility testing

A number of phenotypic antimicrobial susceptibility tests (ASTs), including agar disc diffusion, broth micro-dilution, agar dilution and epsilometer test (E-test), are used to determine resistance of *Campylobacter* isolates to antimicrobial agents. Both agar dilution and E-test methods are often used. The agar dilution method is reliable and highly reproducible and also provides quantitative minimum inhibitory concentrations (MICs). The agar disc diffusion method is simple and low-cost and can provide reproducible results if it is conducted carefully with appropriate standardization and quality controls (Potz *et al*., 2004). However, the disadvantages of conventional phenotypic ASTs are that they require bacterial growth for extended periods

with or without antimicrobials. Additionally, phenotypic ASTs do not provide genotypic information on resistance which can be very valuable in construing surveillance data and conducting outbreak investigations. It is worth noting that, phenotypic antimicrobial resistance may be caused by many different genetic determinants which may present particular epidemiological characteristics (Srinivasan *et al*., 2007).

2.3.1.2 Testing for presence of antimicrobial resistance genes in *Campylobacter* strains using polymerase chain reaction

Genotypic methods detect specific genes that confer antimicrobial resistance. However, even though genotypic tests can rapidly screen presence of specific resistance genes, such tests, depending on existing literature on the resistance mechanisms, are far from complete (Kandavalli *et al*., 2022), owing to the rapid development of new resistance mechanisms. Of particular concern are genetic determinants encoding multi-drug resistance (Magiorakos *et al*., 2012), especially when disseminated with AMR phenotypes. Furthermore, evaluation of genetic determinants of resistance is vital for elucidating and controlling antimicrobial resistance i.e. can be used to reliably predict resistant phenotypes. Additionally, genotypic information on resistance can help trace bacteria to a given source in time, and/or geographic location. Therefore, it is of paramount significance to delve into the genetic mechanisms linked with antimicrobial resistance in *Campylobacter* species. However, non-occurrence of genes enciphering resistance does not imply susceptibility to the respective antimicrobial; it only suggests what antimicrobial not to use and what will work (Kandavalli *et al*., 2022). Also, genes can exist without being expressed or with no gene products.

2.3.2 Antimicrobial use in livestock sector and role in development of AMR

Antimicrobials are used in livestock for therapeutic (treatment of sick animals), prophylactic (administering antimicrobials to animals at high risk of disease) and metaphylactic (treatment of animals in close contact with diseased ones, but themselves not showing signs of disease) purposes. They are also used for non-therapeutic reasons including feed efficiency stimulators and growth promoters in many nations globally (Hosain *et al*., 2021). The most frequently used antimicrobials in livestock are tetracyclines, aminoglycosides, beta-lactams (β-lactams), sulfonamides, amphenicols, lincosamides, quinolones, polypeptides and macrolides (Hosain *et al*., 2021). Data on the exact quantities of antimicrobials consumed in various food animals in Kenya is missing; though a study in Kenya reported antimicrobial consumption in sheep, goats, cows, and camels as follows; 4,168 kg tetracycline, 70kg sulfonamides, 49.7 kg aminoglycosides, 46.4kg β-lactams, 39.4kg macrolides and 0.52kg trimethoprim (Omwenga *et al*., 2020). From this single report, there is obviously widespread consumption of antimicrobials in Kenya and probably in other low-income countries. This is unlike in the past, where high-income countries including USA and some European countries were the main consumers of antimicrobials in livestock (Elliott *et al*., 2017). Worldwide, the approximate yearly usage of antimicrobials is 45 mg/kg (cattle), 148 mg/kg (poultry), and 172 mg/kg (swine), and it is expected that antimicrobial usage will rise by 67% by the year 2030 (van-Boeckel *et al*., 2015).

Apart from the positive role of antimicrobial use in ensuring good health in animals, there are a number of setbacks linked to the practice in livestock. Any application of antimicrobials, whether considered curative or not, deliberate or otherwise, exposes both pathogenic bacteria and gut commensals to varying concentrations for different duration (Weese *et al*., 2015). As such, inappropriate use of antimicrobials in livestock sector and human health contexts precipitates

emergence and spread of resistant bacterial strains through selection pressure (Aarestrup *et al*., 2008; van-Boeckel *et al*., 2015). Selective pressure can result in evolution and spread of resistance or a rise in abundance of resistant bacteria, especially where a resistant subpopulation exists (Weese *et al*., 2015). Frequent use of antimicrobials in livestock as growth promoters, feed conversion enhancers, and preventive therapy is one of the risk factors associated with increasing antimicrobial resistance (Aarestrup *et al*., 2008; van-Boeckel *et al*., 2015). Other deleterious impacts linked with antimicrobial use (AMU) in livestock is presence of antimicrobial residues in meat, eggs and milk (Qamar *et al*., 2023). Consequently, application of antimicrobials in livestock is turning out to be matter of concern for both food safety and public health. As such, there is need to control antimicrobial use in livestock amid the rampant failure in veterinary and/or human medicines.

Even though *Campylobacter* infections are self-restricting; antimicrobial prescription is indicated in: chronic or septicaemic or complicated sickness; severe and prolonged cases of enteritis; immunosuppressed individuals and/or in young children (Chukwu *et al.*, 2019). Macrolides such as erythromycin and fluoroquinolones (FQs) like ciprofloxacin are considered as the last resort drugs in human clinical cases requiring therapy. However, other antimicrobials including; aminoglycosides (gentamicin), tetracyclines, lincosamides (clindamycin) and penicillins (ampicillin) may be prescribed as substitute medication for management of septicaemic campylobacteriosis for human cases (Chukwu *et al.*, 2019). However, the same antimicrobials are extensively used in livestock husbandry. Overuse and illegitimate usage of antimicrobials have led to worldwide upsurge of fluoroquinolone-resistant and macrolide-resistant *Campylobacter* strains (Oporto *et al*., 2009). Consequently, over the decades, several studies in Kenya and beyond, have reported an increase in infections caused by multi-drug resistant

Campylobacter (Uaboi-Egbenni *et al*., 2012; Ewnetu and Mihret, 2010; Kashoma *et al*., 2016; Nguyen *et al*., 2016).

Antimicrobial resistance in thermotolerant *Campylobacter* organisms has drawn attention, particularly due to development of resistance against the few antimicrobials of choice for humans (fluoroquinolones and macrolides), as highlighted by the World Health Organization (2017). Consequently, the WHO enlisted fluoroquinolone-resistant *Campylobacter* under "high priority for research and new drug development responds to urgent public health needs" as presented in Table 2.2 (WHO, 2017).

Table 2.2: WHO's priority pathogens for research and development of new antimicrobials

Bacterial species	**Type of antimicrobials resistance**
Critical priority	
• ***Acinetobacter baumannii***	Carbapenem-resistant
• ***Pseudomonas aeruginosa***	Carbapenem-resistant
• ***Enterobacteriaceae***	Carbapenem-resistant, ESBL-producing
High priority	
• ***Enterococcus faecium***	Vancomycin-resistant
• ***Staphylococcus aureus***	Methicillin- and vancomycin-intermediate and resistant
• ***Helicobacter pylori***	Clarithromycin-resistant
• ***Campylobacter* species**	Fluoroquinolone-resistant
• ***Salmonellae***	Fluoroquinolone-resistant
• ***Neisseria gonorrhoeae***	Cephalosporin-resistant, fluoroquinolone-resistant
Medium priority	
• ***Streptococcus pneumoniae***	Penicillin-non-susceptible
• ***Haemophilus influenzae***	Ampicillin-resistant
• ***Shigella* species**	Fluoroquinolone-resistant

As the world grapples with the AMR scourge, most developed countries began and have been monitoring AMU in livestock; from calls of reducing use to total bans. Developing countries including Kenya also need to take initiatives geared at monitoring antimicrobial use (AMU) and practice, so as to minimize development of AMR. In Kajiado and Kenya at large, significant

knowledge gaps exist on AMU and practices (reasons for use, dosage/exact quantities, frequency and types of antimicrobials) in cattle and chicken production systems. In addition, legislation on AMU in livestock exists in Kenya, but enforcement is poor, hence the livestock owners can easily access veterinary pharmaceuticals and end up administering the animals by themselves. As such, it has become difficult to evaluate the correlation between AMU and AMR. Obtaining such information can possibly assist in making informed decision especially in connection with the framework of national action plans on AMR, formulated by ministry of health and that of agriculture, livestock and fisheries.

2.3.3 Mechanisms of antimicrobial resistance in *Campylobacter* organisms

Antimicrobial resistance can be divided into intrinsic and acquired resistance (Luangtongkum *et al*., 2009). Intrinsic resistance is an innate characteristic of the microorganism and is transmitted to progeny vertically. In contrast, acquired resistance results from changes in the usual genetic makeup of a microorganism and can lead to altered cellular physiology or structure. Intrinsic resistance is considered as natural and a consistently inherited characteristic of either a specific bacterial genus or species; and is therefore predictable once the microorganism is identified. *Campylobacter jejuni* and *C. coli* are naturally resistant to bacitracin, novobiocin, penicillin, most of the cephalosporins, rifampicin, trimethoprim, sulfamethoxazole, and vancomycin (Wieczorek and Osek, 2013a). Unlike intrinsic resistance, acquired resistance may be a trait associated with only some strains of a particular genus or species. Thus, the presence of this type of resistance in any bacterial isolate is neither consistent nor predictable.

Campylobacter acquires resistance determinants by mutations and horizontal gene transfer. Natural transformation, conjugation and transduction can all occur in *Campylobacter* and are likely to contribute to the spread of antimicrobial resistance determinants (Luangtongkum *et al*.,

2009). Information on circulating *Campylobacter*-AMR genotypes is lacking in Kenya at large. However, in other countries, the genetic determinants of antimicrobial resistance in *Campylobacter* have been characterized exquisitely (Wieczorek and Osek, 2013a).

2.3.3.1 Resistance to quinolones and/or fluoroquinolones

First-generation quinolones such as nalidixic acid, cinoxacin and oxolinic acid have low systemic activity and as such are no longer used in clinical cases. The fluorination of first-generation quinolones formed a new class of drugs, the fluoroquinolones, which have a wider and significant/expanded antibacterial activity. These fluoroquinolones include: second-, third- and fourth-generation quinolones exemplified by ciprofloxacin, levofloxacin and trovafloxacin, respectively (King *et al*., 2000).

Quinolones are synthetic antimicrobials that exert their antibacterial action by inhibiting bacterial nuclear enzymes (topoisomerase IV and DNA gyrase, also known as topoisomerase II) which are essential in DNA duplication, transcription, chromosome segregation, and re-integration (Wieczorek and Osek, 2013a). The DNA gyrase gene comprises gyrase A (*gyrA*) and gyrase B (*gyrB*), whereas, topoisomerase IV gene consists of *parC* and *parE* (Payot *et al*., 2004).

Campylobacters' resistance to fluoroquinolones (FQs) is mainly mediated by frameshift transmutations in the quinolone resistance-determining region (QRDR) of *gyrA* (Payot *et al*., 2006). The most often detected mutations in the DNA gyrase A (*gyrA*) gene is the nucleotide 257 (C257T) alteration from ACA to ATA, which leads to the threonine (T86I) replacement to isoleucine, and portrays high level resistance to FQs (Payot *et al*., 2006). Other less common mutations in *gyrA* with low level of resistance to FQs compared to T86I mutation include; T86K, A70T and D90N (Engberg *et al*., 2001; Payot *et al*., 2006). On the contrary, no point mutations

in *GyrB* have been linked with *Campylobacter* resistance to fluoroquinolones (Payot *et al*., 2002; Piddock *et al*., 2003). The topoisomerase IV (*parC* and *parE*) genes are lacking in *Campylobacter*, however, these genes are involved in FQ resistance in Gram-negative bacteria (Luangtongkum *et al*., 2009). Consequently, it is not astounding that amino acid(s) substitution(s) in *parC* and/or *parE* are not associated with *Campylobacter* resistance to FQs.

There are other mechanisms contributing to *Campylobacter* resistance to FQs including multidrug efflux system. The CmeABC multidrug efflux pump activity contributes to resistance by reducing the concentration of antibacterial agents such as FQs and macrolides within *Campylobacter* cells (Lin *et al*., 2002; Ge *et al*., 2005). The CmeABC is enciphered by an operon gene comprising three sub-units coding for; periplasmic fusion protein (*cmeA*), inner membrane drug transporter (*cmeB*), and outer membrane protein (*cmeC*) (Lin *et al*., 2002). Inhibition of CmeABC efflux pump by either deactivating *cmeB* or by blocking the efflux pump leads to increased susceptibility to different antibacterial agents, including those to which *Campylobacter* are inherently resistant (Lin *et al*., 2002; Pumbwe *et al*., 2005; Akiba *et al*., 2006). The cmeABC efflux pump works interactively with the *gyrA* mutations in conferring FQ resistance (Luangtongkum *et al*., 2009); thereby playing an important role in both intrinsic and acquired resistance of *Campylobacter*.

2.3.3.2 Resistance to macrolides

Macrolides exert their bacteriostatic property by binding to the 50S bacterial ribosomal subunit and suppress consecutive addition of amino acid residues to a nascent polypeptide chain, thus interfering with protein synthesis and resultant ribosomal subunit assemblage (Siibak *et al*., 2009; Wilson, 2014). *Campylobacter* resistance to macrolides is due to alteration of binding sites on 23S ribosomal unit (nucleotides 2058 and 2059) as a result of mutation (Wieczorek and Osek,

2018). Mutations by replacements of adenine residues at positions 2074 and 2075 of the 23S rRNA gene (rRNA operon) are the most prevalent mutations, contributing to *Campylobacter* resistance to erythromycin (Jeon *et al*., 2008). Additionally, mutations at positions 2074 [adenine (A) substituted with cytosine (C) and with guanine (G); A→C and A→G] and 2075 (adenine substituted with guanine; A→G) confer increased resistance of *Campylobacter* strains to macrolides (Wieczorek and Osek, 2018).

Another mechanism contributing to *Campylobacter* resistance to macrolides is the mutations of L4 and L22 proteins of 23S ribosomal unit at the binding site; where the adjustments are associated with small level of macrolides resistance (Wieczorek and Osek, 2018). Nevertheless, the precise function of these ribosomal adjustments (in form of insertions and deletions) is unclear (Payot *et al*., 2004; Cagliero *et al*., 2006; Corcoran *et al*., 2006; Caldwell *et al*., 2008).

Finally, more than eight diverse efflux pumps are reported to cause macrolide resistance in *Campylobacter*. Such multidrug efflux system includes CmeABC that works concurrently with 23S rRNA mutations, even without any other mechanism mediating resistance (Payot *et al*., 2004; Cagliero *et al*., 2006). There is data suggesting that high-level macrolide resistance in some *Campylobacter* isolates is as a result of interplay between efflux activity and mutations in the 23S rRNA (Corcoran *et al*., 2006). On the contrary, a reduced level resistance to macrolides was observed in highly resistant *Campylobacter* isolates with 23S rRNA mutations (A2074G or A2075G) as a result of deactivating the CmeABC multidrug efflux pump (Cagliero *et al*., 2005; Cagliero *et al*., 2006; Gibreel *et al*., 2007; Lin *et al*., 2007). These findings confirm existence of synergism between CmeABC multidrug efflux system and specific mutations on 23S rRNA.

2.3.3.3 Resistance to aminoglycosides

Aminoglycosides, such as, streptomycin, kanamycin and gentamycin, exert anti-bacterial properties by binding to the 16S A-site, which forces A1492 and A1493 to flip out of helix 44 and thus, suppressing protein synthesis, resulting into bacterial cell death (Gutierrez *et al*., 2012). Resistance to aminoglycosides is generally on account of: (1) production of several aminoglycoside-modifying enzymes, such as acetyltransferases (AACs); phosphotransferases (APHs) types I, III, IV, and VII; and adenyltransferases (ANTs)]; (2) Diminished intracellular antimicrobial concentration due to major outer membrane changes, two-component or efflux systems; and (3) ribosomal proteins or 16S mutations (Poole, 2005).

Enzymatic modification reduces the attraction of aminoglycosides at the A binding site of the 16S subunit, thus contributing to resistance (Llano-Sotelo *et al*., 2002). The enzymes, phosphotransferases, adenyltransferases and acetyltransferases, act via a similar mechanism. The 3′-aminoglycoside phosphotransferase enzyme coded by aphA-3 gene was the first detected incidence of aminoglycoside resistance in *C. coli* (Khan *et al*., 2019). The aphA-3 gene product is the most common cause of aminoglycoside resistance in *C. jejuni* and *C. coli*. A kanamycin-resistance phosphotransferase, encoded by aphA-1 and aphA-7, has also been detected on the *C. jejuni* plasmids (Khan *et al*., 2019). The aphA-7 is composed of same guanine (G)-cytosine (C) nucleotides content as genomic DNA of *C. jejuni*, supporting the impression that such genes are inherent in *Campylobacter*, whereas the aphA-1 and aphA-3 are believed to be acquired by means of horizontal gene transfer (Khan *et al*., 2019). Some *Campylobacter* strains harbouring aphA-3 gene are also found to possess genes that confer streptomycin resistance, enciphered by acetyl transferase “sat” gene product, a 6′-adenylyl transferase encoded by aadE (Khan *et al*.,

2019). On the other hand, gentamicin resistance could be mediated by occurrence of genes such as; aac/aphD, aacA4, aph-2-IF and aph-2-Ig (Yao *et al*., 2017).

Another mechanism contributing to *Campylobacter* resistance to aminoglycosides is the modification of ribosomal proteins. However, there are scarce studies on ribosomal protein S12 mutations (encoded by gene rpsL) in *C. coli* that confers resistance to streptomycin (Khan *et al*., 2019). In addition, such mutations have not been reported in *C. jejuni* strains (Khan *et al*., 2019).

Finally, the role of efflux system in aminoglycoside resistance is unclear (Khan *et al*., 2019). Inactivation of efflux pump by phenylarginine-β-naphthylamide and 1-(1-naphthylmethyl)-piperazine was shown not to decrease the minimum inhibitory concentration (MIC) of kanamycin in *C. jejuni* isolates (Alfredson and Korolik 2007; Iovine 2013); this suggests that the efflux pump is less significant in aminoglycoside resistance.

2.3.3.4 Resistance to tetracyclines

Tetracyclines are bacteriostatic antibiotics that bind reversibly to the ribosome 30S subunit, and hinder accommodation of the aminoacyl tRNA (aa-tRNA) into the ribosomal A site (Khan *et al*., 2019). This results in inhibition of peptide elongation during protein synthesis (Khan *et al*., 2019). The main resistance mechanisms are efflux systems encoded by CmeABC and CmeG. The (*O*) gene encrypts ribosomal protection proteins (RPPs), and it is located on self-transmissible plasmid (Khan *et al*., 2019). The (*O*) is the most frequently detected tetracycline resistance determinant contributing to very high-levels of resistance in *Campylobacter* strains (Gibreel *et al*., 2004; Dasti *et al*., 2007). These RPPs recognize and adhere on an open A site on the bacterial ribosome resulting in structural change and release of bound tetracycline molecule

(Khan *et al*., 2019). Tetracyclines are liable to RPPs-dependent resistance, including Tet(O), Tet(A) and Tet(M) (Abdi-Hachesoo *et al*., 2014; Khan *et al*., 2019).

Additionally, the multidrug efflux systems such as, CmeABC and CmeG contribute to both acquired and inherent resistance to tetracyclines in *Campylobacter* (Gibreel *et al*., 2007; Lin *et al*., 2002; Jeon *et al*., 2011). CmeABC functions synergistically with Tet(O) to confer high-level resistance to tetracyclines (Lin *et al*., 2002). Deactivation of either CmeABC or CmeG upsurges the sensitivity of *Campylobacter* to tetracyclines (Lin *et al*., 2002; Jeon *et al*., 2011).

2.3.3.5 Resistance to β-lactams

Most of *Campylobacter* species are naturally resistant to β-lactams (e.g., penicillins) and narrow-spectrum cephalosporins. Beta-lactams exert their antibacterial action by adhering to beta-lactam (penicillin) binding proteins and disrupt cross-linking of peptidoglycan units which are a component of bacterial cell wall; which leads to necrobiosis (Martin and Kaye, 2004). The resistance mechanisms of *Campylobacter* to a number of broad-spectrum cephalosporins and β-lactams including ampicillin are diverse and ambiguous (Wieczorek and Osek, 2018). However, campylobacters are intrinsically resistant to most β-lactams but they are sensitive to amoxicillin and ampicillin (Wieczorek and Osek, 2018). Most *Campylobacter jejuni* and *C. coli* strains are capable of secreting β-lactamases (penicillinases), which deactivate the β-lactam molecule by breaking down the basic lactam ring (Wieczorek and Osek, 2018). This contributes to amoxicillin, ticarcillin and ampicillin resistance which can be conquered by using β-lactamase inhibitors, such as, clavulanic acid, tazobactam, and sulbactam (Khan *et al*., 2019). *Campylobacter* strains can produce naturally occurring β-lactamases encoded by $bla_{\text{OXA-193}}$, $bla_{\text{OXA-184}}$, and $bla_{\text{OXA-61}}$ genes (Alfredson and Korolik, 2005; Griggs *et al*., 2009; Raeisi *et al*., 2017). From the existing published data, $bla_{\text{OXA-61}}$ is the most frequently reported β-lactamase. In

addition, alleles of other genes associated with aminoglycoside resistance [e.g., aminoglycoside 3'-phosphotransferase gene (aph-3-1)] have also been reported (Obeng *et al*., 2012).

Additionally, changes in major outer membrane porin proteins and efflux pump or in plasma membrane structure could also contribute to *Campylobacter* resistance to β-lactams through elimination most β-lactams which are negatively charged in general (Khan *et al*., 2019).

2.3.3.6 Resistance to other antibacterial agents

Chloramphenicol exerts anti-bacterial properties by suppressing protein synthesis. The plasmid carries the gene that codes for chloramphenicol acetyltransferase (*cat*) which prevents the drug from binding to the ribosomes leading to resistance (Schwarz *et al*., 2004).

Microbes require PABA to form dihydrofolic acid, a precursor of folic acid. Sulphonamides act as competitive antagonists by competing with para-aminobenzoic acid (PABA) for dihydropteroate synthetase (DHPS) in microbial cells (Aarestrup and Engberg, 2001). *Campylobacter jejuni* resistance to sulphonamides is due to substitutional mutation in in the gene coding for DHPS enzyme (Wieczorek and Osek, 2018).

2.3.4 Drivers and impacts of antimicrobial resistance

Evolution and transmission of AMR have been hastened by indiscriminate use and/or misuse of antimicrobials in man, animals and plants; poor sanitary and unhygienic conditions; lack of biosecurity measures; and inadequate infection prevention and control strategies in hospitals, communities, livestock and food production systems. Inadequate implementation of legislation and lack of cognizance and information has also been cited as drivers of AMR. Additionally, unequitable access to inexpensive and quality-assured antimicrobials, vaccines and diagnostics have also been linked with the AMR scourge (WHO, 2021).

In Kajiado County and Kenya at large, AMR scourge is further compounded by collapse of public veterinary services in the 1980s. With privatization of veterinary services, delivery of animal health services, more so in arid and semi-arid counties, have become a nightmare. Alternatives to this new reality include engaging community-based animal health workers (CAHWs) (Riviere-Cinnamond and Eregae, 2003). The CAHWs lack continuous training on/or up-to-date know-how on antimicrobial use (AMU) and treatment guidelines; and may end up prescribing inappropriate antimicrobial therapy including those controlled for humans and animals. While in some developed countries including Australia and Korea, use of fluoroquinolones (FQs) and gentamicin in livestock including poultry was banned over a decade ago (Ku *et al*., 2011; Obeng *et al*., 2012), the same antimicrobials continue to be used in livestock in Kenya. Furthermore, Kajiado County is dominated by the Maasai, one of Kenya's major pastoralists, who are known to self-treat and/or engage unskilled persons to treat their sick animals with antimicrobials; which is considered the source of resistance. Antimicrobial resistance crisis has greatly impacted on; food security, human, environmental and animal health. Figure 2.3 summarizes the adverse effects of AMR.

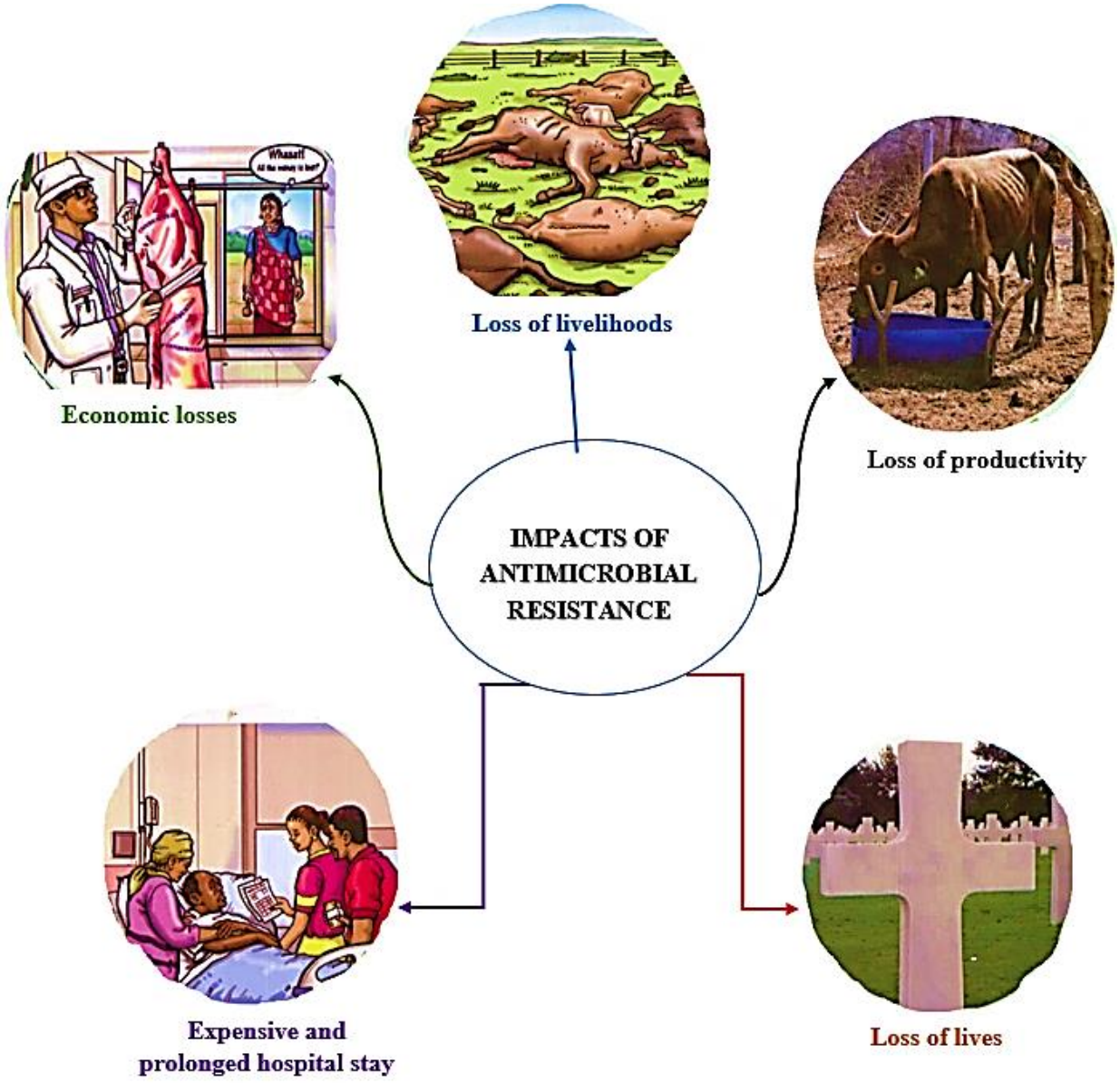


Figure 2.3: Pictorial diagram summarizing impacts of antimicrobial resistance crisis

New resistance means are unfolding and disseminating internationally. The increasing resistance threatens to erode the gains made by medical science over diseases that were once treatable. The latter has rendered antimicrobials ineffective for treatment of diseases including campylobacteriosis, leading to increased mortality and morbidity. Drug resistance in livestock has a major impact on animal health and may be associated with human illnesses that are difficult to treat. These illnesses are associated with increased morbidity and deaths, lengthy and

expensive hospital stays due to failing antimicrobials (MacVane, 2017), greater direct health care bills and indirect economic losses. For instance, infections due to antimicrobial-resistant bacteria (super bugs) are reported to kill 700, 000 patients every year globally (Taylor *et al*., 2014; O'Neill, 2016), and the numbers are anticipated to rise to 28 million persons, with majority of them in third world countries, including Kenya. The resultant economic implications are projected to be around $100 trillion globally by the year 2050 (O'Neill, 2016; WHO, 2021). Moreover, infections caused by antimicrobial-resistant bacteria have also led to reduced productivity and deaths of livestock, with dire consequences on livelihoods and food safety and security (WHO, 2021). Leading threats in developing countries are Methicillin Resistant *Staphylococcus aureus* (MRSA) and extended-spectrum beta-lactamase(s) [(ESBL(s)] producing *Escherichia coli*, which have already led to human morbidity, mortality and financial losses.

CHAPTER THREE: SEASONAL PREVALENCE OF THERMOPHILIC *CAMPYLOBACTER* SPECIES FROM CHICKEN, CATTLE AND WATER IN KAJIADO COUNTY, KENYA

3.1 Introduction

In Kenya, several studies have focused on chicken and their products as the major reservoir of *Campylobacter* infection (Chepkwony, 2016; Nguyen *et al*., 2016; Carron *et al*., 2018; Mageto *et al*. 2018; Abubakar *et al*., 2019; Kariuki *et al*., 2020). However, minimal information is available on thermophilic *Campylobacter* epidemiology in cattle in Kenya, with literature search indicating that only three studies had been conducted by the time of inception of this study, on occurrence of thermotolerant *Campylobacter* in cattle and cattle products (Turkson *et al*., 1988; Osano and Arimi, 1999; Chepkwony, 2016). This is further compounded by lack of surveillance data on incidence of thermophilic *Campylobacter* infections as most laboratories do not routinely test for the bacterium; it is seen as a silent threat. More-over, seasonal effect and molecular characterization has not been conducted in Kenya. The seasonal effect on *Campylobacter* carriage and/or colonization in cattle and poultry has not been documented in tropical low and middle-income countries (LMICs), perhaps due to lack of studies in these settings (Carron *et al*., 2018) and/or inadequate surveillance.

Given the above data gaps, the probable association among thermophilic *Campylobacter* spp. harbored by cattle, chicken and environment (water) and human illness, justifies more research on the same. This study aimed at investigating the prevalence and seasonality of thermophilic *Campylobacter* species (with emphasis to *C. jejuni*, *C. coli* and other

thermophilic campylobacters) in chicken, cattle and cattle trough water samples from Kajiado County, Kenya.

3.2 Materials and Methods

3.2.1 Study area

The study was conducted in Kajiado County particularly in Kajiado North sub-county (areas of Ongata Rongai, Ngong), Kajiado West sub-county (Kiserian) and Kajiado East sub-county (Kitengela, Isinya, Mashuru) as indicated in Figure 3.1. Kajiado County borders Nairobi, and spreads to Tanzania border further South and lies between latitude -2° 00’S and longitude 36° 52’E.

Figure 3.1: Map of Kajiado County and its location in Kenya (shaded green) and sites where sampling and interviews were conducted

The county has a distinct bi-modal rainfall pattern: October to December's insubstantial and/or short rains and March to May's substantial and/or long rains. However, rainfall is inconsistent across the county: the long (March to May) rains are more distinct in the Kajiado West

(Keekonyokie, Magadi, Iloodokilani, Ewuaso and Mosiro) while the short (October to December) rains are substantial in Kajiado East (Kitengela, Kenyawa-Poka, Imaroro, Kaputei North and Oloosirkon Sholinke). The amount of rainfall increases with altitude and ranges from 300 mm in Amboseli basin to 1250 mm in Ngong.

The months of January-February are usually hot and dry; June to August are cool and dry; while November to April is hot. The temperatures fluctuate with both season and altitude; and range from 10°C in Loitokitok to 34°C in Lake Magadi. The county is however vulnerable to climate change (vulnerability index of 0.426) especially rainfall fluctuations and increasing temperatures.

The county was purposively selected owing to its weather variability and vulnerability to climate change, especially rainfall fluctuations. The sampling sites were selected because of their distinct variation in climatic patterns (for comparison), but also for their potential for livestock farming.

Kajiado County is preponderantly semi-arid and inhabited by Maasai ethnic group; however, persons from other regions in Kenya as well as foreigners have since migrated there. While Maasai are traditionally pastoralists, the dire need to sustain food security has necessitated a focus on both livestock including cattle, shoats and poultry keeping as well as crop farming. Most pastoralists (and/or agro-pastoralists) are not enlightened on the improved livestock farming practices. Loss of livelihoods as a result of diseases could be extenuated if good livestock husbandry practices are adopted at county and national levels (Mutua *et al*., 2022).

3.2.2 Study design

A two-season-based cross-sectional study design was carried out among small-holder cattle farms in Kajiado County, Kenya, between October 2020 and May 2022. The study involved

collection of faecal samples (cattle rectal and chicken cloacal swabs) and animal trough water samples from the enrolled farms.

3.2.3 Study animals

Target population of interest consisted cattle and chicken. Households rearing cattle and/or chicken were used as the sampling units, which in this study were considered as study farms. A list of farms (which forms the sampling frame) was obtained from local livestock production offices.

3.2.4 Study farms and sample size determination

Farms were enrolled in this study based on the following criteria: (i) smallholder to medium farms raising multiple species rearing cattle (≥200 cows) and other ruminants with or without chicken or farms keeping chicken (≥300 birds) with other ruminants with or without cattle; (ii) free-roaming cattle under outdoor grazing; (iii) and farm-fed cattle under zero grazing. In addition, farm owners' willingness and availability to participate was also considered. The minimum number of farms enrolled was guided by the formula, $n = \frac{\mathbf{N}}{1+\mathbf{N}\,(e)^2}$ (Ryan, 2013); where **N** is the overall number of farmers in the county, while **e** is the error permitted for the population. Based on National Farmers Information Service (NAFIS, 2014); there are around 223 farms in Kajiado County. By applying the above formula at 12% error for N = 223, the number of farms enrolled was 50. However, with assistance from the local animal health providers, a total of 55 farms were recruited by simple randomization. Global positioning system (GPS) co-ordinates for each farm enrolled in this study were recorded to enable investigator to make a follow-up (Appendix 2).

3.2.5 Sample size determination and sampling strategy

The minimum number of samples was calculated by applying the formula by Thrusfield (2007), $n = \frac{Z^2 P(1-P)}{d^2}$; where n is the sample size, **Z** is the Z statistic for 95% confidence (1.96), **P** is anticipated *Campylobacter* prevalence while **d** is the precision. The target sample size for cattle samples with presumed prevalence of 50% and a precision of 8% (0.08) was 150 animals. The expected prevalence for poultry samples was set at 69% (Nguyen *et al*., 2016) and a precision of 9% (0.09); this gave a sample size of 100 poultry samples. The number of individual faecal samples collected ranged from 1–7 per animal species, depending on herd/flock size per recruited farm. One (1) unstirred water sample (unstirred water was preferred so as to minimize gross dirt during processing/filtration) was collected from bovines' water troughs and/or watering points in each of the enrolled farms.

3.2.6 Sampling plan

The sampling scheme attempted to assess seasonal variations in occurrence of thermophilic *Campylobacter*; such that sampling coincided with: October-December and March-May (rain season); January-February and June-September (dry season). In addition, climatic data (minimum and maximum daily temperatures, relative humidity and precipitation) spanning over the sampling period for two seasons were retrieved from local meteorological stations.

Proportional stratified sampling was conducted for each farm. Strata were based on livestock species (chicken vs. cattle). Cattle derived samples were further stratified into either farm-fed/confined (zero-grazing) or free-roaming (outdoor grazing either settled or transhumant pastoral systems). Thus, the representative sampling plan entailed sampling of 407 faecal samples (comprising 265 cattle rectal swabs and 142 chicken cloacal swabs) and 50 surface water samples (from troughs and common watering points); from 55 households (described as

"smallholder farms" in this study). Thus, a total of 457 samples distributed across two seasons (cold-wet and warm-dry season) were collected as tabulated in Table 3.1.

Table 3.1: Sample types and distribution per season for the different production system

Sample type	Production system	Seasonal sampling		Total
		Cold and wet season	Dry and warm season	
Cattle rectal swabs	Farm-fed/confined (zero grazing)	96	44	140
	Free roaming (Outdoor grazing)	24	101	125
Chicken cloacal swabs	Housed	97	45	142
Surface water	Bovine's water troughs and/or designated watering point	29	21	50
Total		246	211	457

3.2.7 Sample collection

Animal (cattle and chicken) sampling was done by a veterinarian (the principal investigator) in strict conformity with animal welfare standards and aseptic technique. On each farm visit, cattle were restrained in a crush, and samples were collected by swabbing the recto-anal mucosa with cotton swabs. Swabbing was done using ethylene sterilized cotton-tipped swab sticks following a protocol described by Khaitsa *et al*. (2005) as shown in Figure 3.2. Briefly, the swab was inserted into the rectum by holding onto applicator stick end without inserting the hand in the rectum. The swab was aimed at the dorsal mucosa of the rectum, which was swiftly swabbed. Immediately after swabbing, swabs were separately placed in bijou bottles containing Stuart's® transport medium (Hi-media, Mumbai, India) and labeled accordingly.

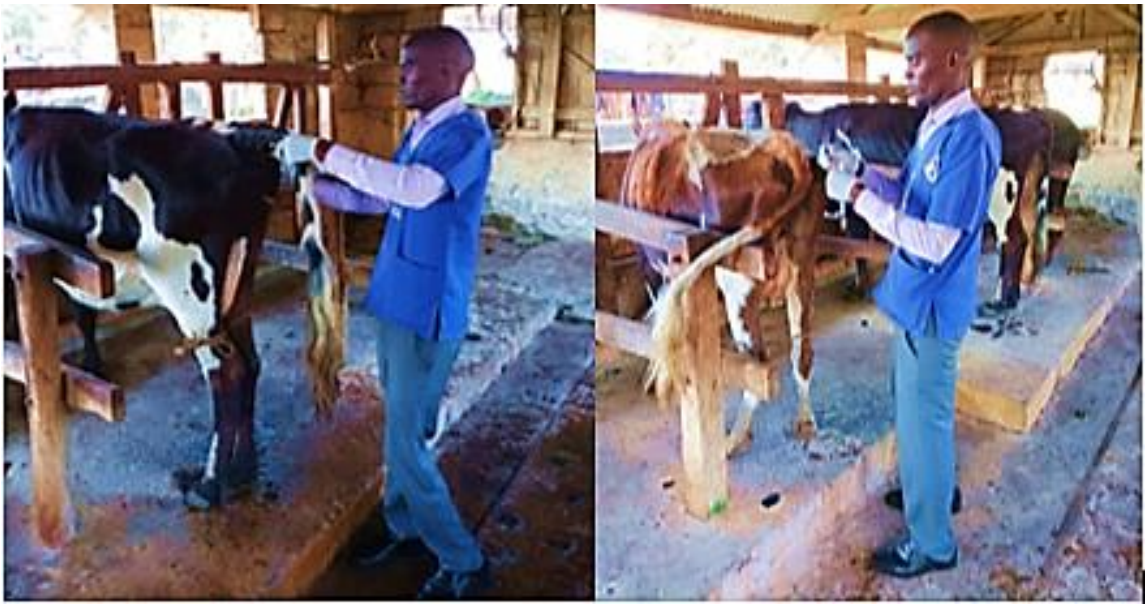


Figure 3.2: Principal investigator taking rectal swabs from cattle restrained in a crush in one of the surveyed farms in Ngong in Kajiado County

Chicken cloacal specimens were collected from 38 of the 55 participating farms. The other 17 farms were used as negative control in assessing effect of keeping chicken as a risk factor for *Campylobacter* positivity in cattle (Chapter 4). Live chicken was restrained manually, using minimal force. Cloacal swabs were then collected by introducing the whole tip of commercially obtained ethylene oxide sterilized cotton swab into the cloaca and swabbing with two to four circular motions while applying gentle pressure against the mucosal surfaces; gently shaking any faecal residues from the swab before transferring it into bijou bottles containing Stuart's® transport medium (Hi-media, Mumbai, India) and labeled accordingly.

Unstirred animal trough water samples were collected aseptically by partially immersing a 250 mL-sodium thiosulphate sterilized sampling bottle into the surface of water in cattle troughs and/or other watering points like dam or river (Figure 3.3), from the same cattle pen(s) from where faecal sampling had been done.

Figure 3.3: A 250 ml-water sample collected from cattle's water trough in Mashuru sub-county, Kajiado County

Water samples from different study cattle pens in a farm (pens in which sampling was done) were pooled and taken as one sample. For cattle under outdoor grazing/transhumant; surface waters from watering points (dams and rivers) were sampled. A total of 50 water samples (42 water samples from privately owned cattle water troughs in 42 farms, and, 8 water samples from communal animal watering points that were being shared by 13 farms) were collected.

All samples (rectal swabs, cloacal swabs and water samples) were labeled accordingly, placed in cooler boxes packed with ice packs and were immediately transferred to the microbiology laboratory in the department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi, for *Campylobacter* culture within 3 hours of collection.

3.2.8 Isolation and culture conditions for *Campylobacter* species

For isolation, faecal samples were cultured using conventional methods optimized for the detection of thermophilic *Campylobacter* species (Jokinen *et al*., 2012). Briefly, swabs were

loaded aseptically into 7 ml-bijou bottles containing *Campylobacter* enrichment broth, Bolton broth without the addition of blood and selective supplement (Oxoid). The bijou bottles were almost filled with the broths leaving a minimal headspace, to prevent aerobiosis. After 24 hours incubation at 42 °C, the broths were streaked aseptically onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) plates (incorporated with *Campylobacter* selective supplement containing cefoperazone, vancomycin, trimethoprim, and cycloheximide (SR0167E, Oxoid®) which were further incubated micro-aerobically at 42 °C for 48 hours.

All water samples were processed by filtration method; following procedures described by Horman *et al*. (2004) and Jokinen *et al*. (2012) with slight modifications. Briefly, a 100-ml water sample was filtered through a sterile 0.45µm-pore-size cellulose nitrate filter membrane (CHMLAB®), and the filter was then placed in a universal bottle containing 20 ml of Bolton enrichment broth for *Campylobacter* without antibiotics. After 3 hours of incubation at 42° C, 0.2 ml of a selective supplement containing cefoperazone, vancomycin, trimethoprim, and cycloheximide (SR0167E, Oxoid®) was added. Incubation was continued microaerobically for a further 24 hours at 42° C. After the selective enrichment phase, a 10^{l} portion of broth was spread onto the surface of a modified CCDA agar and incubated microaerobically at 42 °C for 48 hours.

Incubations of both broths and plates were done at 42 °C under microaerobic conditions which were provided by burning candles in air-tight jars (Ghimire *et al*., 2014). Afterwards, the plates were examined and the colony morphology recorded. The flow chart representing laboratory isolation and identification of thermophilic *Campylobacter* from faecal and water samples is shown in Figure 3.4.

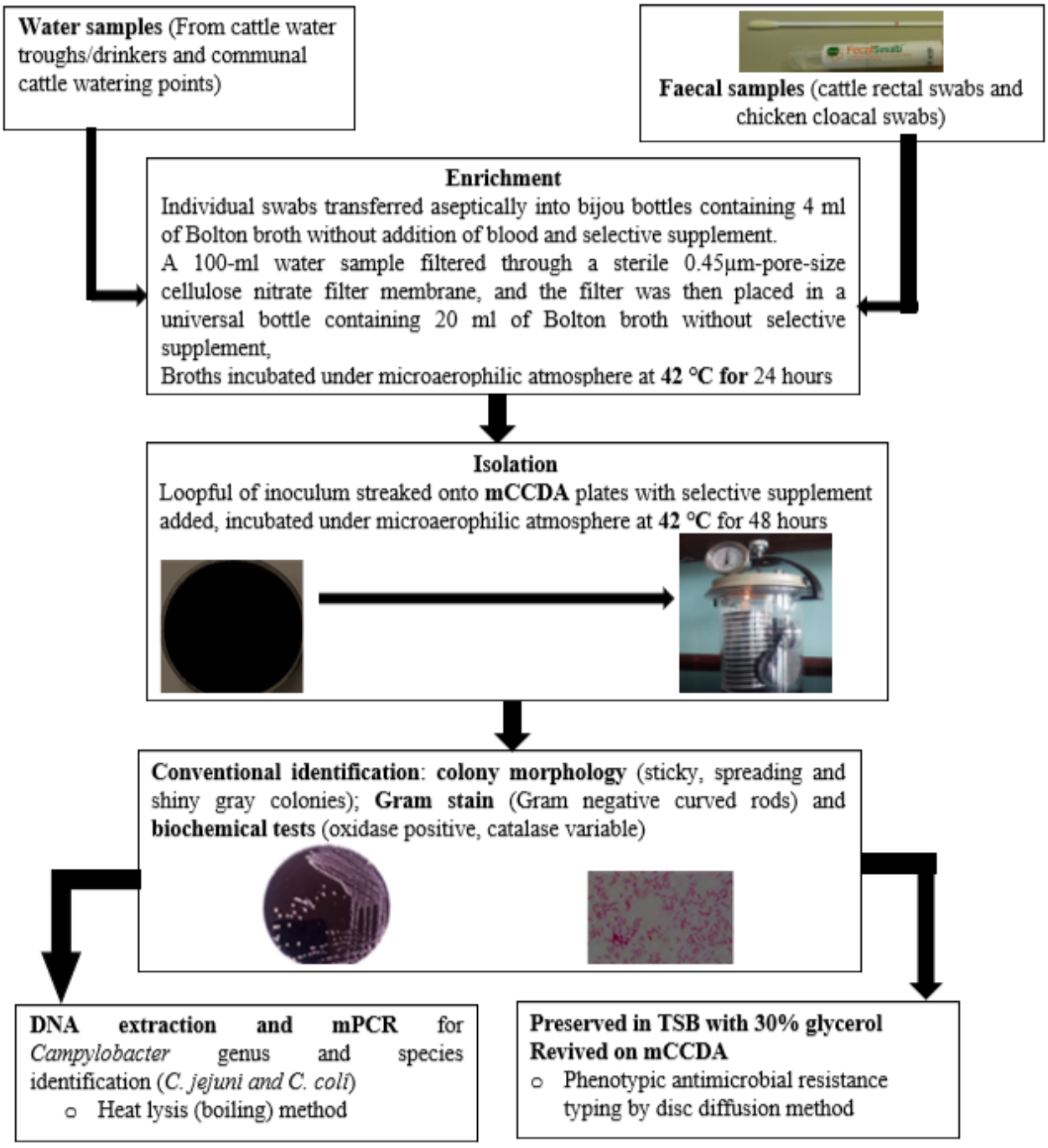


Figure 3.4: A flow chart representing laboratory isolation and identification of *Campylobacter* isolates from faecal and water samples

3.2.9 Conventional identification of *Campylobacter* species

Distinct colonies were sub-cultured to obtain pure colonies by re-streaking onto blood agar plates (with selective supplement). Presumptive identification of the *Campylobacter* suspect colonies was done by culture characteristics (growth at 42° C), colony morphology, Gram staining characteristics and biochemical reactions that is, oxidase, catalase and hippurate hydrolysis reactions; following criteria given by Hendriksen *et al*. (2003).

3.2.9.1 Gram staining and microscopic examination

A smear of a single bacterial colony from fresh pure culture was prepared on a clean microscope slide. The smear was then heat fixed by passing the slide, smear side up, swiftly through the Bunsen flame. For Gram staining, the method described by Markey *et al*. (2013) was followed, where the smear was flooded with 2.5% crystal violet solution for 1 minute, Gram's iodine solution for 1 minute, 50/50 acetone alcohol for 30 seconds and 1% safranine for 1 minute. Between each staining reagent, the smear was rinsed under a gentle stream of tap water. The stained smear was washed and then air dried. Microscopic examination was done using ×100 objective immersion lens on a light microscope, using immersion oil. From the Gram-reaction, size and shape of cells were observed and recorded.

3.2.9.2 Biochemical testing

3.2.9.2.1 Oxidase and catalase tests

These were carried out following the methods given by Hendriksen *et al*. (2003). Commercially available oxidase strips (Oxoid) were used to test ability of the isolates to produce cytochrome oxidase enzyme. Oxidase reaction was carried out by touching a well-isolated colony of fresh young pure culture with the oxidase strip. Results were recorded within 5-10 seconds. Positive

result was indicated by colour changing to purple. Formation of a purple colour confirms a positive result. *Pseudomonas aeruginosa* was used as a positive control organism.

A 3 percent hydrogen peroxide solution was used to detect ability of the isolates to produce catalase enzyme. A loopful of pure colony of fresh young culture was placed on a clean slide and a drop of 3% hydrogen peroxide added. Positive reaction was indicated by effervescence or bubbling within a few seconds. Non-typhoidal *Salmonella* was used as positive control in this procedure.

3.2.9.2.2 Hippurate hydrolysis test

Hippurate hydrolysis test determines ability of bacteria to enzymatically hydrolyze sodium hippurate to benzoic acid and glycine. It is used to differentiate *Campylobacter jejuni* from other *Campylobacter* species (Markey *et al*., 2013). Hippurate impregnated disks (Remel®) were used as per the manufacturer's instructions. Briefly, a loopful of a fresh isolate was emulsified in 0.1 mL of sterile distilled water in plastic vials. The disk was then placed into the suspension and incubated aerobically at 37° C for two hours. Then 2 drops of a Ninhydrin reagent (prepared by adding 3.5 g of Ninhydrin to 100 mL of a 1: 1 mixture of acetone and butanol) was added to the tubes, mixed, and re-incubated aerobically for further 30 minutes at 37° C. A positive reaction was indicated by development of a purple colour after 10 minutes. *Streptococcus agalactiae* and *Streptococcus pyogenes* were used as positive and negative controls, respectively.

3.2.10 Preservation of *Campylobacter* isolates

Pure cultures of suspect *Campylobacter* isolates were preserved in duplicate in Tryptose soya broth (TSB, HiMedia®) supplemented with 30% (v/v) glycerol in deep freezer at –20° C, until

further analysis. The latter included species differentiation using polymerase chain reaction (PCR) and phenotypic antibiotic susceptibility tests using Kirby-Bauer disc diffusion method.

3.2.11 Polymerase chain reaction assays

3.2.11.1 Extraction of bacterial deoxyribonucleic acid

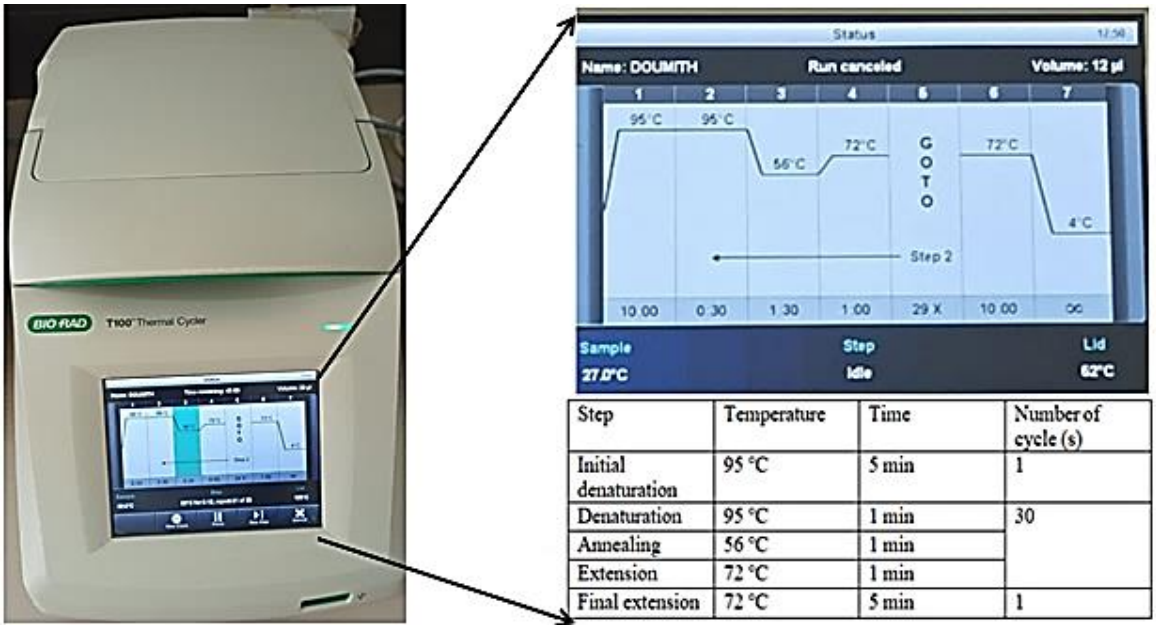
Campylobacter DNA was extracted using a heat lysis or boiling technique (Best *et al*., 2003). Briefly, previously preserved *Campylobacter* isolates were revived by sub-culturing on blood agar plates with selective supplement (BASs) at 42 °C for 24 hours. The colonies (3-5) were then suspended in sterile distilled water in an Eppendorf tube. The resulting bacterial suspension was boiled in a water-bath at 100 °C for 30 minutes; then allowed to cool and later centrifuged at 15,000 gravitational force for 5 minutes. The supernatant containing DNA was transferred into a sterile Eppendorf tube; which was then preserved at -80°C

3.2.11.2 Polymerase chain reaction identification of the genus *Campylobacter*

A singleplex PCR assay was initially undertaken to detect 857bp portion of 16 sub-unit ribosomal RNA (16S rRNA, a highly ubiquitous and extremely conserved region within the *Campylobacter* genome) specific for identification of *Campylobacter*-genus, using forward (F)-TCTAATGGCTTAACCATTAAAC and reverse (R)-GGACGGTAACTAGTTTAGTATT primers (Denis *et al*., 1999). The choice of 16S rRNA was largely due to the fact that it is ever-present in members of the genus *Campylobacter* and therefore ideal for primary identification. The primer sequences were subjected to BLAST analysis against the entire microbial genome database in GenBank (https://www.ncbi.nlm.nih.gov) to confirm primer specificity. A singleplex PCR assay was carried out in a 25 µL reaction volume comprising: 5 µL of template DNA, 12.5 µL master mix (New England Biolabs), 2 µL of each of forward and reverse primer (Inqaba

Biotechnologies, Pretoria, South Africa), and 3.5 µL of nuclease-free water (BioConcept, Switzerland).

The PCR tubes, containing 25 µL amplification mixture were then transferred to a pre-heated 96 wells thermal cycler (Bio-Rad T100^{TM}). The DNA was amplified using a program of initial heating at 95° C for 10 minutes followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 56° C for 30 seconds, extension at 72° C for 1 minute with a final extension of 72°C for 10 minutes as shown in Figure 3.5.



Step	Temperature	Time	Number of cycle (s)
Initial denaturation	95 °C	5 min	1
Denaturation	95 °C	1 min	30
Annealing	56 °C	1 min	
Extension	72 °C	1 min	
Final extension	72 °C	5 min	1

Figure 3.5: Thermal cycler conditions used for amplification protocol

The amplicons were verified by gel electrophoresis on Ethidium Bromide stained 1.5% agarose gel prepared by adding 3 Grams agarose (Cleaver Scientific Limited, Rugby, UK) to 200 mL Tris acetate EDTA (10xTAE) buffer (Glentham Life Sciences, Corsham, UK) containing 1 µg/ml ethidium bromide (Sigma, Dorset, UK) at 250 volts for 45 minutes. Thereafter, the bands were visualised on an ultra violet (UV) transilluminator connected to the imaging system (UVP BioDoc-It™ imaging-system, Cambridge, UK). Hyperladder IV (Bioline, London, UK) was used

as the molecular weight marker and band positions were determined visually against the molecular weight marker. *Campylobacter jejuni* (ATCC 33560) were used as positive controls for this protocol.

3.2.11.3 Polymerase chain reaction identification of *Campylobacter jejuni* and *Campylobacter coli* isolates

All the PCR-confirmed thermophilic *Campylobacter* isolates were further subjected to a singleplex PCR assay to delineate the isolates to species level for the detection of *C. jejuni* and *C. coli*. The 600bp fragment of hippurate hydrolase (*hipO*) gene of *C. jejuni* was amplified by PCR using F-TGATGGCTTCTTCGGATAG and R-CTAGCTTCGCATAATAACT primers (Han *et al*., 2016). In order to confirm *C. coli* isolates, the gene encoding siderophore transport protein (*ceuE*) was amplified using the F-ATTGAAAATTGCTCCAACTATG and R-GATTTTATTATTTGTAGCAGCG primers (Denis *et al*., 1999). The amplification was done separately using the respective primer sequences and thermal cycler conditions as for genus *Campylobacter* assay above. *Campylobacter coli* (ATCC 33559) and *C. jejuni* (ATCC 33560) were used as positive controls, whereas sterile nuclease-free water was used as negative control. Amplicons were analyzed by gel electrophoresis and then observed under ultraviolet light against the DNA ladder.

3.2.12 Data handling and statistical analysis

The data collected was cleaned, validated and then entered into Microsoft excel (which was also used to calculate proportions) and then validated prior to descriptive and inferential statistical analyses on EPI INFO software. Chi-square (χ^2) test was used to assess significance of association between isolation rates of thermophilic *Campylobacter* species and seasons and cattle grazing system. Confidence intervals at 95% level were analyzed for proportions, using the

Clopper and Pearson exact method using IBM SPSS software version 21. *P*-value ≤ 0.05 was considered significant.

3.3 Results

3.3.1 Cultural characteristics

The major culture conditions (growth at 42 ºC), phenotypic (Gram stain, oxidase and catalase activity) and biochemical (hippuricase activity) characteristics of the isolates obtained in this study were typical of thermophilic *Campylobacter* spp. as tabulated in Table 3.2.

Table 3.2: Gram stain and biochemical characteristics of the isolates

Isolate(s)	Gram stain reaction	Growth at 42 ºC	Colony morphology	Oxidase test	Catalase test	Hippurate hydrolysis
Campylobacter jejuni	Gram-negative short rods to curved appearing as coccobacilli	positive	Small flat colonies with entire margin, glistening grey to off-white/creamy pigmentation spreading and sticky appearance	positive	positive	positive
***Campylobacter coli* and other thermophilic *Campylobacter* species (OTCs)**	Gram-negative short rods to curved appearing as coccobacilli	positive	Small to medium sized, flat with entire margin, glistening grey to off-white/creamy pigmentation, spreading and sticky appearance	positive	variable	negative

All the suspect *Campylobacter* isolates demonstrated small to medium, gray, glistening and spreading colonies on mCCDA plates containing selective supplement, after 48 hours of micro-aerobic incubation at 42 ºC (Figure 3.6). Colony morphology on BAss medium was as shown on Figure 3.7.

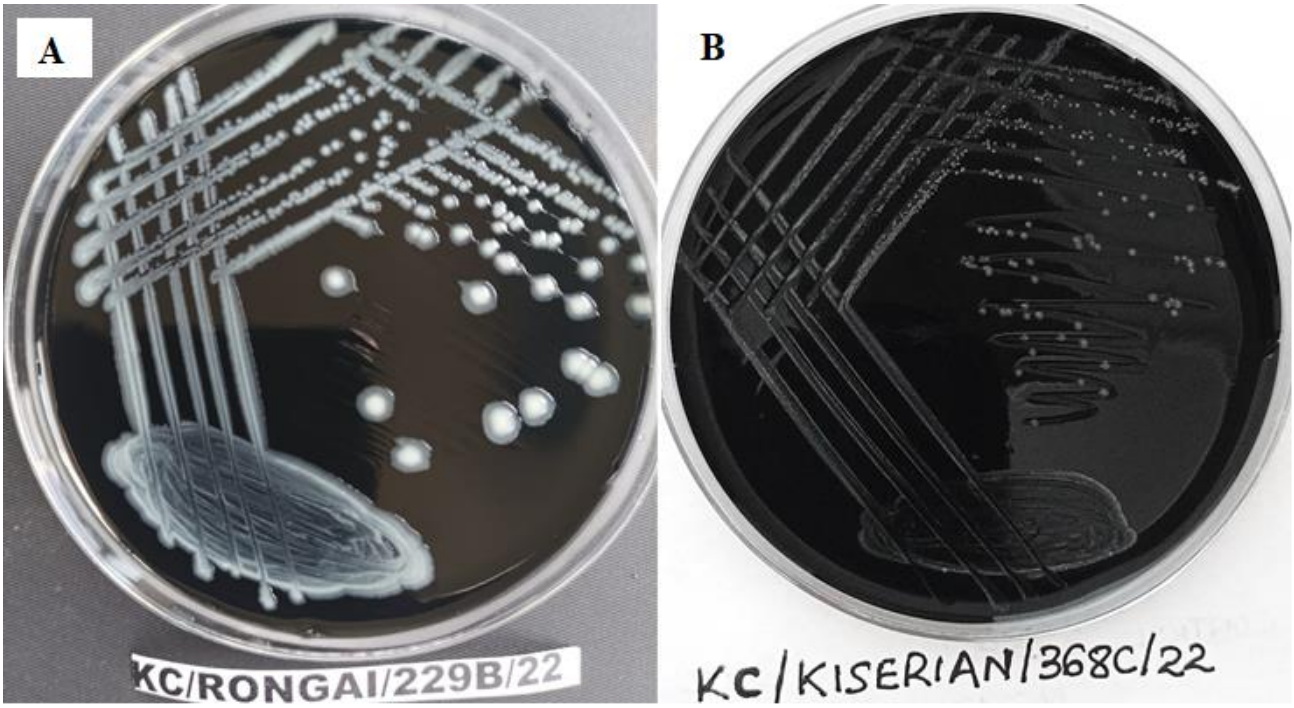


Figure 3.6: *Campylobacter* colonies on mCCDA plate, after 48 hours of microaerobic incubation at 42 ºC. Medium off-white glistening/shiny and spreading colonies (plate A) and the small gray colonies on the media (Plate B)

Figure 3.7: Colony morphology of thermophilic *Campylobacter* isolate on BAss media, that are glistening and have spreading appearance on the slightly moist plates

All of the presumptive *Campylobacter* isolates tested were positive oxidase reaction and showed variable catalase reaction (effervescence). The *Campylobacter* isolates were further subjected to

Gram-staining, where they exhibited Gram negative small curved rods to coccobacilli (Figure 3.8).

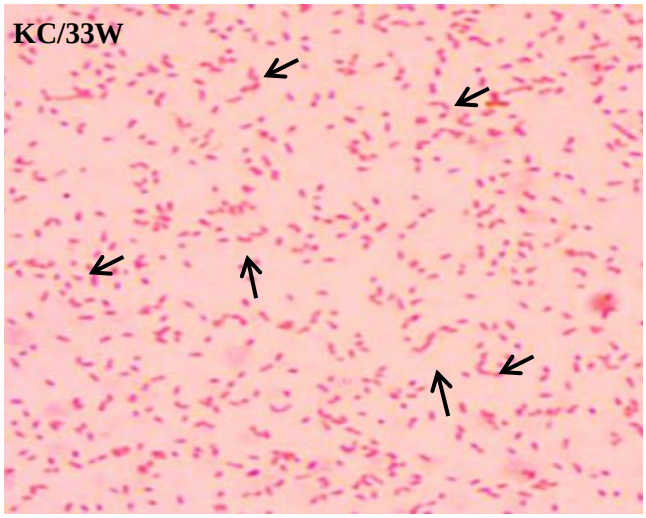


Figure 3.8: Small Gram-negative short or curved rods to coccobacilli of *Campylobacter* isolate (X1000) with characteristic "seagull" shaped arrangement

Some (48%) suspect *Campylobacter* isolates (putative *C. jejuni* isolates) showed ability to hydrolyze sodium hippurate (Figure 3.9).

Figure 3.9: Hippurate hydrolysis reaction differentiating *C. jejuni* (positive reaction; purple colour) from other *Campylobacter* species including *C. coli*. Positive results for the *C. jejuni* isolate 328B1, 330C, 48W and negative results for *Campylobacter* isolate 325B2. *Streptococcus pyogenes* was used as a negative control

Out of the 457 analyzed samples, 213 (46.6%) were presumptively positive based on conventional culture-identification-dependent methods (Table 3.3). However, a significant proportion of the samples (20.8%, 95/457) were overgrown by non-campylobacter background flora on mCCDA plate with selective supplement. These non-campylobacters were oxidase-negative rods. This finding was serendipitous; i.e., growth of non-campylobacter organisms on selective modified charcoal-cefoperazone-deoxycholate agar (mCCDA) under ideal culture conditions for *Campylobacter* species. A subset of these non-campylobacter isolates was commercially analysed using matrix-assisted laser desorption/ionisation time-offlight (MALDITOF) at National Public Health Laboratories, Nairobi; where they were confirmed as *Escherichia coli* and *Klebsiella* species. The rest of the samples (149 samples) produced no observable growth after 72 hours of micro-aerobic incubation at 42º C.

Table 3.3: Summary of culture-based results per individual sample source

Sample source	**Production system**	**Culture based identification**		
		Presumed thermophilic *Campylobacter* (n, %)	NC (n, %)	No observable growth**
Cattle rectal swabs (n=265)	Farm-fed/confined (Zero grazing) (n = 140)	66 (47.1%)	33 (23.6%)	41 (29.3%)
	Free-roaming (Outdoor) grazing systems (n = 125)	33 (26.4%)	16 (12.8%)	76 (60.8%)
Chicken cloacal swabs (n= 142)	Housed chicken	91 (64.1%)	32 (22.5%)	19 (13.4%)
Surface water sample (n=50)		23 (46.0%)	14 (28.0%)	13 (26.0%)
Total (n= 457)		213 (46.6%)	95 (20.8%)	149 (32.6%)

Key: ** Produced no observable growth after 72 hours of micro-aerobic incubation at 42°C; NC = Non-campylobacter (negative for putative *Campylobacter* spp)

3.3.2 Molecular detection of *Campylobacter* isolates; overall and sample-source level prevalence of *Campylobacter* isolates

Amplification of 857 bp of 16S ribosomal RNA (16S rRNA) genes for the genus *Campylobacter* produced bands corresponding to the target molecular size (Figure 3.10), where the target amplicon (857 bp) was compared with a 100-bp DNA marker.

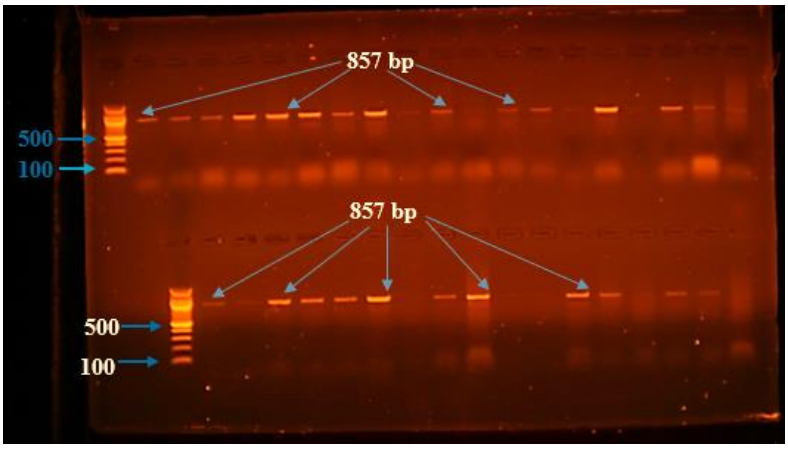


Figure 3.10: Agarose gel electrophoresis visualization of amplification of 857 bp 16S rRNA gene for genus *Campylobacter* identification

Out of the 213 culture positive samples, 162 were confirmed as belonging to *Campylobacter* genus by singleplex 16S rRNA PCR; giving an overall sample-level prevalence of 35.4% (162/457, 95% CI= 31.0–39.8%). The highest prevalence was observed in cloacal swabs of live chicken at 44.4% (63/142, 95% CI=36.2–52.6%), followed by rectal swabs from live cattle at 30.9% (82/265, 95%CI=25.3–36.5%). Water samples from cattle drinkers/troughs were found to be contaminated at 34% (17/50, 95% CI=20.9–47.1%). The isolation rate was higher in cattle under confinement system at 44.3% (62/140, 95%CI=36.1–52.5%), compared to those under free-roaming grazing system at 16.0% (20/125, 95%CI=9.6–22.4%) as presented in Figure 3.11.

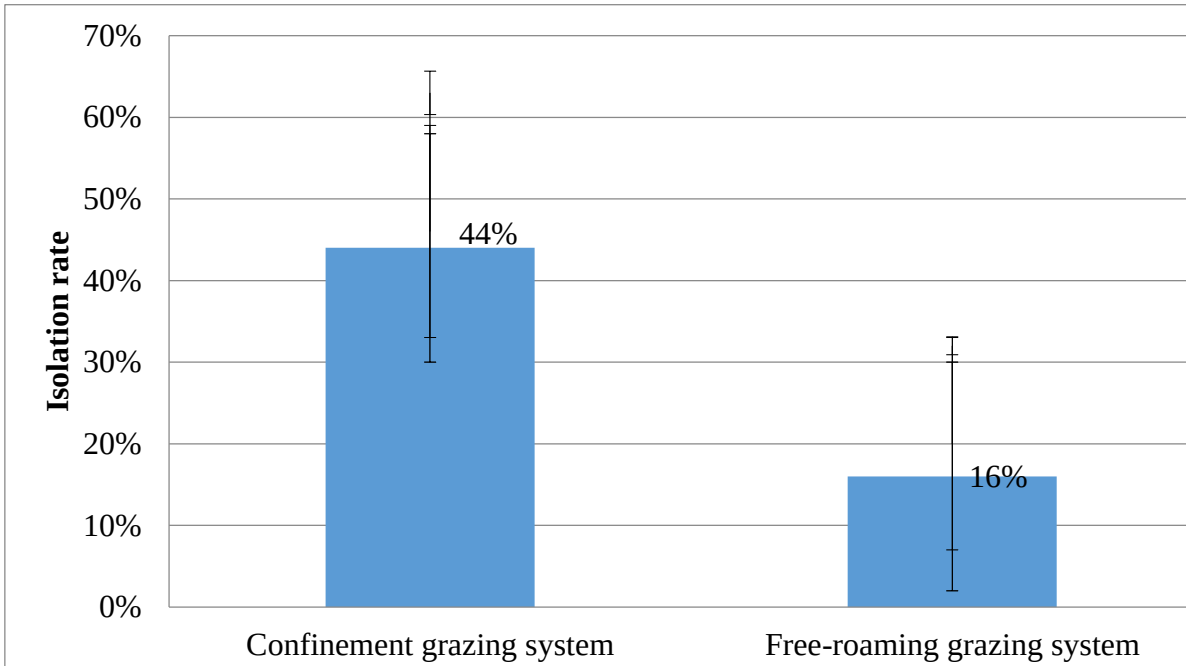


Figure 3.11: Thermophilic *Campylobacter* isolates in confinement and free-roaming cattle grazing system as confirmed through 16S rRNA

3.3.3 Seasonal prevalence of *Campylobacter* isolates and assessment of associated climatic variables

Thermophilic *Campylobacter* species were isolated in both seasons; with higher prevalence recorded during rainy and cold season in all sample types except for water (Table 3.4). There was a significant association between season and thermophilic *Campylobacter* carriage (χ=24.726, p=0.000). The cumulative prevalence was higher during the cold-wet/rainy season at 39.8% (98/246, 95%CI = 33.6–45.9), compared to warm-dry season (30.3% (64/211, 95%CI = 24.1–36.5), though statistically insignificant (P – value > 0.05).

Table 3.4: Seasonality of thermophilic *Campylobacter* isolates from different sample types

Sample type	Distribution of thermophilic *Campylobacter* isolates			
	Cold-wet (rainy) season		Warm-dry season	
	% ($^n/_N$)	95% CI	% ($^n/_N$)	95% CI
Cattle rectal swabs	40.8% (49/120)	32.0–49.6	22.8% (33/145)	16.0–29.6
Chicken cloacal swabs	46.4% (45/97)	36.5–56.3	40.0% (18/45)	25.7–54.3
Surface water samples	13.8% (4/29)	1.2–26.4	61.9% (13/21)	41.1–82.7
Total	39.8% (98/246)	33.6–45.9	30.3% (64/211)	24.1–36.5

N = Total number of isolates in the given category; n = Proportion of positive isolates in the given category (sub-total); CI = Confidence interval

The mean ± SEM and range of selected continuous climatic variables assessed over the sampling period across the two seasons are shown in Table 3.5. The lowest temperature, rainfall and humidity recorded in different seasons were 10.94 ℃, 0.03 mm and 42.3% respectively.

Table 3.5: Mean ± SEM and range of selected climatic variables collected during field survey conducted from October 2021 to May 2022 in Kajiado County

Climatic variables	Warm-dry season		Wet-rainy season	
	Mean ± SEM	Range	Mean ± SEM	Range
Average rainfall amount (mm)	11.22 ± 3.35	0.03 – 53.60	76.47 ± 16.16	8.4 - 305.4
Daily maximum temperature (℃)	23.35 ± 0.62	19.39-27.57	24.41 ± 0.33	19.97 – 25.2
Daily minimum temperature (℃)	12.93 ± 0.35	10.94 – 15.42	14.73 ± 0.26	11.04 – 14.83
Relative humidity (%)	55.86 ± 2.22	47.68 – 65.00	54.52 ± 2.20	42.30 – 69.57

3.3.4 Molecular confirmation of *Campylobacter coli* and *Campylobacter jejuni*

Singleplex polymerase chain reaction (sPCR) for speciation of *Campylobacter jejuni* and *C. coli* produced amplicons corresponding to 600bp for hippurate hydrolase (*hipO*), and 462bp for siderophore enterochelin (*ceuE*) genes respectively (Figure 3.12). Of the 162-genus specific PCR positive isolates, (comprising 82 cattle-derived, 63 chicken-derived and 17 water-derived samples), *C. jejuni* was the predominant species [55.6% (n = 90); 95%CI=47.9-63.3%], followed by *C. coli* [17.9% (n = 29); 95%CI=19.7-33.3%]; while 43 isolates (26.5%; 95%CI=19.7-33.3)

were categorized as other thermophilic *Campylobacter* species (Table 3.6). Similarly, *Campylobacter jejuni* was the most frequently confirmed thermophilic *Campylobacter* species in all sample types at; 66.7% (n = 42) in chicken, followed by 51.2% (n = 42) in cattle and lastly 35.3% (n = 6) in water samples. A total of Surprisingly, other thermophilic *Campylobacter* species (OTCs) had the highest frequency, with *C. jejuni* and *C. coli* appearing less frequently in water samples.

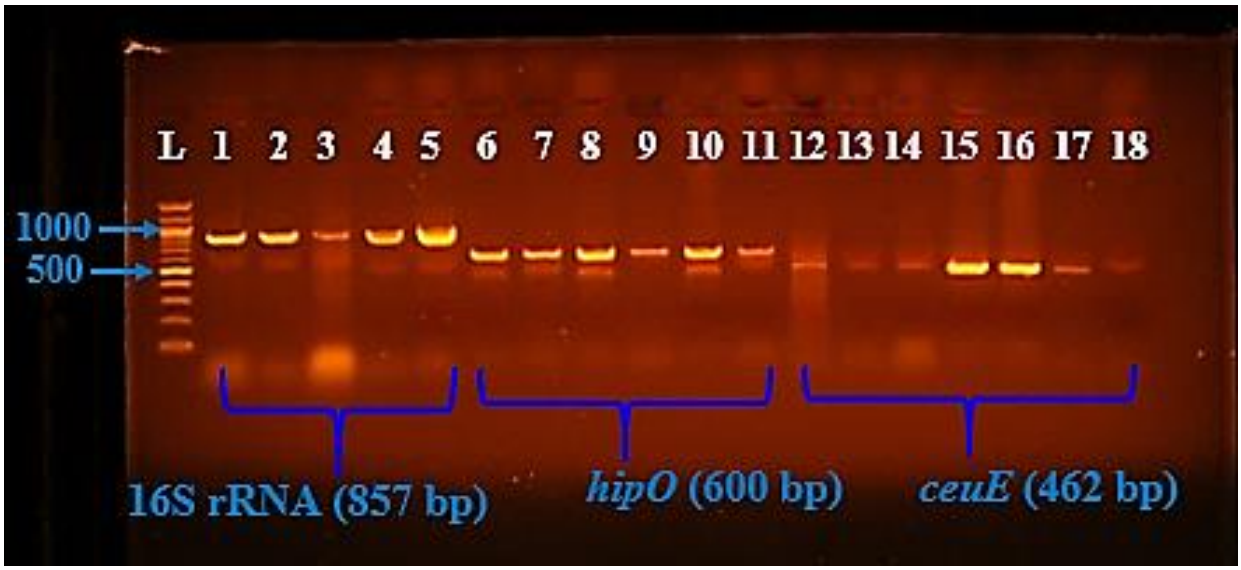


Figure 3.12: Agarose gel electrophoresis visualization of positive amplicons of 857bp 16S rRNA gene for *Campylobacter* genus (wells 1-5), 600 bp *hipO* gene for *C. jejuni* (wells 6-11) and 462 bp *ceuE* gene for *C. coli* (wells 12-18). L: DNA ladder, where each band represent 100bp

Table 3.6: Molecular typing of *Campylobacter* species per sample type

Source of isolate	Number analysed by PCR for genus assay	PCR positive (N)	Frequencies of *Campylobacter* species					
			C. jejuni		***C. coli***		**OTCs**	
			n (%)	95%CI	n (%)	95%CI	n (%)	95%CI
Cattle rectal swabs	99	82	42 (51.2%)	40.4–62.0	16 (19.5%)	10.9–28.1	24 (29.3%)	19.4–39.2
Chicken cloacal swabs	91	63	42 (66.7%)	55.1–78.3	9 (14.3%)	5.7–22.9	12 (19.0%)	9.3–28.7
Drinking/trough water	23	17	6 (35.3%)	12.6–58.0	4 (23.5%)	3.3–43.7	7 (41.2%)	17.8–64.6
Total	213	162	90 (55.6%)	47.9–63.3	29 (17.9%)	12.0–23.8	43 (26.5%)	19.7–33.3

OTCs = other thermophilic *Campylobacter* species that were not identified; 95% CI = 95% Confidence intervals for the proportions; N = Total number of isolates in the given category; n = Proportion of positive isolates in the given category (sub-total)

3.4 Discussion

Even though *Campylobacter* species are considered commensals in poultry; they have been isolated from cases of bovine abortion, bovine mastitis, enteritis and/or diarrhoea in calves. In addition, *Campylobacter* poses a public health risk through faecal contamination of milk or drinking water or meat at slaughter. The overall sample-level prevalence of thermophilic *Campylobacter* recorded in this study was 35.4%; whereas prevalence for chicken, cattle and water samples were 44.4%, 30.9% and 34%, respectively. Similar findings were obtained by Uaboi-Egbenni *et al*. (2012), in their study of the prevalence of thermophilic *Campylobacter* species in cattle and chickens in rural areas of Limpopo province, South Africa. In Kenya, there is paucity of studies on the prevalence of *Campylobacter* from different live farm animals and their natural environment (water, soil or even feeds/pastures) with which to compare the findings of this study. Even though, this study's finding is in discrepant with an earlier study conducted in informal settlements in Nairobi, Kenya, by Chepkwony (2016), who documented an overall prevalence of thermophilic campylobacters at 21.2% in livestock (with no clear contribution of each of the studied livestock including; cattle, goat, pigs, sheep, rabbits and chicken).

In this study, some colonies on mCCDA plates were overgrown by non-campylobacter organisms some of which were confirmed to be *Klebsiella* species and *E. coli*. The rate for non-campylobacter flora on this medium was 20.8%. These results indicate that mCCDA medium is not 100% sensitive and selective for isolation of thermophilic campylobacters. A similar finding has been reported by Chon *et al*. (2012); who reported presence and overgrowth of background/non-fastidious flora that interfered with isolation of *Campylobacter*. There is, therefore, need to enhance selectivity of the culture medium so as to maximize isolation rate. However, these were sorted-out when 16S ribosomal RNA typing and speciation PCR were done; some culture positive isolates were neither amplified using 16S rRNA nor with speciation PCR probes for *C. jejuni* and *C. coli.* These were taken to be neither of the two species, even though it is possible that the negative PCR assay could be ascribed to genetic disparities in these isolates; for example, point alterations in the regions complementary to this study's target sequence, thus altering binding by PCR probes and preventing amplification (Abu-Madi *et al*., 2016). The quality and quantity of genomic bacterial DNA could also lead to negative PCR results.

Among the 162 isolates that were genus-specific PCR-positive, 55.6% were confirmed to be *C. jejuni*, followed by *C. coli* (17.9%) and the rest were other thermophilic *Campylobacter* species. *Campylobacter jejuni* was the most confirmed species in all the sample types [chicken cloacal swabs (66.7%), cattle rectal swabs (51.2%) and trough water samples (35.3%)]. The predominance of *C. jejuni* over *C. coli* in all the sample types agrees with previous studies in Kenya and beyond (Häkkinen and Hänninen, 2009; Chepkwony, 2016; Carron *et al*., 2018; Kuria *et al*., 2018; Thépault *et al*., 2018; Karama *et al*., 2020). However, some studies elsewhere reported *C. coli* as the most confirmed species in bovine (Sanad *et al*., 2013; Karikari *et al*.,

2017a; Smith *et al*., 2019). Other thermophilic *Campylobacter* species (OTCs) comprise infrequently isolated ones and have also been reported in both cattle and chicken (Costa and Iraola, 2019).

The relatively high *Campylobacter* prevalence in chicken of 44.4% found in this study is comparable to that reported by other studies carried out in Kenya. Poultry are documented as asymptomatic reservoirs for thermophilic *Campylobacter*; with studies in Kenya reporting a prevalence of 29–44% (Carron *et al*., 2018; Mbai *et al*., 2022). However, a number of studies in this country have reported prevalence higher than 44% [Nguyen *et al*., 2016 (91%); Kuria *et al*., 2018 (50.7%); Mageto *et al*., 2018 (69.5%); Kariuki *et al*., 2020 (82-98%). Several studies in other sub-Saharan African countries have reported both lower and higher prevalences: 69.8% in Tanzania (Mdegela *et al*., 2006); 77.6% in Nigeria (Salihu *et al*., 2012); 22.5% in Ghana (Karikari *et al*., 2017b); and 28.9% in Ethiopia (Nigatu *et al*., 2015). Notably, the prevalence of chicken in the current study is moderately lower, which might be due to various differences: in isolation techniques, in sampling units (breeds and production systems) or in identification methods. In this study, the cloacal swabs were pre-enriched in Bolton broth; which has been shown to affect both the number and species of thermophilic *Campylobacter* isolated from naturally contaminated samples (Williams *et al*., 2012). A higher prevalence was observed in chicken compared to cattle. Chicken in smallholder systems are often confined to undesignated houses in the evenings in close interaction with other farm animals, including, cattle, where they scavenge for feed leftovers (Dlamini, 2002). Subsequently, chicken may play a role in epidemiology of campylobacter infections in cattle.

Thermophilic *Campylobacter* isolates were recovered from 30.9% of the 265 cattle rectal swabs analysed. This finding is consistent with studies conducted in other countries (Kashoma *et al*.,

2015; Karama *et al*., 2020; Hoque *et al*., 2021). There are variations in isolation rate of thermophilic *Campylobacter* in bovine ranging from 4 to 89.4% (Harvey *et al*., 2004); depending on isolation protocols (direct streaking or enrichment), herd characteristics (age, breed and production system), seasonal management practices, and sample type (rectal swabs vs dung or gastro-intestinal contents). The prevalence reported in this study might reflect where the cattle were sampled; with higher prevalence (44.3%) recorded under zero-grazing/confinement production systems than in outdoor grazing system. However, Grove-White *et al*. (2010) observed a higher prevalence among outdoor grazing cattle. A probable explanation to this study's finding is that, there are higher risk of acquiring thermophilic *Campylobacter* from a herd-mate (close contact) especially if under group housing and/or under high stocking density. More-over, in most integrated confined farming systems, there was tendency of spreading slurry (or poorly dried manure) on pastures/fodder crops. Cattle under outdoor grazing system are known to evade grazing on faecal contaminated pasture (Michel, 1955), thus risk of exposure to the organisms is minimal.

Although it is clear that both cattle and chicken are reservoirs, it is likely that water samples collected from water troughs (and/or animal watering points) are a significant *Campylobacter* contaminant at 34%, and therefore plays a key role in transmission to livestock. Contaminated water has been incriminated as a significant source of thermophilic *Campylobacter* contagion for bovine (Häkkinen and Hänninen, 2009; Bianchini *et al*., 2014; Rapp *et al*., 2014; Merialdi *et al*., 2015). Other thermophilic campylobacters had the highest frequency, with *C. jejuni* and *C. coli* appearing less frequently. Similar findings were reported in a study on diverse *Campylobacter* species in water samples from river Bø (Rosef *et al*., 2009). Besides *C. jejuni* and *C. coli*; *C. hyointestinalis* and *C. lari* have also been isolated from water (Brown *et al*., 2004; Rosef *et al*.,

2009). Carriage in water samples is an indication of *Campylobacter* contamination rather than colonization *per se*. However, the carriage maybe driven by the ability of the organism to survive outside the host and/or environmental factors e.g. presence of different reservoirs. Therefore, the higher isolation rate for OTCs probably suggest a high degree of contamination involving multiple faecal-shedder besides cattle and/or poultry.

Seasonality effect on occurrence of thermophilic *Campylobacter* in Kenya have not been described previously, though a survey by Shimotori *et al*. (1986) reported varying thermophilic *Campylobacter* colonization in children at 17%, 5.4% and 12.2% in July (cold and dry), September (hot and dry) and November (wet and cold) respectively. In this study, peaks in prevalence of thermophilic campylobacters in both cattle, and chicken were observed during cold and rainy seasons. Yet, Nwankwo *et al*. (2018) reported higher prevalence during cold-dry season (during the months of October-February, which are partly rainy and dry in Kajiado) in free range chickens. The current study also recorded a higher prevalence of thermophilic campylobacters in water samples during the dry and warm period. A probable hypothesis for this finding is that transhumant activities (movement of both livestock and to some extent wildlife migration in search of water and pasture especially during drought) inside and outside the region, may contribute to faecal contamination of water, more so for natural watercourses or reservoirs (dams). Even though a small seasonal effect (prevalences differed insignificantly) was observed in this study; the minimal variations may be due to animal husbandry practices, presence of reservoirs (rodents, birds and flies) or other confounding factors. Indeed, the reported summer and autumn peaks of *Campylobacter* infections in both humans and livestock in Europe and North America is attributable to distinct seasons having discrete climatic conditions. Unlike in Kajiado County where the climatic conditions (ambient temperature, relative humidity and

precipitation) were more or less the same during the two seasons. Therefore, the small seasonal effect could be attributable to the minimal disparities in climatic variables across the seasons. Kajiado-specific climatic factors that may influence *Campylobacter's* persistence in the environment at the time of sampling include: rainfall of 0.03 mm, ambient temperature above 10.94 ℃ and humidity which was above 42.3% during the two seasons. In almost comparable findings, Carron *et al*. (2018) suggest that a constant temperature above 16°C, or a precipitation 80 to 191 mm may favour *Campylobacter* survival in the environment. Consequently, this calls for further investigations especially on the biological mechanisms.

3.5 Conclusions

- Results of this study demonstrate that cattle, chicken and water harbour potentially pathogenic thermophilic campylobacters; with higher prevalence observed in chicken This suggests that cattle and chicken are important reservoirs of *Campylobacter* spp.; potentially posing public health hazard
- *Campylobacter jejuni* was widely distributed among water and faecal samples from cattle and chicken
- The isolation rate was higher in cattle under confinement system 44.3% (95% CI=36.1–52.5%) than those under free-roaming grazing system
- There were minimal seasonal variations with respect to occurrence of thermophilic *Campylobacter* carriage. The organisms were isolated in both seasons; with higher prevalence [39.8% (95%CI=33.6–45.9)] recorded during rainy and cold season in all sample sources except for water

3.6 Recommendations

- Selective modified charcoal-cefoperazone-deoxycholate agar (mCCDA) has shown poor sensitivity and selectivity for isolation of *Campylobacter*; it is, therefore recommended to use a more selective medium for respective isolations
- there is need for definitive identification for the non-campylobacter (NC) and other thermophilic *Campylobacter* species (OTCs) isolates and may be detect virulence genes that they harbour; so as to investigate their role in disease process, if any
- Further epidemiological and phylogenetic studies comparing livestock, environmental samples and human thermophilic *Campylobacter* isolates are needed to establish source-attribution and zoonotic potential of thermophilic campylobacters

CHAPTER FOUR: RISK FACTORS ASSOCIATED WITH OCCURRENCE OF THERMOPHILIC *CAMPYLOBACTER* IN CATTLE HERDS RAISED ON INTEGRATED SMALL-SCALE FARMS IN KAJIADO COUNTY, KENYA

4.1 Introduction

Farm management practices and environment influence occurrence and persistence of *Campylobacter*, which may be crucial for cattle re-infection, and for human infections (An *et al*., 2018). Although, farm management and animal health practices have received more attention as risk factors influencing transmission of livestock diseases; there has been relatively less extensive research on predisposing factors associated with occurrence of campylobacteriosis in livestock, particularly, in developing countries (Uddin *et al*., 2021). In Kenya especially in Kajiado County, the epidemiology of campylobacters in cattle and the likely transmission dynamics (i.e. the likely sources of contamination and transmission mechanisms) is lacking or not fully understood.

Owing to the potential role of husbandry practices, environment and chicken in the epidemiology of *Campylobacter*; there is need to establish their causal role/relationship in *Campylobacter* colonization in cattle. Preventive strategies such good sanitation practices and biosecurity measures can prevent *Campylobacter* colonization in farm animals and contribute in the control of this zoonotic bacteria in farms. Therefore, this study was undertaken to assess farm characteristics and/or management practices and climatic factors as potential risk factors associated with *Campylobacter* positivity in integrated smallholder cattle herds in Kajiado County. The findings of this study will form a reference point for designing practical solutions.

4.2 Materials and Methods

4.2.1 Study area and design

A two-season based descriptive cross-sectional study design was carried out among integrated small-holder cattle farms in Kajiado County, Kenya, between October 2020 and May 2022. The study involved faecal sampling (from cattle and chicken) and collection of water from cattle troughs and other watering points. Additionally, a semi-structured questionnaire designed to collect farm characteristics and management practices that might predispose cattle to thermophilic campylobacters was also administered and the hygienic status of the surrounding environment assessed in farms that consented to this study. Climatic variables including: rainfall, daily ambient temperature (minimum and maximum values) and relative humidity were included in the model as environmental factors. Respective data for the study sites was retrieved from Ngong and Wilson Airport weather stations and from the archives of AccuWeather (https://www.accuweather.com). Faecal and water samples were specifically processed for campylobacter isolation (Hitchins *et al*., 1998) and molecular techniques (PCR) for identification. Thermophilic *Campylobacter* positivity status of chicken cloacal swabs and water samples for the given cattle farm were then included in the model as potential risk factors for *Campylobacter* positivity.

4.2.2 Sample size determination (for study farms and faecal samples) and sampling strategy

As described in **Chapter 3, subsection 3.2.5 and 3.2.6**; enrolment of integrated small-scale farms was based on: farms primarily rearing ≥200 heads of cattle, farms rearing multiple species of livestock including poultry and farmers consent. The sample size for the study farms was determined as 55 integrated small-scale farms in Ngong, Isinya, Ongata Rongai, Kitengela, Mashuru and Kiserian sub-counties.

4.2.3 Sample collection

The cattle rectal swabs, chicken cloacal swabs and water samples were collected as described in **Chapter 3, subsection 3.2.7**.

4.2.4 Isolation and identification of thermophilic *Campylobacter* species

Isolation and identification of thermophilic *Campylobacter* species were achieved using culture and molecular techniques as described in **Chapter 3, subsection 3.2.8** to **subsection 3.2.11.2**

4.2.5 *Campylobacter* status in cattle

Using the enrolled integrated farms (households keeping cattle) as sampling units, a site with at least one PCR-positive cattle rectal isolate was classified as positive for thermophilic *Campylobacter* spp. The dependent variable was defined as thermophilic *Campylobacter* status of a herd of cattle.

4.2.6 Independent variables

4.2.6.1 *Campylobacter* status in chicken and water samples

Thermophilic *Campylobacter* status of chicken and water samples from sampled farms were included in the model as potential risk factors for *Campylobacter* colonization in cattle. A farm with at least one PCR-positive chicken isolate and one PCR-positive water isolate were classified as positive for thermophilic *Campylobacter* species.

4.2.6.2 Questionnaire administration and assessment of environmental hygiene

A semi-structured questionnaire was administered through face-to-face interviews during farm visit (Figure 4.1). The questionnaires were pre-tested and revised accordingly for clarity and time management. The questionnaire was themed to collect information on farm characteristics (herd size, breed type, other animals in the farm); management practices (housing, water supply,

feeding regime and manure disposal) and biosecurity measures in place (Appendix 3). Hygienic practices were assessed through direct researcher observation; where dampness and cleanliness of the housing unit and/or enclosure were scored (1 = dry and clean 2 = wet and dirty) following Hughes (2001).

Figure 4.1: The principal investigator (with a clip board) conducting a questionnaire interview in Illaimiror area in Mashuru sub-county in Kajiado County

4.2.6.3 Climatic variables

Climatic data [rainfall/precipitation, daily ambient temperature (minimum and maximum values) and relative humidity)] during the sampling period was retrieved from Ngong and Wilson Airport weather stations (nearest weather stations with similar microclimate as other parts of Kajiado County). Any missing data was retrieved from the archives of AccuWeather (https://www.accuweather.com). Also, FAO's country pasture and forage resource zoning which is based on average annual rainfall, was included as a proxy for the microclimates in Kajiado County (FAO, 2006). Only two zones were used to classify the surveyed farms: zone III/semi-humid (800-1400 mm) and V/semi-arid (450-900 mm).

4.2.7 Statistical data analysis and model building

The data collected was entered, validated, and stored in Microsoft Excel® 2019 spreadsheet. Descriptive and inferential statistics were performed using the 'base', 'epiDisplay' and 'aod' packages of the R software version 4.1.3 (R Foundation for Statistical Computing 2017, Vienna, Austria, http://www.R-project.org/). The proportions and/or counts of categorical-independent variables were stratified into a dichotomous table of thermophilic *Campylobacter* positivity and negativity groups in cattle herd. Chi-square (χ^2) test of independence was applied to assess for association between independent variables and outcome/dependent variable (Thermophilic *Campylobacter* status based on PCR). Fisher's exact test was applied in variables with counts of less than six (6) in at least one stratum.

All variables with *P*-value < 0.2 in the Chi square tests were used to develop the univariate and multivariate binary logistic regressions. A stepwise regression was applied in the saturated logistic model and two-way interaction of terms tested. The final model was compared to saturated model using likelihood ratio test (LRT) and a *P*-value < 0.05 signifying improved performance of model. Akaike Information Criterion (AIC) values for the two models were compared and final model chosen only when the value was lower than that of saturated model. Test for multicollinearity was performed in the final model and model covariates with variance inflation factor (VIF) above 5 discarded. A correlogram was plotted to investigate the actual correlations in the covariates violating the multicollinearity assumption. Correlated variables were individually added to the model and picked if the overall model performance was enhanced, as measured using the AIC value. Inspection of standardized residuals was also done to check for potential outliers in the dataset. The performance of the final selected model in predicting outcome of thermophilic *Campylobacter* in cattle herds, given the set of covariates

was done using confusion matrix. The model was used to predict outcome status in each of the 265 samples and the predicted values compared to actual values as indicated in the dataset. Summation of the true positives and true negatives was divided by summation of all the predicted values to get the model efficiency, represented by the quotient. The proportion (quotient) illustrated the variation in outcome variable (*Campylobacter* status) that could be explained by the model, hence its performance. In terms of levels within each categorical risk factor, for comparison purposes, the risk factor associated with the least *Campylobacter* spp. positivity was used as a reference. Adjusted OR that considered all cofounder variables were calculated. Odds ratio significance was computed using the Wald's test. A 5% level of significance (95% confidence interval) was used.

4.3 Results

4.3.1 Characteristics of farms surveyed

Fifty-five small-scale farms were enrolled in this study and were located in six regions of Kajiado County: Kitengela (n = 4), Isinya (n = 4), Kiserian (n = 7), Ongata Rongai (n = 9), Mashuru (n = 13) and Ngong (n = 18). Seventeen of the 55 farms (30.9%, 95%CI: 18.7-43.1) raised cattle and small ruminants only, while the rest (69.1%, 95%CI:56.9-81.3) raised a combination of poultry and livestock (mainly cattle co-reared with either goats or sheep). The main sources of cattle drinking water were untreated borehole water at 81.8% (45/55, 95%CI: 71.6-92.0), followed by treated tap water at 10.9% (6/55, 95%CI: 2.7-19.1); dams and/or groundwater and seasonal river water were used at 5.5% (3/55, 95%CI: -0.5-11.5) and 1.8% (1/55, 95%CI: -1.7-5.3) respectively. A significant number of farms (34.5%, 19/55, 95%CI:21.9-47.1) shared the source water with animals from other farms (Figure 4.2).

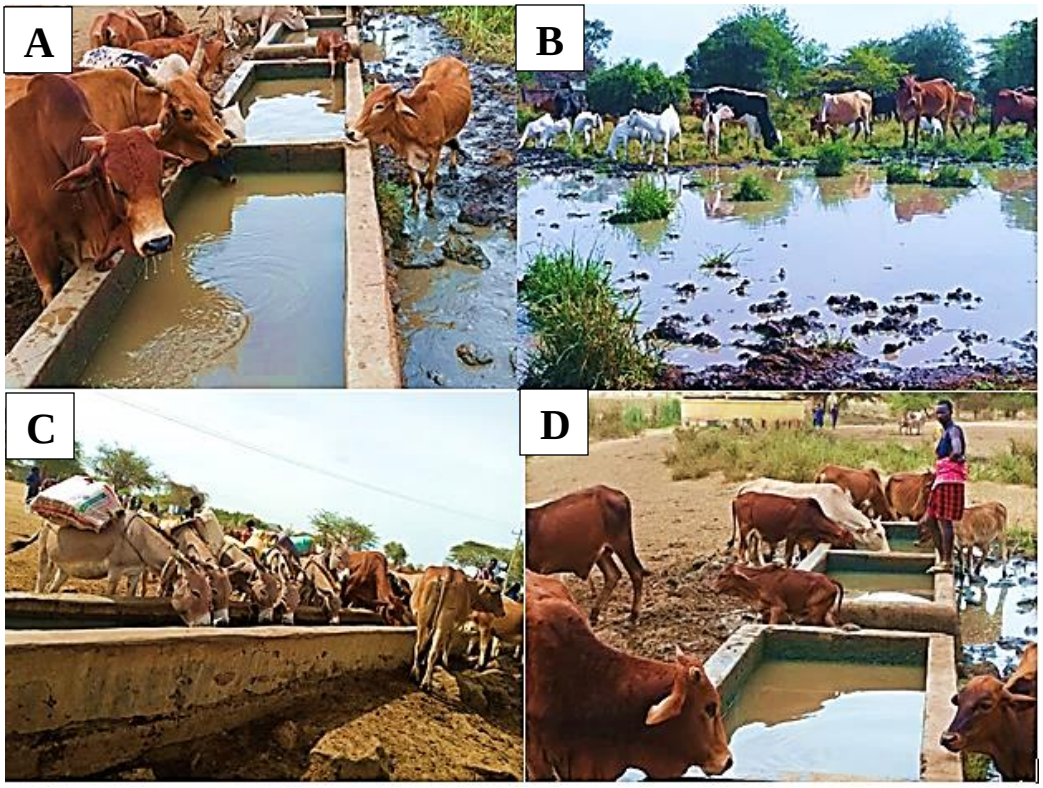


Figure 4.2: A communal animal watering point "*oltinka*" with cattle co-grazing with other animals (Figures A-D). Note some animals are drinking ground water (Figure B)

Sixteen farms practiced beef keeping (mainly Borana, Sahiwal and their crosses) under settled outdoor grazing or transhumant pastoral systems. The rest practiced dairy farming (mainly Friesian and their crosses, some Ayrshire and Jersey) under zero grazing unit (Figure 4.3). Forty-two percent (n = 23) of the farmers kept between 1 and 10 heads, whereas the rest owned more than 10 heads of cattle. Free range and backyard chicken farming was a common practice in most of the farms surveyed.

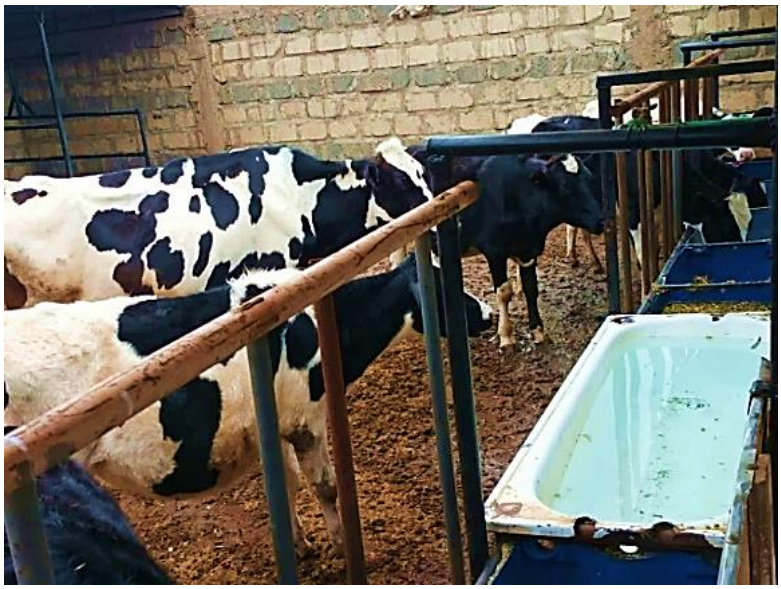


Figure 4.3: Housing enclosure stocked with Friesian and their crosses under zero grazing unit. Note the mixed housing with sub-optimal conditions

4.3.2 Farm-level status of thermophilic *Campylobacter* species in cattle herds

Farm-level and/or herd-level prevalence of thermophilic *Campylobacter* spp., as established in **Chapter 3**, was 72.7% (40/55, 95%CI=60.9-84.5) representing 72.2% (13/18, 95%CI=51.5–92.9) and 73% (27/37, 95%CI=58.7–87.3) in the agroecological zone III (Ngong region) and agroecological zone V (Mashuru, Kitengela, Rongai, Isinya and Kiserian), respectively. However, there was no significant difference in the farm/herd-level positivity of thermophilic *Campylobacter* among the zones (P-value >0.05). The proportion of positive samples within each surveyed farm ranged between 12.5% (1/8) and 100% (8/8). However, samples from 27.27% (15/55, 95%CI=15.5-39.1) of the surveyed farms were *Campylobacter* negative. Seventy-two-point seven three percent (72.73%; 40/55) of the *Campylobacter* positive farms had a mean prevalence of 48.14%, and median of 50%.

4.3.3 Farm-level status of thermophilic *Campylobacter* species in chicken

As established in **Chapter 3**, among the 38 farms that kept chicken, 28 were found to be positive with *Campylobacter* spp. overall via molecular assays (PCR). Therefore, a flock-/farm-level prevalence was confirmed as 73.7% (95% CI: 59.7–87.7%). Of the 28-*Campylobacter* positive farms, the prevalence ranged between 20% and 100%; with a mean and median of 61% and 55% respectively.

4.3.4 Farm-level status of thermophilic *Campylobacter* species in water samples

As established in **Chapter 3**, thermophilic campylobacters were detected in 17 water samples; which represented 87.5% (7/8, 95%CI: 64.5-110.4) and 23.8% (10/42, 95%CI: 10.9-36.7) in the communal watering points and privately-owned cattle water troughs, respectively. There was a significant variation observed in the water-level *Campylobacter* contamination (*P*-value = 0.000). Water samples from borehole had the highest level of *Campylobacter* positivity at 36.4% (16/44, 95%CI: 22.2-50.6), followed by treated tap water at 50% (1/2, 95%CI: -19.3-119.3). Water samples obtained from both dams and/or groundwater and seasonal river were *Campylobacter* negative.

4.3.5 Climatic variables assessed for the participating farms

The mean and range of selected continuous variables assessed for the participating farms over the sampling period were as shown in Table 4.1. There was no statistical difference in means of: daily maximum temperature, daily minimum temperature, humidity and amount of rainfall across the seasons and agro-ecological zones (minimal variations in terms of seasonal and agro-ecological zones).

Table 4.1: Mean and range of selected climatic variables assessed for model building and retrieved from local weather stations in Kajiado County

Zone	Climatic variables	Warm-dry season		Wet-rainy season	
		Range	Mean ± SEM	Range	Mean ± SEM
ACZ III	Rainfall (mm)	1.83 – 53.60	17.10 ± 4.71	18.7 - 305.4	122.20 ± 19.28
	Daily maximum temperature (℃)	19.39 -27.57	22.54 ± 0.82	21.35 – 25.20	23.39 ± 0.27
	Daily minimum temperature (℃)	10.94 – 14.46	12.08 ± 0.34	12.88 – 14.83	13.73 ± 0.15
	Relative humidity (%)	47.68 – 65.00	55.86 ± 2.22	42.30 – 69.57	54.51 ± 2.20
ACZ V	Rainfall (mm)	0.03 – 4.18	1.97 ± 0.64	1.11 – 13.38	7.88 ± 1.48
	Daily minimum temperature (℃)	21.71 -27.16	24.63 ± 0.80	24.03 – 27.36	25.94 ± 0.36
	Daily maximum temperature (℃)	13.32 – 15.42	14.27 ± 0.30	15.74 – 16.73	16.23 ± 0.11
	Relative humidity (%)	M	M	M	M

[a]FAO's agroecological zones (ACZ) were used to categorize participating farms: ACZ III or semi-humid region (800-1400 mm annual rainfall) and ACZ V or semi-arid (450-900 mm annual rainfall); M = Missing data

4.3.6 Questionnaire (independent variables) data and association with *Campylobacter* positivity in cattle herds

A correlogram identified variable linearly correlated to likelihood of *Campylobacter* positivity in cattle is given in Figure 4.4. From the questionnaire data and the results of statistical analysis, 17 of the 23 factors tested showed significant associations with thermophilic *Campylobacter* species positivity in cattle in the surveyed farms (Chi-square *P*-value <0.05) (Table 4.2).

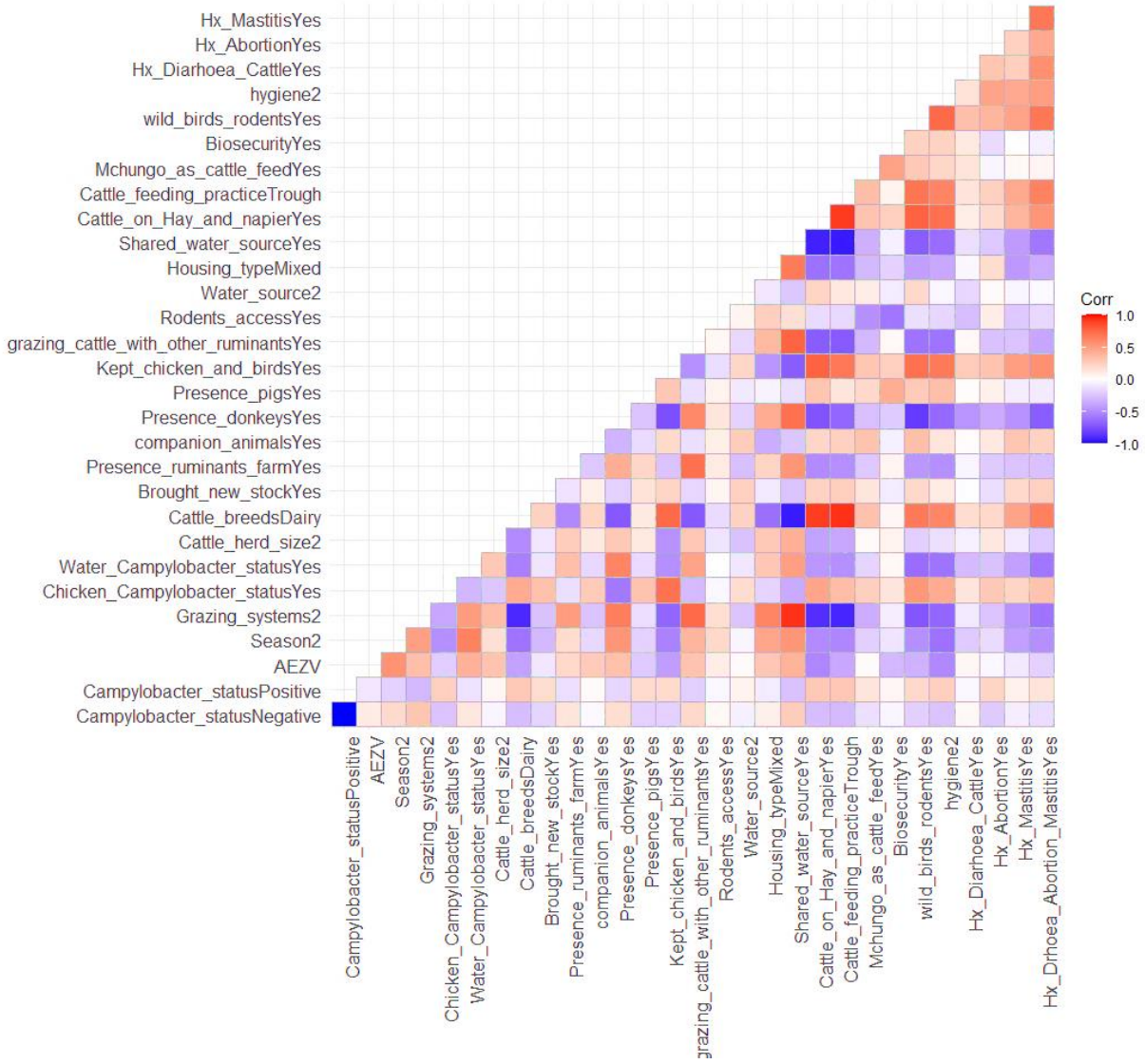


Figure 4.4: Correlogram projecting the relationship between each pair of variables associated with *Campylobacter* positivity in cattle. Positive correlations are shown in red and negative correlations in blue. Color intensity is proportional to the correlation coefficients. On the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding color

Table 4.2: Dichotomous analysis for selected categorical variables stratified by thermophilic *Campylobacter* status of cattle rectal swabs from cattle from small-scale farms in Kajiado County

Variable	Modality	Thermophilic *Campylobacter* positivity (%)	Thermophilic *Campylobacter* negativity (%)	95% CI		P Value	χ2 (df=1)
				Lower	Upper		
Geographical location of the farm in Kajiado County based on FAO's climate zones[a]	ACZ III	29 (39.2)	45 (60.8)	0.338	1.060	0.078	3.20
	ACZ IV	53 (27.7)	138 (72.3)				
Sampling season	Wet rainy season	49 (40.8)	71 (59.2)	1.138	3.99	**0.002[b]**	10.04
	Dry-warm season	33 (22.8)	112 (77.2)				
Farm herd size	≤ 10 animals	62 (32.1)	131 (67.9)	0.677	2.237	0.496	0.47
	≥ 10	20 (27.8)	52 (72.2)				
Cattle breed	Dairy	64 (42.1)	88 (57.9)	2.111	6.980	**0.000[b]**	21.87
	Beef	18 (15.9)	95 (84.1)				
Cattle grazing system	Indoor/confined (zero grazing)	62 (44.3)	78 (55.7)	0.134	0.429	**0.000[b]**	25.72
	Free-roaming	20 (16.0)	105 (84.0)				
Cattle housing and/or enclosure type	Mixed housing	50 (28.6)	125 (71.4)	0.422	1.246	0.245	1.34
	Group housing	32 (35.6)	58 (64.4)				
Farm Biosecurity e.g. a footbath	Yes	8 (36.4)	14 (63.6)	0.525	3.244	0.566	0.32
	No	74 (30.5)	169 (69.5)				
Kept and co-graze cattle with other ruminants (shoats)	Yes	36 (23.1)	120 (76.9)	0.241	0.699	**0.001 [b]**	10.90
	No	46 (42.2)	63 (57.8)				
Presence of companion animals in the farm	Yes	14 (32.6)	29 (67.4)	0.543	2.199	0.802	0.063
	No	68 (30.6)	154 (69.4)				
Kept chicken that were *Campylobacter* positive	Yes	50 (44.6)	62 (55.4)	1.78	5.23	**0.000[b]**	16.98
	No	32 (20.9)	121 (79.1)				
Kept pigs in the farm	Yes	16 (55.2)	13 (44.8)	1.445	6.951	**0.004[b]**	8.27
	No	66 (28.0)	170 (72.0)				
Keeping chicken and other birds irrespective of their *Campylobacter* status	Yes	61 (38.4)	98 (61.6)	1.418	4.476	**0.002[b]**	10.62
	No	21 (19.8)	85 (80.2)				
Kept donkeys in the farm	Yes	18 (20.0)	72 (80.0)	0.238	0.791	**0.006 [b]**	7.99
	No	64 (36.6)	111 (63.4)				
Brought new cattle stock in the last 6 months	Yes	11 (61.1)	7 (38.9)	1.452	10.451	**0.007 [b]**	7.50
	No	71 (28.7)	176 (71.3)				
Source drinking water for cattle	Untreated borehole water	75 (30.2)	173 (69.8)	0.227	1.689	0.349	0.85
	Treated tap water	7 (41.2)	10 (58.8)				
Cattle drinking water shared with animals from other farms	Shared	21 (17.5)	99 (82.5)	0.164	0.519	**0.000 [b]**	19.24
	Individual	61 (42.1)	84 (57.9)				
Cattle drinking contaminated with *Campylobacter*	Yes	32 (25)	96 (75.0)	0.341	0.986	**0.044 [b]**	4.12
	No	50 (36.5)	87 (63.5)				
Enclosure condition and hygiene	Wet and dirty	59 (41.8)	82 (58.2)	1.799	5.548	**0.000 [b]**	17.24
	Moderately dry and clean	23 (18.5)	101 (81.5)				
Housing unit (feed store and feed trough) accessible to rodents and wild birds	Yes	64 (37.9)	105 (62.1)	1.450	4.809	**0.001 [b]**	10.99
	No	18 (18.8)	78 (81.3)				
Feeding practice	Floor	20 (16.4)	102 (83.6)	2.181	6.988	**0.000 [b]**	23.32
	Built-in trough	62 (43.4)	81 (56.6)				
Fed cattle on hay and napier grass	Yes	65 (41.9)	90 (58.1)	2.117	7.147	**0.000 [b]**	21.74
	No	17 (15.5)	93 (84.5)				
Fed cattle on chicken feed leftover and/or litter	Yes	15 (46.9)	17 (53.1)	1.032	4.628	**0.041[b]**	4.07
	No	67 (28.8)	166 (71.2)				
Histories of diarhoea, abortion and/or mastitis in the last 6 months	Yes	55 (36.9)	94 (63.1)	1.119	3.323	**0.018[b]**	5.77
	No	27 (23.3)	89 (76.7)				

[a]FAO's agroecological zones (ACZ) were used to categorize participating farms: ACZ III or semi-humid region (800-1400 mm annual rainfall) and ACZ V or semi-arid (450-900 mm annual rainfall; [b] Denotes independent variables considered significant ($P < 0.05$) at 95% confidence interval; χ2 = Chi-square; df = degrees of freedom

4.3.7 Univariate logistic regression models

A number of risk factor covariates were considered in unadjusted/crude (univariate logistic) models as tabulated in Table 4.3. Several risk factors were found to have significant association with thermophilic *Campylobacter* positivity in cattle in the unadjusted or univariate logistic regression model. The risk factors included: cattle breeds [crude odds ratio (COR)=3.8, 95%CI=2.1–7.1]; thermophilic *Campylobacter* positivity in chicken (presence chicken harbouring *Campylobacter* spp.) (COR=3.0, 95%CI=1.8–5.3); thermophilic *Campylobacter* positivity in water (cattle drinking water contaminated with *Campylobacter* spp.) (COR=0.6, 95%CI=0.3–1.0); brought new stock without isolation (COR=3.9, 95%CI=1.5–11.0); keeping small ruminants in the farm (COR=0.5, 95%CI=0.3-0.9); presence of donkeys (COR=0.4, 95%CI=0.2–0.8), presence of pigs (COR=3.2, 95%CI=1.4–7.1); keeping chicken and other birds irrespective of their *Campylobacter* status (COR=2.5, 95%=1.4–4.6); co-grazing cattle with other ruminants (COR=0.4, 95%CI=0.2–0.7); housing unit (feed store and feed trough) accessible to rodents and wild birds (COR=2.6, 95%CI=1.5–4.9); condition and hygiene status of the enclosure (COR=3.2, 95%CI=1.8–5.6); histories of diarrhoea, abortion and mastitis in the last 6 months (COR=1.9, 95%CI=1.1–3.4); and cattle fed on poultry litter and/or leftovers (locally known as “mchungo”) were about twice as much likely to be *Campylobacter* positive compared to those that didn’t (COR=2.2, 95%CI=1.0–4.6).

Table 4.3: Univariable logistic regression analysis of potential risk factors covariates for thermophilic *Campylobacter* positivity in cattle swabs from small-scale farms in Kajiado County

Variable	Modality	COR	95%CI (COR)	*P*-value
Geographical location of the farm in Kajiado County based on FAO's climate zones[a]	AEZ III (reference) (n=45)	–	–	–
	AEZ V (n=138)	0.6	0.3-1.1	0.0721
Thermophilic *Campylobacter* positivity in chicken (keeping chicken harbouring *Campylobacter* spp.)	No (ref) (n=121)	–	–	–
	Yes (n=62)	3.0	1.8-5.3	<0.0001[b]
Thermophilic *Campylobacter* positivity in water (cattle drinking water contaminated with *Campylobacter* spp.)	No (ref) (n=86)	–	–	–
	Yes (n=97)	0.6	0.3-1.0	0.0362[b]
Cattle breeds	Beef (ref) (n=95)	–	–	–
	Dairy (n=88)	3.8	2.1-7.1	<0.0001[b]
Brought new cattle stock in the last 6 months	No (ref) (n=176)	–	–	–
	Yes (n=7)	3.9	1.5-11.0	0.0069[b]
Presence of small ruminants in the farm	No (ref) (n=40)	-	-	-
	Yes (n=143)	0.5	0.3-0.9	0.0216[b]
Presence of companion animals in the farm	No (ref) (n=154)	–	–	–
	Yes (n=29)	1.1	0.5-2.2	0.8024
Presence of donkeys in the farm	No (ref) (n=111)	–	–	–
	Yes (n=72)	0.4	0.2-0.8	0.0064[b]
Presence of pigs in the farm	No (ref) (n=170)	-	-	-
	Yes (n=13)	3.2	1.4-7.1	0.0039[b]
Keeping chicken and other birds irrespective of their *Campylobacter* status	No (ref) (n=85)	–	–	–
	Yes (n=98)	2.5	1.4-4.6	0.0016[b]
Co-grazing cattle with other ruminants	No (ref) (n=63)	–	–	–
	Yes (n=120)	0.4	0.2-0.7	0.0011[b]
Feeding cattle on poultry litter and/or leftovers (locally known as "mchungo")	No (ref) (n=166)	–	–	–
	Yes (n=17)	2.2	1.0-4.6	0.0409[b]
Housing unit (feed store and feed trough) accessible to rodents and wild birds	No (ref) (n=78)	–	–	–
	Yes (n=105)	2.6	1.5-4.9	0.0015[b]
Condition and hygiene status of the enclosure	Moderately clean and dry (ref) (n=101)	–	–	–
	Wet and dirty (n=82)	3.2	1.8-5.6	<0.0001[b]
Histories of diarrhoea, abortion and mastitis in the last 6 months	No (ref) (n=89)	–	–	–
	Yes (n=94)	1.9	1.1-3.4	0.0180[b]

[a]FAO's agroecological zones (ACZ) were used to categorize participating farms: ACZ III or semi-humid region (800-1400 mm annual rainfall) and ACZ V or semi-arid (450-900 mm annual rainfall); [b] Independent variables considered significant ($P < 0.05$) at 95% confidence interval; **ref**= Reference group; **N**=265; **COR**= Crude odds ratio (ratio of the sides**)**; **95% CI (COR)**= Confidence interval for crude odds ratio to 95%

4.3.8 Multivariate logistic regression models

In the adjusted (multivariate logistic) model: thermophilic *Campylobacter* positivity in chicken (keeping chicken harbouring *Campylobacter* spp.); cattle breeds; presence of donkeys and presence of pigs in the farm; keeping chicken and other birds irrespective of their *Campylobacter* status; were significantly ($P<0.05$) found to be linked with *Campylobacter* positivity in cattle (Figure 4.5; Table 4.4).

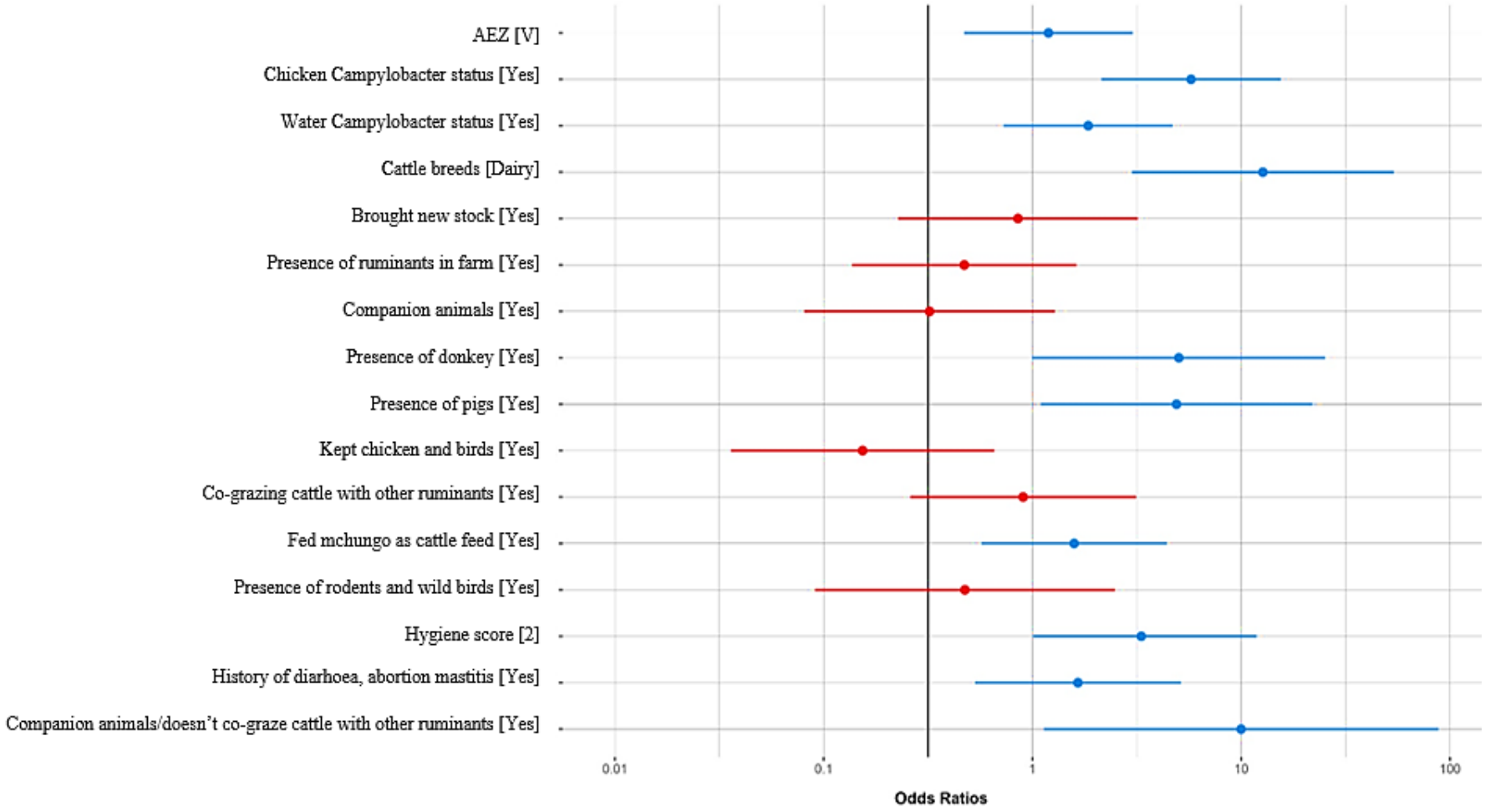


Figure 4.5: Adjusted model plot showing the final multivariable logistic model. For each category the variables, levels, odds ratios (OR) with 95% confidence interval (95% CI), are provided. Horizontal lines in blue and red colors depict the significant and insignificant associations respectively

Table 4.4: Multivariable logistic regression analysis of potential risk factors covariates for thermophilic *Campylobacter* positivity in cattle swabs from small-scale farms in Kajiado County

Variable	Modality	AOR	95%CI (AOR)	*P*-value
Geographical location of the farm in Kajiado County based on FAO's climate zones[a]	AEZ III (reference) (n=45)	–	–	–
	AEZ V (n=138)	1.2	0.5-3.1	0.7092
Thermophilic *Campylobacter* positivity in chicken (keeping chicken harbouring *Campylobacter* spp.)	No (ref) (n=121)	–	–	–
	Yes (n=62)	5.8	2.2-16.2	**0.0005[b]**
Thermophilic *Campylobacter* positivity in water (cattle drinking water contaminated with *Campylobacter* spp.)	No (ref) (n=86)	–	–	–
	Yes (n=97)	1.9	0.7-4.9	0.1959
Cattle breeds	Beef (ref) (n=95)	–	–	–
	Dairy (n=88)	12.7	3.2-60.0	**0.0006[b]**
Brought new cattle stock in the last 6 months	No (ref) (n=176)	–	–	–
	Yes (n=7)	0.9	0.2-3.3	0.8122
Presence of small ruminants in the farm	No (ref) (n=40)	-	-	-
	Yes (n=143)	0.5	0.1-1.6	0.233
Presence of companion animals in the farm	No (ref) (n=154)	–	–	–
	Yes (n=29)	0.3	0.1-1.2	0.107
Presence of donkeys in the farm	No (ref) (n=111)	–	–	–
	Yes (n=72)	5.0	1.1-27.4	**0.0498[b]**
Presence of pigs in the farm	No (ref) (n=170)	-	-	-
	Yes (n=13)	4.9	1.2-23.5	**0.0373[b]**
Keeping chicken and other birds irrespective of their *Campylobacter* status	No (ref) (n=85)	–	–	–
	Yes (n=98)	0.2	0.03-0.6	**0.0114[b]**
Co-grazing cattle with other ruminants	No (ref) (n=63)	–	–	–
	Yes (n=120)	0.9	0.3-3.2	0.8709
Feeding cattle on poultry litter and/or leftovers (locally known as "mchungo")	No (ref) (n=166)	–	–	–
	Yes (n=17)	1.6	0.6-4.5	0.3779
Housing unit (feed store and feed trough) accessible to rodents and wild birds	No (ref) (n=78)	–	–	–
	Yes (n=105)	0.5	0.1-2.5	0.3767
Condition and hygiene status of the enclosure	Moderately clean and dry (ref) (n=101)	–	–	–
	Wet and dirty (n=82)	3.3	**0.9-12.6**	**0.0638[c]**
Histories of diarhoea, abortion and mastitis in the last 6 months	No (ref) (n=89)	–	–	–
	Yes (n=94)	1.7	0.5-5.2	0.3867
Presence of companion animals in farms that did not co-graze cattle with other ruminants	No (ref) (n=241)			
	Yes (n=24)	10.0	1.2-95.9	**0.0379[b]**

[a]FAO's agroecological zones (ACZ) were used to categorise participating farms: ACZ III or semi-humid region (800-1400 mm annual rainfall) and ACZ V or semi-arid (450-900 mm annual rainfall); [b] Independent variables considered significant ($P < 0.05$) at 95% confidence interval**;** [c] Independent variables considered significant ($P < 0.1$) at 90% confidence interval; **ref**= Reference group; **N**=265; **AOR**= Adjusted odds ratio (ratio of the sides**); 95% CI (AOR)**: Confidence interval for adjusted odds ratio to 95%

Additionally, the wetness with dirt enclosure/cattle pens was significantly associated with *Campylobacter* positivity in cattle at $P<0.1$. Thermophilic *Campylobacter* positivity in chicken (presence of chicken harbouring *Campylobacter* spp.) increased the odds of thermophilic *Campylobacter* positivity in cattle by 5.8 times (95%CI=2.2–16.2). Keeping chicken and other birds irrespective of their *Campylobacter* status offered 80% protective effect to thermophilic *Campylobacter* positivity in cattle [adjusted odds ratio (AOR)=0.2, 95%CI=0.03–0.6]. Presence of multiple animals in the farm seemed to increase the odds of thermophilic *Campylobacter* positivity in cattle, with the highest exposure seen in farms stocking dairy cattle breeds (AOR= 12.7; CI: 3.2–60.0). The presence of donkeys (AOR=5, 95%CI: 1.1–27.4) and pigs (AOR=4.9, 95%CI: 1.2–23.5) were also individually associated with increased odds of acquiring thermophilic *Campylobacter* by 5 times, as compared to farms that neither kept donkeys nor pigs. The condition and hygiene status of the enclosure recorded a significant association at 10% level of significance; with 3.3 odds of exposure of outcome in the wet and dry category compared to the moderately dry category (95%CI=0.9–12.6).

There was a significant interaction between farms that kept companion animals and those that co-graze cattle with other ruminants; modifying the odds of acquisition of thermophilic *Campylobacter* in cattle by 10 times (95%CI=1.2–95.9). The wide confidence intervals can be attributed to the low sample size in this category.

4.4 Discussion

This study describes the unique characteristics of small-scale farms in Kajiado County while ascertaining significant associations between risk factors and thermophilic *Campylobacter* positivity in cattle. This study detected thermophilic *Campylobacter* species in over 72.7% (40/55) of the participating small-scale farms. However other studies in different geographical

regions have reported both higher and lower farm and/or herd level prevalence (Ellis-Iversen *et al.,* 2009b; Klein *et al*., 2013; Ocejo *et al*., 2019a; Hoque *et al*., 2021). Animal husbandry practices among other predisposing factors might increase the likelihood of *Campylobacter* shedding by either enhancing the risk of introduction or maintaining the existing *Campylobacter*. Maintenance of *Campylobacter* within a herd and/or a flock can be achieved by: lowered host's immunity, close contact to a contaminated source; increased faecal shedding or by heightened environmental endurance (Ellis-Iversen *et al.,* 2009b).

Although thermotolerant *Campylobacter* prevalence in livestock particularly poultry has been investigated frequently in previous studies, documentation on assessment of *Campylobacter* positivity and associated risk factors in integrated smallholder farms is scanty. Risk factors for the transmission and/or occurrence of campylobacter pathogens on smallholder farms may be diverse, especially if they only rear one livestock type, unlike in integrated farms with multiple animal species. This study investigated farm management practices and characteristics, animal-level and geo-environmental factors which might influence transmission pathways; without elaborating the precise biological mechanisms of *Campylobacter* involvement in cattle. As such, the explanations given thereafter are hypothetical and require further investigation. But even then, an understanding of probable risk factors enhancing occurrence and/or transmission of thermophilic campylobacters in farms, can be instrumental in designing practical control measures.

Cattle are also common reservoirs of campylobacters. The odds of thermophilic *Campylobacter* species positivity were 12.7 times higher with farms which kept dairy cattle over those that kept beef cattle. This finding is consistent with other studies conducted elsewhere (Ellis-Iversen *et al.,* 2009b; Thépault *et al*., 2018). The variation in thermophilic *Campylobacter* carriage in beef

and dairy cattle observed in this study may, however, reflect the husbandry practices and/or conditions of the animals rather than the type of breed. Nearly all the dairy cattle sampled were under zero-grazing (confinement) systems. An interaction between breed and sub-optimal housing conditions (dirty conditions) may increase faecal shedding of *Campylobacter* species.

The final multivariable model also included presence of (or rather coming into contact /co-graze with) other farm animals including: keeping chicken harbouring *Campylobacter* spp. (AOR=5.8, 95%CI=2.2-16.2, *P*-value = 0.0005); presence of donkeys (AOR=5.0, 95%CI=1.1-27.4, *P*-value = 0.0498); presence of pigs (AOR=4.9, 95%CI=1.2-23.5, *P*-value = 0.0373) were significantly associated with occurrence of thermophilic *Campylobacter* species in cattle. This agrees with other studies conducted elsewhere (Ellis-Iversen *et al.*, 2009b; Pires *et al*., 2019; Hoque *et al*., 2021; Patterson *et al.*, 2022). Domestic animals including pigs, chicken and donkey are known reservoirs of thermophilic campylobacters (Nguyen *et al*., 2016; Carron *et al*., 2018; Conrad *et al*., 2018). *Campylobacter* species shed in faeces from all the carriers/reservoirs may end up contaminating farm environment including pasture, soil and water sources. Consequently, this facilitates the introduction of *Campylobacter* into the farm. The effect of companion animals, that is dogs and cats, on *Campylobacter* positivity in cattle was amplified/modified by failure to co-graze cattle with small ruminants; increasing the AOR from 0.3, (95%CI=0.1-1.2, *P*-value = 0.107) to 10 (95%CI=1.2-95.9, *P*-value = 0.0379). Thermotolerant *Campylobacter* have been isolated from the gastrointestinal tracts of both healthy and diseased dogs and cats (Karama *et al*., 2019; Conrad *et al*., 2018; Thépault *et al*., 2020). Companion animals and particularly dog may scavenge offal's of already infected mammalian species from other farms; thereby introducing the organisms within the farm.

Ironically, there was inverse association between keeping chicken and other birds irrespective of their *Campylobacter* status and *Campylobacter* positivity in cattle (AOR=0.2, 95%CI=0.03-0.6, *P*-value = 0.0114); i.e., keeping chickens seemed to be protective against thermophilic *Campylobacter* positivity in cattle. The protective effect of keeping chicken and other birds may suggest the organism's ecological niche; *Campylobacter* thrives optimally at 42 ℃, similar to the normal body temperature of poultry and therefore, more likely ('preference') to colonize/inhabit the gut of birds over other animals, including cattle. The protective effect could also suggest protective immunity due to constant or frequent exposure. Nonetheless, there is need to investigate this biological 'protective' effect plausibly.

Farms where cattle were housed in persistently wet and dirty environmental conditions appeared to have a higher probability (AOR=3.3, 95%CI=0.9–12.6, *P- value* = 0.0638) to being positive for thermophilic *Campylobacter* colonization. However, the association between the two was not found to be significant at *P* = 0.05. This finding is supported by Hoque *et al*. (2021), who also reported that floor wetness would potentially increase the likelihood (AOR=2.0, 95%CI=0.1–56.3, *P*– value = 0.67) of thermophilic *Campylobacter* positivity in cattle. However, the finding was not intuitively easy to construe. A probable hypothesis is that this being a cross-sectional study, the observation (hygiene and condition of the establishment) reported was probably a one-time case and not a long-term one. As such, a much-controlled experimental study design or longitudinal studies are recommended.

In the current study, some of the potential risk factors were found to be insignificant (*P-value*>0.05) for farm-level thermophilic *Campylobacter* positivity in cattle in the multivariable logistic regression model. These included: geographical location of the participating farms; thermophilic *Campylobacter* positivity in cattle drinking water; history of diarrhoea, abortion

and mastitis in the last 6 months; accessibility of housing unit (feed store and feed trough) by rodents and wild birds; co-grazing cattle with other ruminants and/or presence of small ruminants in the farm, and bringing new cattle stock in the last 6 months without temporary isolation. The disparity in seasonal means of daily ambient temperatures, humidity and rainfall in different agro-ecological zones in Kajiado County were minimal; which possibly led to the insignificance of geographical distribution effect on the variation of thermophilic *Campylobacter* positivity in cattle herds. Presence of *Campylobacter* organisms in cattle drinking water from the respective farm is not an indication of colonization but rather a case of faecal contamination. Subsequently, the question would be: "how long the organisms can persist in water under the given environmental conditions to cause re-infection". Furthermore, if water troughs are cleaned on daily basis and source is potable; then trough water cannot be considered as a consistent reservoir. Therefore, water positivity status reflects the general level of cleanliness and contamination at sampling. Subsequently other transmission pathways in cattle are probably involved. Even though, *Campylobacter* are one of the aetiological agents for diarhoea, abortion and mastitis in cattle; the latter could be due to other causes which need to be investigated. Rodents and wild birds (Sanad *et al*., 2013; Battersby, 2015; Hald *et al*., 2015) are known *Campylobacter* carriers, and therefore carry the risk of contaminating cattle-environment as they scavenge for feed-left overs. Presence of potentially pathogenic bacteria is one of the hazards associated with feeding poultry litter to cattle. However, presence of rodents and wild birds in cattle environment and feeding poultry litter to cattle were found to be insignificant in this study. An explanation to this is probably low survival of *Campylobacter* in fecal material and secretions from wild birds and rodents.

4.5 Conclusions

- The findings of this study suggest that some farm characteristics, livestock husbandry practices and geo-environmental factors may be associated with occurrence and/or transmission of campylobacters
- Thermophilic *Campylobacter* organisms were detected in 72.7% of the surveyed cattle farms, suggesting that cattle may be reservoir for *Campylobacter*, which may be spread to or may have been acquired from other animals
- A number of independent variables were identified as potential risk factors including; stocking dairy breeds, presence of companion animals including cats and dogs, thermophilic *Campylobacter* positivity status in chicken (keeping chicken harbouring campylobacters), presence of donkey, pigs, and companion animals and low levels of hygiene in the farm. These factors need to be eliminated or minimized while designing on-farm mitigation strategy to minimize faecal shedding of *Campylobacter* in cattle

4.6 Recommendations

- More research to be carried-out on the disease so as to help further understand its transmission dynamics and biological interactions
- It is recommended that authorities pay more attention to campylobacteriosis, with respect to control and creation of awareness

CHAPTER FIVE: ANTIMICROBIAL USAGE, SUSCEPTIBILITY PROFILES AND RESISTANCE GENES IN *CAMPYLOBACTER* ISOLATED FROM WATER, CATTLE AND CHICKEN FAECAL SAMPLES IN KAJIADO COUNTY, KENYA

5.1 Introduction

The World Health Organization formulated the Global Action Plan on Antimicrobial resistance (AMR) as an urgency because of the snowballing danger posed to animal and human health. Countries all over the world have been urged to devise individual National Action Plans (NAPs) to assist in fighting AMR. Most of the national action plans deatail an outline of activities underway to combat AMR globally; however, developing countries have shown little progress in realizing their objectives (**Willemsen *et al.*, 2022**). Despite *Campylobacter* being one of the sentinel organisms targeted for AMR surveillance, there is little information on its: phenotypic antimicrobial susceptibility profiles; presence of antimicrobial resistance genes in strains emanating from food animals in Kenya. The few available studies are on humans, while the animal-based studies conducted in other regions in Kenya have only focused on resistance profiles displayed by chicken *Campylobacter* isolates (Chepkwony, 2016; Nguyen *et al*., 2016; Kariuki *et al*., 2020); without investigating the resistance situation in cattle and their respective environment. More-over, no previous studies have been conducted in Kajiado County on these aspects, with respect to thermotolerant *Campylobacter* species from food animals, despite the high dependency and/or consumption of animal protein in this county.

As a result of the widespread resistance to multiple antimicrobials, it is not surprising that the World Health Organization has enlisted priority pathogens including fluoroquinolone-resistant-*Campylobacter*; with the objective of more research and development of new antimicrobials

(WHO, 2017). In the wake of these glaring realities and scarce published data on antimicrobial use (AMU) and antimicrobial resistance (AMR) in Kenya: this study aimed at investigating antimicrobial use, susceptibility profiles and presence of resistance genes in *Campylobacter* isolates from chicken, cattle and respective water in Kajiado County, Kenya.

5.2 Materials and Methods

5.2.1 Study area, design and selection of production systems

A field and laboratory-based cross-sectional study design was conducted between October 2020 and May 2022 in Kajiado County, located South of Nairobi, Kenya. The county has well-established smallholder mixed-livestock (cattle and poultry) production systems. These production systems were chosen based on the fact that: (1) poultry production is the highest consumer of antimicrobials; (2) there is sketchy information on antimicrobial use in cattle production systems; (3) and resistance profile in environmental samples (water).

5.2.2 Bacterial isolates

All the 119 PCR-confirmed *Campylobacter* isolates: 29 *C. coli* (16 from cattle rectal swabs, 9 from chicken cloacal swabs and 4 from water samples), and 90 *C. jejuni* (42 from bovine, 42 from chicken and 6 from water) as described in **Chapter 3** were assayed for phenotypic antimicrobial susceptibility (using Kirby–Bauer disk-diffusion method) and detection of selected resistant genes by PCR and amplicon sequencing. These isolates were cryopreserved as pure colonies in Tryptone soya broth (Hi-media) with 30% glycerol in a deep-freezer at -20 °C. Genomic DNA extracted was extracted using the boiling method and preserved at -20 °C as described in **Chapter 3**, **sub-section 3.2.11.1**.

5.2.3 Survey on antimicrobial use and antimicrobial resistance awareness

Data on antimicrobial use was collected by requesting farm owners/respondents to avail any drugs or used drug containers/sachets kept at the house/farm; these were then recorded accordingly. In farms that indicated to have used antimicrobials but had disposed the container/sachet, the respondents were asked if they could recall the drugs used by its trade name. The survey also concentrated on: local disease histories, animal health-seeking behaviors and AMR awareness using a semi-structured questionnaire (Appendix 3).

5.2.4 Phenotypic antimicrobial susceptibility profiling using Kirby-Bauer diffusion method

The antimicrobial susceptibility of *C. jejuni* and *C. coli* isolates was established using the Kirby-Bauer disc diffusion technique on plates containing Mueller Hinton-agar augmented with 10% defibrinated ovine blood (MHBA), in strict conformity with the procedures of the Clinical and Laboratory Standards Institute (CLSI, 2015). Standard antimicrobial impregnated disks (Hi-media Mumbai, India) containing six different classes of antimicrobials at the given concentration were used. The drug discs included: (1) β-lactam [25 µg ampicillin (AMP)]; (2) aminoglycoside [10 µg gentamicin (GEN)]; (3) fluoroquinolone [5 µg ciprofloxacin (CIP)]; (4) quinolone [30 µg nalidixic acid (NA)]; (5) macrolide [15 µg erythromycin (E)]; and (6) tetracycline [30 µg tetracycline (TE)].

Polymerase chain reaction-confirmed *C. jejuni* and *C. coli* isolates from cryopreserved stocks in Tryptone soya broth (Hi-media) with 30% glycerol were defrosted and then revived by direct plating onto blood agar plates augmented with selective supplement (SR0167E, Oxoid®) and 10% lysed ovine blood. Then, the inoculated plates were incubated for 36 hours at 42 °C under microaerobic conditions. Out of 119 stored *Campylobacter* isolates; 103 [29 *C. coli* (16 isolates from cattle, 9 isolates from chicken and 4 isolates from water samples) and 74 *C. jejuni* (38

isolates from bovine, 30 isolates from chicken and 6 isolates from water)] were recovered. Sixteen (16) *C. jejuni* isolates (4 from bovine and 12 from chicken) could not be recovered from TSB-glycerol stocks.

Colonies of previously revived *Campylobacter* isolates were emulsified in physiological saline and then diluted to a turbidity equivalent to that of 0.5 McFarland standard. Fresh uninoculated MHBA plates were initially dried in an incubator at 35ºC with the lid removed for 15 minutes prior to inoculation. Sterile swabs were then used to seed the suspension onto MHBA plates; to produce confluent growth. The inoculum was allowed to dry for 5 minutes, then antimicrobial discs were placed on the plate. The seeded plates were incubated micro-aerobically overnight at 42 °C. The positive controls included *C. coli* (ATCC 33559) and *C. jejuni* (ATCC 33560).

The inhibition zone diameters around antimicrobial (ciprofloxacin, erythromycin and tetracycline) discs were measured, recorded and then construed as sensitive and/or resistant; following CLSI (2015) breakpoints guideline for infrequently isolated or fastidious organisms (M45) including *C. jejuni* and *C. coli*. Since CLSI's M45 (third edition) had no provisions for interpretive criterion for inhibition diameters for ampicillin, nalidixic acid and gentamicin for *C. jejuni* and *C. coli*, the breakpoints provided by CLSI (2016) (M100S) for the family *Enterobacteriaceae* were used instead.

5.2.5 Detection of genes conferring resistance to antimicrobials

Previously extracted DNA samples of 103 *Campylobacter* isolates were screened for five genes conferring antimicrobial resistance as follows: multi-drug efflux pump *cmeB* gene, aminoglycoside 3'-phosphotransferase *aph-3-1* gene, tetracycline resistance *tet*(O) gene, ampicillin ($bla_{\text{OXA-61}}$) gene and quinolone resistance topoisomerase gene (*gyrA*). The forward

and reverse primers specific for the antimicrobial resistance genes used in this study were designed based on the gene sequence in previously published studies (Pratt and Korolik, 2005; Obeng *et al*., 2012; Chatur *et al*., 2014) (Table 5.1).

The specificity of the primers was assayed by subjecting the sequences to basic nucleotide BLAST at NCBI (National centre for biotechnology information; https://www.ncbi.nlm.nih.gov).

Table 5.1: Primer sequences, amplicon sizes and annealing temperature for the *Campylobacter* specific oligonucleotides conferring antimicrobial resistance genes

Name of Primer	Primers Sequence (5'- 3')		Amplicon size (bp)	Annealing temperature	Reference
	Forward (F)	Reverse (R)			
***tet*(O)**	F-GGC GTT TTG TTT ATG TGC G	R-ATG GAC AAC CCG ACA GAA GC	559	54°C	Pratt and Korolik, (2005)
***Bla*$_{OXA-61}$**	F-AGAGTATAATACAAGCG	R-TAGTGAGTTGTCAAGCC	372	54°C	Obeng *et al*., (2012)
cmeB	F-TCCTAGCAGCACAATATG	R-AGCTTCGATAGCTGCATC	241	54°C	Obeng et al., (2012)
aphA-3-1	F-TGCGTAAAAGATACGGAAG	R-CAATCAGGCTTGATCCCC	701	54°C	Obeng *et al*., (2012)
gyraA	F-GAAGAATTTTATATGCTATG	R-TCAGTATAACGCATCGCAGC	235	53°C	Chatur *et al*., (2014)

The cryopreserved DNA was defrosted and then amplified in a final reaction volume of 25 µL in a BIO-RAD, T100™ Thermal Cycler (Singapore). The reaction mixture contained: 12.5 µl of OneTaq® 2x PCR Master Mix (New England Biolabs), 0.2 µl of each forward and reverse primer, 5 µl of template DNA and the rest topped up with nuclease free water (BioConcept). The primers used were synthesized and purchased from Inqaba Biotechnologies (Pretoria, South Africa).

Multiplex PCR (m-PCR) conditions for *tet*(O), *aph-3-1*, *cme*B, and *bla*OXA-61 consisted: an initial primary denaturation for 5 minutes at 94 °C, a further 39 cycles of secondary denaturation at 94 °C for 30 seconds, annealing at 54 °C for 45 seconds, extension at 72 °C for 1 minute, and

final extension at 72 °C for 10 minutes (Kashoma *et al*., 2016). The amplification conditions for the *gyrA* gene (a 235 bp product) were as follows: an initial primary denaturation at 95 °C for 5 minutes, 30 cycles at 95 °C for 50 seconds, annealing at 53 °C for 30 seconds and 72 °C for 1 minute, followed by a final extension at 72 °C for 7 minutes (Reddy and Zishiri, 2017).

DNAse/RNAse free water (BioConcept) was used as a negative control. Amplicons were resolved by electrophoresis on a 1.5% agarose gel stained with ethidium bromide in Tris-Borate-EDTA (TBE) buffer; run at 60 V for 60 minutes, and then visualised under ultraviolet (UV) light using the GelMax® 125 imager (UVP, Cambridge UK).

5.2.6 Deoxyribonucleic acid sequencing

A representative of positive amplicons (*two C. jejuni* and one *C. coli* for each antimicrobial resistance gene) generated with each primer was purified using QIAquick PCR Purification Kit (Qiagen) and commercially Sanger-sequenced in both directions at Inqaba Biotechnologies, Pretoria, South Africa. The forward and reverse sequences were edited, aligned and assembled in consensus sequences using BioEdit software. Nucleotide sequences were subjected to BLAST search tool (www.ncbi.nlm.nih.gov/BLAST), for confirmation of genes detected. Sequences were deposited in the GenBank.

5.2.7 Data handling and analysis

Data were analyzed with statistical software R version 3.6.1. The difference was significant when $p < 0.05$. Cohen's kappa coefficient was used to assess concordance between phenotypic antimicrobial susceptibility and genotypic detection of resistance genes. According to McHugh (2012), a Kappa value of: 0-0.2 indicates non-agreement, 0.21-0.39 indicates minimal level of agreement, 0.4-0.59 weak level of agreement, 0.60-0.79 moderate level of agreement, 0.80-0.90 strong level of agreement and above 0.90 almost perfect level of agreement. A kappa value of 1

(100%) indicates total concordance between the two antimicrobial susceptibility tests. Correlation between AMU and occurrence of resistance was determined by Pearson's correlation coefficient (r) method. Moreover, a 95% confidence interval was also determined for antibiotic resistance rates. All the analyses were considered statistically significant at $P < 0.05$.

5.3 Results

5.3.1 Animal health service seeking behavior and antimicrobial use among farmers in Kajiado County

When animals (cattle/chicken) were sick, majority of farmers (56.4%, 31/55) treated their animals themselves, 43.6% (24/55) sought services from a veterinarian or animal health assistant and/or community-based animal health workers. Those who self-treated their animals sought information on antimicrobial usage from other farmers and agro-vet shop owners. Seventy-five percent (41/55) of the farm owners interviewed were unaware/incognizant of the failing trend in antimicrobial therapy response.

Based on recall of antimicrobial usage in the last 6 months; 76.4% (42/55) of the farmers reported to have used antimicrobials mainly for treatment and prevention. Tetracyclines, aminoglycosides (streptomycin and gentamicin) and β-lactams based antimicrobials were the most commonly used antimicrobials to treat sick cattle and/or chicken (Table 5.2; Figure 5.1). Antimicrobial use was generally higher in chicken production systems (54.5% of sampled farms reported to have used antimicrobials in chicken) than in cattle for most of antimicrobials apart from aminoglycosides and β-Lactams (penicillins).

Table 5.2: Antimicrobials commonly used by farmers for treatment of sick chicken and cattle in Kajiado County, Kenya

Antibiotic class	Trade name (s)	Active ingredient (s)	Animal use	Proportions of farms using the drug
Tetracyclines	Aliseryl™	Oxytetracycline, erythromycin, streptomycin, colistin, vitamins	Chicken	39/42
	Tylodoxy 200™	Doxycline and tylosin	chicken	
	Vetoxy™	Oxytetracycline	chicken	
	Tetracolivit™	Oxytetracycline, colistin, vitamins	chicken	
	Ulticycline 10%™/Oxytetra 10%™/Oxymet-10/Adamycin 10%™/AlamycinLA 300™/Twigamycin™	Oxytetracycline	Cattle	
Aminoglycosides	Pen &Strep™/Penistrep™	Dihydrostreptomycin sulphate and procaine penicillin	Cattle	24/42
	Vetgenta™	Gentamicin	Cattle	
	Terrexine™	Kanamycin sulphate and Cephalexin	Cattle	
	Gentamast™	Gentamicin	Cattle	
	Aliseryl™	Streptomycin, oxytetracycline, erythromycin, colistin, vitamins	Chicken	
β-Lactams (Penicillins)	Bimoxyl LA™	Amoxicillin	Cattle	19/42
	Bovaclox™	Cloxacillin and ampicillin	Cattle	
	Pen &Strep™/Penistrep™	Procaine penicillin and dihydrostreptomycin sulphate	Cattle	
Macrolides	FosBac™/Fostyl plus ™	Tylosin sulfate and calcium fostomycin	Chicken	11/42
	Tylodoxy 200™	Tylosin tartrate and doxycline	Chicken	
	Marolan WS	Tylosin tartrate	Chicken	
	Tylosin injection	Tylosin tartrate	Cattle	
	Aliseryl™	Erythromycin, streptomycin, oxytetracycline, colistin, vitamins	Chicken	
Sulphonamides	BIOSOL™/TRIMOVET™	Trimethoprim-sulfamethoxazole	Chicken	9/42
	Esb3™	Sulphachloropyrazine	Chicken	
	Diseptoprim™	Trimethoprim-sulfamethoxazole	Cattle	
Polymyxins	Colistin sulphate	Colistin sulphate	Chicken	9/42
	Tetracolivit™	Oxytetracycline, colistin, vitamins	Chicken	
	Aliseryl™	Erythromycin, streptomycin, oxytetracycline, colistin, vitamins	Chicken	
Cephalosporins	Terrexine™	Kanamycin sulphate and Cephalexin	Cattle	3/42

Figure 5.1: Drugs commonly used in chicken and cattle production systems by small-scale farmers in Kajiado County, Kenya

The most commonly reported diseases in cattle 6 months prior to the study were: mastitis (21/55, 38.2%), foot and mouth disease (14/55, 25.5%), contagious bovine pleuropneumonia (12/55, 21.8%), East Coast Fever (11/55, 20%), anaplasmosis (6/55, 10.9%) and lumpy skin disease (2/55, 3.6%). Clinical syndromes such as diarrhoea and abortion were also common in 16/55 (29.1%) and 12/55 (21.8%) of the surveyed farms respectively. In poultry, most farms generally reported sick-bird syndromes such as ruffled feathers and/or drooping of wings, anorexia, diarrhoea, head tucked under wing, squinting or half-closed eyes and solemnness of unknown cause.

Some farmers (10/55, 18.2%) indicated using non-conventional medications such as herbs like, *Aloe vera*, leaves of *Tithonia diversifolia* (Figure 5.2) and chilli pepper among other "***miti shamba***" and/or "***dawa za kienyenji***" to relieve respiratory distress, diarrhoea and other related sick-bird-syndrome cases in chicken.

Figure 5.2: Herbal decoction from *Tithonia diversifolia* leaves used against sick bird syndromes in chicken production systems in Kajiado County

5.3.2 Antimicrobial susceptibility profiles of *Campylobacter jejuni* and *Campylobacter coli*

The test isolates showed varying degrees of inhibition zones to: ampicillin (AMP), tetracycline (TE), erythromycin (E), nalidixic acid (NA); gentamicin (GEN) and ciprofloxacin (CIP) on Mueller Hinton blood agar (MHBA) plates (Figure 5.3).

Figure 5.3: A representative photograph of antimicrobial susceptibility test for a *Campylobacter* isolate on Mueller Hinton blood agar culture plate

The diameters of inhibition zones were construed as either susceptible (S) or resistant (R) using the CLSI breakpoint criterion (Table 5.3).

Table 5.3: Guidelines adopted for interpreting antimicrobial susceptibility breakpoints for *Campylobacter* species

Antibiotic Class	**Antibiotic agent tested**	**Disc concentration**	**Inhibition zone diameter (mm) interpretation criteria**		**Reference**
			Susceptible	Resistance	
Macrolide	Erythromycin	15 µg	≥ 16	≤ 12	CLSI (2015)
Fluoroquinolone	Ciprofloxacin	5 µg	≥ 24	≤ 20	CLSI (2015)
Tetracycline	Tetracycline	30 µg	≥ 26	≤ 22	CLSI (2015)
β-Lactam	Ampicillin	25 µg	≥ 17	≤ 13	CLSI (2016)
Quinolone	Nalidixic acid	30 µg	≥ 19	≤ 13	CLSI (2016)
Aminoglycoside	Gentamicin	10 µg	≥ 15	≤ 12	CLSI (2016)

The findings of antimicrobial resistance phenotypes performed on the 29 *C. coli* and 74 *C. jejuni* isolates are tabulated in Table 5.4. Overall, all the 103 *Campylobacter* species were resistant to ampicillin (100%), followed by resistance to tetracycline (97.1%), erythromycin (75.7%), and to ciprofloxacin (63.1%). The least resistance was observed against gentamicin (11.7%).

Table 5.4: Phenotypic antimicrobial resistance profiles for *Campylobacter coli* and *Campylobacter jejuni* isolates

Antimicrobial agent	No. of resistant isolates (%)	Source and number of isolates showing resistance (%)							
		Campylobacter coli (N=29)				*Campylobacter jejuni* (N=74)			
		Bovine rectal swabs (n=16)	Chicken cloacal swabs (n=9)	Water samples (n=4)	Total (N=29)	Bovine rectal swabs (n=38)	Chicken cloacal swabs (n=30)	Water samples (n=6)	Total (N=74)
Ampicillin	103 (100)	16 (100)	9 (100)	4 (100)	29 (100)	38 (100)	30 (100)	6 (100)	6 (100)
Tetracycline	100 (97.1)	15 (93.8)	9 (100)	4 (100)	28 (96.6)	36 (94.7)	30 (**100**)	6 (100)	72 (97.3)
Gentamicin	12 (11.7)	1 (6.3)	2 (22.2)	0	3 (10.3)	6 (15.8)	3 (10)	0	9 (12.2)
Erythromycin	78 (75.7)	15 (93.8)	9 (100)	3 (75)	27 (93.1)	29 (76.3)	19 (63.3)	3 (50)	51 (68.9)
Ciprofloxacin	65 (63.1)	9 (56.3)	7(77.8)	4 (100)	20 (69)	16 (42.1)	25 (**83.3**)	4 (66.7)	45 (60.8)
Nalidixic acid	37 (35.9)	1 (6.3)	1 (11.1)	1 (25)	3 (10.3)	15 (39.5)	16 (53.3)	3 (50)	34 (45.9)

N = Total number of isolates in the given category; n = Proportion of resistant isolates in the given category (sub-total)

As for *C. coli*, all the isolates were resistant to ampicillin (100%), followed by resistance to tetracycline (96.6%), erythromycin (93.1%) and ciprofloxacin (69%); few strains were resistant to nalidixic acid and gentamicin (each at 10.3%). Tetracycline resistance in *C. coli* was seen more frequently in isolates from chicken and water samples (each at 100%). Similarly, *C. coli* resistance to ciprofloxacin was prevalent in isolates from water samples and chicken at 100% and 77.8%, respectively. The *C. coli* isolates from chicken and cattle swabs displayed the highest resistance to erythromycin at 100% and 93.8%, respectively. While no resistance to gentamicin was observed in any of the *Campylobacter* isolates from water samples, *C. coli* isolates from chicken recorded higher resistance to gentamicin at 22.2%.

Likewise, ampicillin resistance was the most prevalent in *C. jejuni*, at 100%; followed by resistance to tetracycline (97.3%), erythromycin (68.9%), ciprofloxacin (60.8%) and nalidixic

acid (45.9%); few strains were resistant to gentamicin (1.3%). *Campylobacter jejuni* isolates from chicken showed a high rate of resistance to tetracycline and ciprofloxacin at 100% and 83.3%, respectively. Conversely, *C. jejuni* from cattle were highly resistant to erythromycin (76.3%), followed by gentamicin (15.8%), whereas, those from water samples were 100% resistant to tetracycline, 66.7% resistant to ciprofloxacin and 50% resistant to nalidixic acid.

5.3.3 Multiple drug resistance in *Campylobacter coli and Campylobacter jejuni* isolates

Campylobacter isolates that were resistant to three or more classes of antibacterial agents were designated multidrug resistant (MDR). Ninety-nine of 103 (96.1%) isolates [29 (100) *C. coli* and 70 (94.6%) *C. jejuni*] displayed MDR. In addition, the highest MDR, irrespective of drug tested and/or *Campylobacter* species was found among isolates from chicken, at 100% (n = 39). Overall, a total of 14 different multiple drug resistance profiles were exhibited by the *Campylobacter* isolates from cattle, chicken and water samples (Table 5.5). The most frequent MDR combinations demonstrated by the isolates were: ampicillin-tetracycline-erythromycin-ciprofloxacin (AX-TE-E-CIP) (29.1%), ampicillin-tetracycline-nalidixic acid-ciprofloxacin (AX-TE-NA-CIP) (18.4%), and ampicillin-tetracycline-erythromycin (AX-TE-E) (16.5%).

Table 5.5: Multiple resistance patterns of *Campylobacter jejuni and Campylobacter coli* isolated from cattle, chicken and water samples

Antimicrobial resistance pattern	No. of resistant isolates (%)	Source and number of isolates showing resistance (%)							
		Campylobacter coli (N=29)				*Campylobacter jejuni* (N=74)			
		Cattle (n=16	Chicken (n=9)	Water samples (n=4)	Total	Cattle (n=38)	Chicken (n=30)	Water samples (n=6)	Total
AX-TE	2 (1.9)	0	0	0	0	2 (5.3)	0	0	2 (2.7)
AX-E	2 (1.9)	0	0	0	0	1 (2.6)	0	1 (16.7)	2 (2.7)
AX-TE-E	17 (16.5)	7 (43.8)	2 (22.2)	0	9 (31)	6 (15.8)	1 (3.3)	1 (16.7)	8 (10.8)
AX-TE-CIP	4 (3.9)	1 (6.3)	0	1 (25)	2 (6.9)	0	1 (3.3)	1 (16.7)	2 (2.7)
AX-E-CIP	1 (1)	1 (6.3)	0	0	1 (3.4)	0	0	0	0
AX-E-NA	1 (1)	0	0	0	0	1 (2.6)	0	0	1 (1.4)
AX-TE-E-NA	9 (8.7)	0	0	0	0	5(13.2)	4 (13.3)	0	9 (12.2)
AX-TE-E-CIP	30 (29.1)	5(31.3)	4 (44.4)	2 (50)	11 (37.9)	9(23.7)	10(33.3)	0	19 (25.7)
AX-TE-NA-CIP	19 (18.4)	0	0	0	0	7 (18.4)	10 (33.3)	2 (33.3)	19 (25.7)
AX-TE-E-GEN	3 (2.9)	0	0	0	0	3 (7.9)	0	0	3 (4.1)
AX-TE-E-NA-CIP	6 (5.8)	1 (6.3)	1 (11.1)	1 (25)	3 (10.3)	1 (2.6)	1 (3.3)	1 (16.7)	3 (4.1)
AX-TE-E-NA-GEN	1 (1)	0	0	0	0	1 (2.6)	0	0	1 (1.4)
AX-TE-E-GEN-CIP	7(6.8)	1(6.3)	2 (22.2)	0	3 (10.3)	2 (5.3)	2 (6.7)	0	4 (5.4)
AX-TE-E-NA-GEN-CIP	1 (1)	0	0	0	0	0	1 (3.3)	0	1 (1.4)

AMP = ampicillin; TE = tetracycline; E = erythromycin; NA = nalidixic acid; GEN = gentamicin; CIP = ciprofloxacin; N = Total number of isolates in the given category; n = Proportion of isolates in N in the given category (sub-total)

5.3.4 Correlation between usage of various antimicrobials and phenotypic resistance among the *Campylobacter* isolates

Pearson correlation demonstrated highly significant ($p < 0.01$) positive correlations between antimicrobial usage at farm level and phenotypic antimicrobial resistance profiles for various drugs investigated in this study (Table 5.6). The highest positive correlation was between tetracycline usage and its resistance at 31.4%. Beta-lactams and macrolide usage showed positive correlation against erythromycin resistance at 29.6% and 25.6%, respectively.

Table 5.6: Pearson correlation between antimicrobial use at farm level and occurrence of respective resistance

Antimicrobial usage at farm level	Comparison	Phenotypic resistance using the disk-diffusion method				
		TE	E	NA	GEN	CIP
Tetracycline usage	Pearson Correlation (R)	**.314****	.006	-.110	.131	-.085
	Sig. (2-tailed)	.001	.950	.270	.190	.397
Aminoglycoside usage	Pearson Correlation	-.053	.099	-.032	.150	-.033
	Sig. (2-tailed)	.613	.345	.759	.149	.75
Macrolide usage	Pearson Correlation	.022	**.256***	-.199	-.022	-.01
	Sig. (2-tailed)	.830	.013	.055	.831	.923
Beta-lactam usage	Pearson Correlation	-.106	**.296****	-.138	.054	-.163
	Sig. (2-tailed)	.309	.004	.186	.608	.117

Sig.: Significance; **: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed); TE: tetracycline; E: erythromycin; NA: nalidixic acid; GEN: gentamicin; CIP: ciprofloxacin

5.3.5 Detection of genes conferring resistance, and concordance between resistance phenotypes and genotypes

The occurrence of assayed genes conferring resistance to tetracyclines [*tet*(O)], β-lactams/ampicillin (bla_{OXA-61}), aminoglycoside 3'-phosphotransferase gene (*aph*-3-1), fluoroquinolones (*gyrA*) and multi-drug efflux pump (*cmeB*) were confirmed by PCR, by comparing respective amplicon size with a 100-bp DNA marker (Figure 5.4).

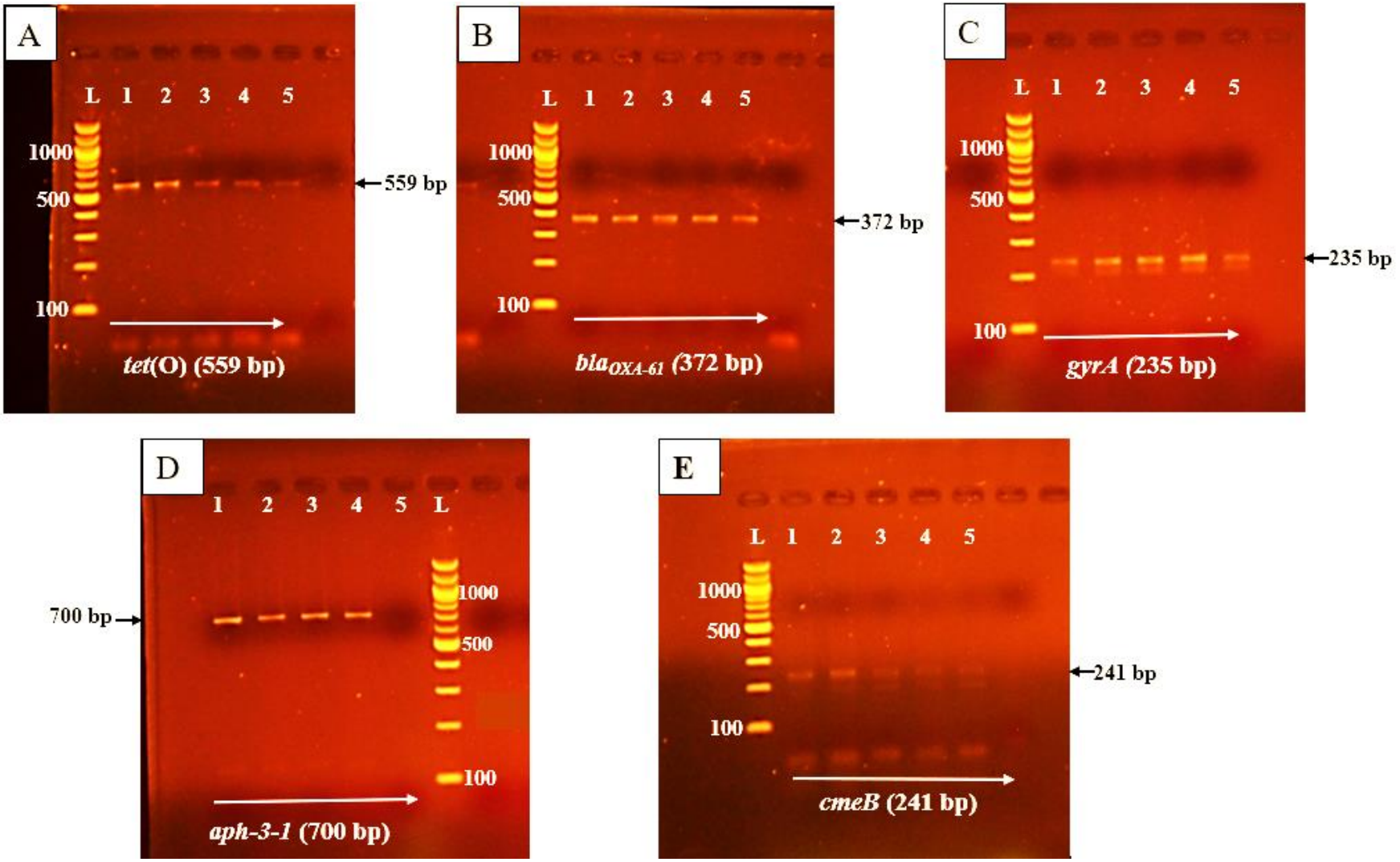


Figure 5.4: Exemplar of agarose gel electrophoresis of antimicrobial resistance genes: L: 100 bp ladder/marker; 559 base pair (bp) *tet*(O) (A); 372 bp bla_{OXA-61} (B); 235 bp *gyrA* (C); 700 bp *aph*-3-1 (D) and 241 bp *cmeB* (E)

Figure 5.5 illustrates the findings which indicate that *C. coli* isolates, as well as *C. jejuni* isolates, demonstrated more or less similar occurrence of antimicrobial resistance genes. Overall, *tet*(O), *gyrA*, *cmeB*, bla$_{OXA-61}$ and *aph*-3-1 genes were detected at 93.2%, 61.2%, 54.4%, 36.9% and 22.3% in all *Campylobacter* isolates, respectively, irrespective of source and *Campylobacter* species. The *tet*(O) (93.1% and 93.2%), *gyrA* (62.1% and 60.8%), *cmeB* (69% and 48.6%), bla$_{OXA-61}$ (44.8% and 33.8%) and *aph*-3-1 (17.2% and 24.3%) genes were detected in *C. coli* and *C. jejuni* isolates, respectively.

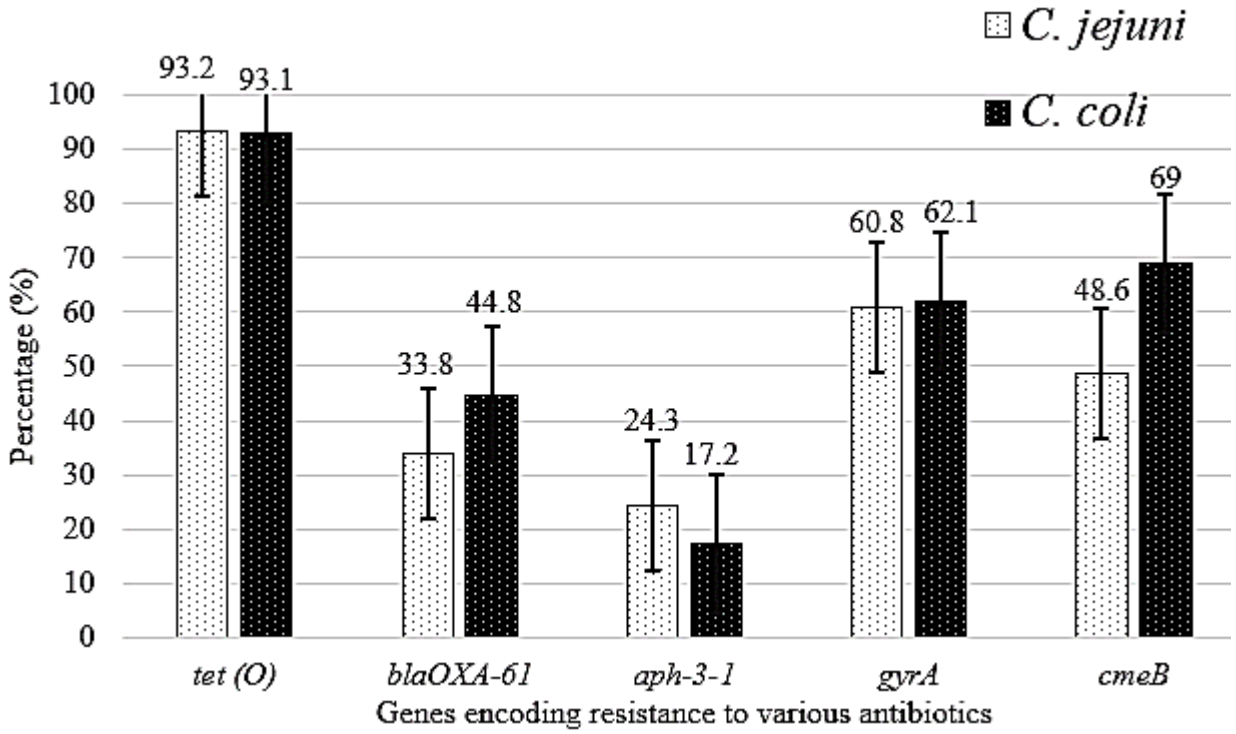


Figure 5.5: Percentage of *Campylobacter* isolates harbouring antimicrobial resistance genes

Figure 5.6 shows percentage distribution of *C. jejuni* isolates [cattle (n=38), chicken (n=30) and water samples (n=6)], *C. coli* isolates [cattle (n=16), chicken (n=9) and water samples (n=4)] that tested positive to each of the five antimicrobial resistance genes evaluated.

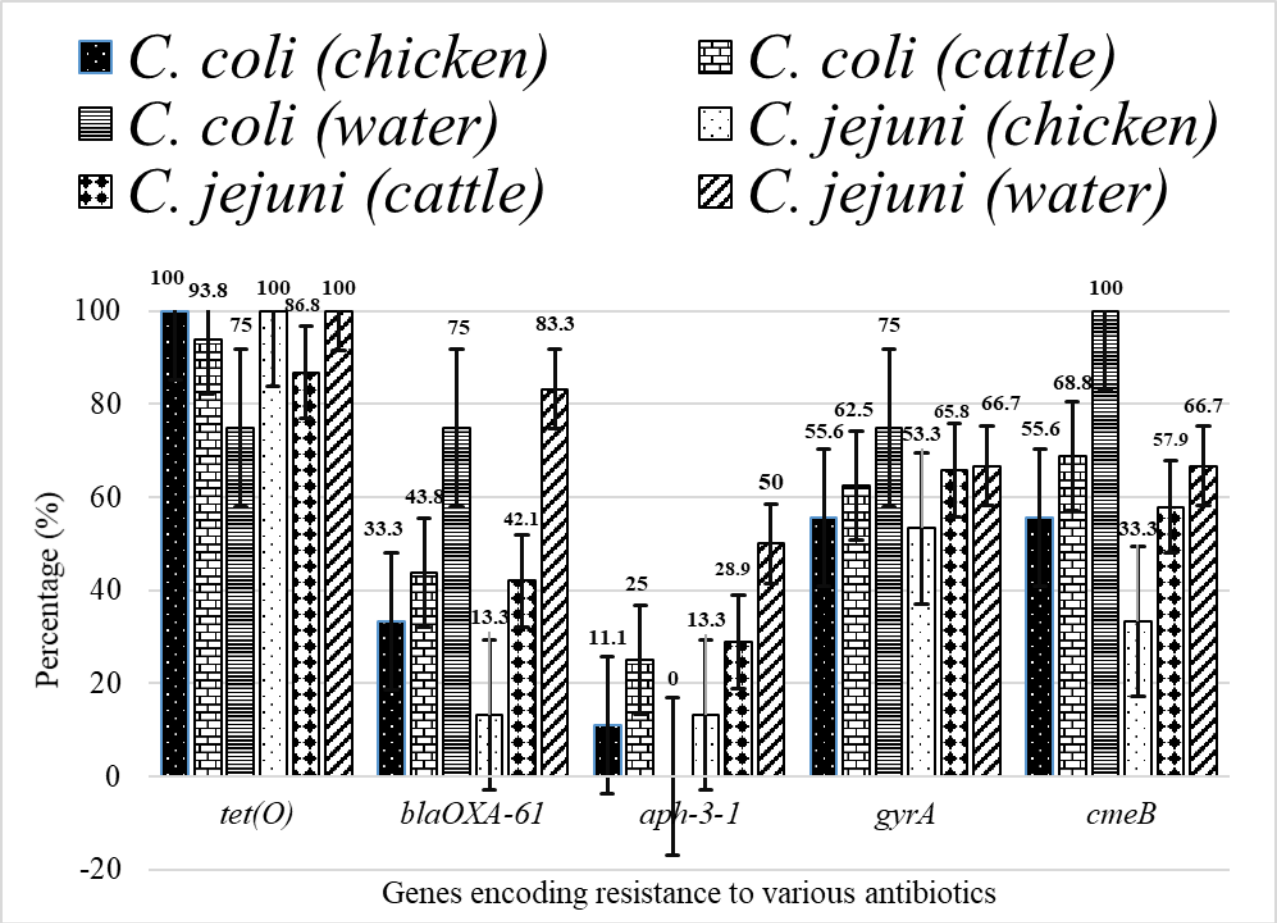


Figure 5.6: Proportion of *Campylobacter jejuni* and *Campylobacter coli* from cattle rectal swabs (n=54), chicken cloacal swabs (n=39) and water samples (n=10) that resulted positive to each of the four antimicrobial resistance genes tested

The *tet*(O) gene was detected in 100%, 93.8% and 75% of the *C. coli* isolates from the chicken, cattle and water samples, respectively. The β-lactams/ampicillin resistance gene (bla_{OXA-61}) was detected at 75%, 43.8% and 33.3% in C. *coli* isolates from water, cattle and chicken samples, respectively. Aminoglycoside 3'-phosphotransferase gene (*aph*-3-1) recorded lower detection; being detected in *C. coli* isolates from cattle (25%) and chicken (11.1%) only. Detection rates for *gyrA* were 75%, 62.5% and 55.6% among *C. coli* isolates from water, cattle and chicken samples, respectively. C. *coli* isolates from water, cattle and chicken samples were also found to harbour multi-drug efflux pump genes (*cmeB*) at 100%, 68.8% and 55.6%, respectively.

Nearly all the *C. jejuni* isolates from chicken (100%), water (100%) and cattle samples (86.8%) were found to harbour *tet*(O) genes. Beta-lactam resistance-conferring genes (bla_{OXA-61}) were present at 83.3%, 42.1% and 13.3% in *C. jejuni* isolates from water, cattle and chicken samples, respectively. The *aph*-3-1 gene was detected in *C. jejuni* at 50% of the water strains, 28.9% of the cattle strains and 13.3% of the chicken strains. The *gyrA* gene in *C. jejuni* was detected in 66.7% of water isolates, in 65.8% of cattle isolates and in 53.3% of the isolates from chicken. Sixty-six-point seven percent (66.7%), 57.9% and 33.3% of *C. jejuni* strains from water, cattle and chicken samples respectively, were found to possess *cmeB* genes.

5.3.6 Correlation between the two methods of testing for antimicrobial resistance in bacteria: phenotypic antimicrobial susceptibility testing (AST) and detection of resistance genes

The highest correlations were found between the tetracycline resistance gene [tet(O)] and tetracycline-resistant phenotypes for *C. coli* (96.4%) and *C. jejuni* (95.8%) (Table 5.7). The findings showed significant associations ($p<0.05$) among tetracycline, gentamicin and ciprofloxacin resistant phenotypes and their corresponding resistance genes for *C. jejuni* and *C. coli*. Interestingly, few nalidixic acid-resistant phenotypes harboured *gyrA* gene (27% *C. jejuni* and 3.4% *C. coli*), compared to ciprofloxacin-resistant phenotypes harbouring gyrA gene (48.3% *C. jejuni* and 41.9% *C. coli*).

In addition, using the Cohen's Kappa coefficient, a moderate level of concordance between Kirby-Bauer disk diffusion method (phenotypic assay) and PCR (genotypic assay) was observed for tetracycline in both *C. coli* (Kappa coefficient = 0.65) and *C. jejuni* (Kappa coefficient = 0.55), while non-agreement was noted for nalidixic acid in both *C. coli* (Kappa coefficient = 0.10) and *C. jejuni* (Kappa coefficient = -0.036) (Table 5.7).

Table 5.7: Correlations between phenotypic antimicrobial susceptibility testing and detection of resistance genes among *Campylobacter* isolates

Drug tested phenotypically using disc diffusion method	Antimicrobial resistance gene detected using PCR method	*Campylobacter* species	No. of isolates with resistant phenotype (%)	No. of isolates possessing resistance genes or mutations corresponding to resistance phenotype (%)	Correlation between genotypes and phenotype (%)	Measure of agreement between the methods
						Kappa value
Ampicillin	*bla*$_{OXA-61}$	*Campylobacter coli*	29 (100)	13 (44.8)	44.8	
		Campylobacter jejuni	74 (100)	25 (33.8)	33.8	
Tetracycline	*tet*(O)	*Campylobacter coli*	28 (96.6)	27 (93.1)	96.4	0.65*
		Campylobacter jejuni	72 (97.3)	69 (93.2)	95.8	0.55*
Gentamicin	*aph*-3-1	*Campylobacter coli*	3 (10.3)	2 (6.9)	66.7	0.43
		Campylobacter jejuni	9 (12.2)	8 (10.8)	88.9	0.51
Erythromycin	-	*Campylobacter coli*	27 (93.1)	-	-	-
		Campylobacter jejuni	51 (68.9)	-	-	-
Ciprofloxacin	*gyrA*	*Campylobacter coli*	20 (69)	14 (48.3)	70	0.24
		Campylobacter jejuni	45 (60.8)	31 (41.9)	68.9	0.21
Nalidixic acid		*Campylobacter coli*	3 (10.3)	1 (3.4)	33.3	-0.10
		Campylobacter jejuni	34 (45.9)	20 (27)	58.8	-0.04

No.: Number; PCR: polymerase chain reaction; *aph-3-1*: aminoglycoside 3'-phosphotransferase; *tet*(O): tetracycline resistance gene; *bla*$_{OXA-61}$: beta-lactamase (ampicillin resistance); *gyrA*: quinolone resistance topoisomerase gene

5.3.7 GenBank Accession Numbers

The partial sequences for some of the isolates from this study have been deposited in the GenBank database and assigned accession numbers: OQ389471 (*Campylobacter jejuni* strain 354B1), OQ389472 (*C. jejuni* strain 254B) and OQ389473 (*C. jejuni* strain 376B) for the *gyrA* gene; OQ390085 (*C. jejuni* Strain 376B) and OQ390086 (*C. coli* Strain 368C1) for *tet* (O) gene; OQ421183 (*Campylobacter jejuni* strain 342B1) and OQ421184 (*Campylobacter jejuni* strain 398B) for *bla*$_{OXA-61}$ gene. Consensus sequences obtained from *cmeB* and *aph-3-1* genes were too short with many gaps and as such were rejected on submission to GenBank (Appendix 4).

5.4 Discussion

The world is at the verge of tipping over due to the adverse effects of AMR; with the latter emerging and spreading at a rate that by far surpasses development of newer drugs. It is notable

that macrolide-fluoroquinolones-resistant bacterial pathogens, particularly *Campylobacter* spp, have dramatically increased (Wieczorek and Osek, 2013a). Fluoroquinolones and macrolides are prescribed as the first priority drugs for management of human campylobacteriosis, and as such increasing trends in resistance poses a public health hazard.

Campylobacter species are naturally resistant to β-lactam antimicrobials, including ampicillin (Kashoma *et al*., 2016). None of the *Campylobacter* isolates in this study were susceptible to ampicillin, translating to 100% resistance. Previous studies in other African countries including Tanzania and Morocco have reported resistance rate to this antimicrobial of 63% and 95.2%, respectively (Kashoma *et al*., 2016; Asmai *et al*., 2020). The high ampicillin-resistant phenotypes in this study might be due to the reported usage of β-lactams (including amoxicillin or a combination of procaine penicillin and dihydrostreptomycin sulphate or cloxacillin and ampicillin) among farmers in therapy of bacterial infections such as mastitis in cattle.

Tetracycline is relatively inexpensive and highly effective against a wide range of microorganism; thus, it has been frequently used in livestock husbandry practices (Chopra and Roberts, 2001). It is therefore not surprising that about 97% of the isolates (96.6% for *C. coli* and 97.3% for *C. jejuni*) in this study were resistant to tetracycline. The results found in this study are comparable to a study conducted recently in intensively managed commercial broiler production systems in various counties in Kenya including Kajiado County (Kariuki *et al*., 2020). Beyond Kenya, similar findings were reported in studies carried out in Spain (Lopez-Chavarrias *et al*., 2021), Tunisia (Gharbi *et al*., 2022), South Korea (Gahamanyi *et al*., 2021) and China (Han *et al*., 2016).

The results further demonstrated that the resistance rate among the *Campylobacter* isolates recovered from livestock and water samples to erythromycin was 75.7%, including 93.1% for *C. coli* and 68.9% for *C. jejuni*. Such resistance rate is somewhat worrying in contrast to preceding findings from the outskirts of Thika, a town in Central Kenya (Nguyen *et al*., 2016). The finding agrees with the study of Asmai *et al*. (2020) who also reported high phenotypic *Campylobacter* resistance rate of 92.8% to erythromycin. Going by the findings of this study, macrolide (erythromycin) would no longer be considered as an alternative therapy in systemic campylobacter infections in man.

Ciprofloxacin, a fluoroquinolone, is one of the first line antimicrobials in treatment of clinical campylobacteriosis in man. Notably were the significant 63.1% ciprofloxacin-resistant isolates (69% *C. coli* and 60.8% *C. jejuni*) compared to 35.9% nalidixic acid-resistant strains (10.3% *C. coli* and 45.9% *C. jejuni*) reported in this study. The observed resistance to ciprofloxacin is comparable to other studies in Kenya (Nguyen *et al*., 2016) and beyond [Ethiopia (Chala *et al*., 2021), Poland (Maćkiw *et al*., 2012)]. The relatively low resistance to nalidixic acid observed in this study is in contrast with what was reported on *Campylobacter* isolates from backyard chicken in Central Kenya, where resistance to nalidixic acid was observed at 77.4% (Nguyen *et al*., 2016). The level of resistance to nalidixic acid observed in this study, is however concordant with findings found in studies from other regions: Poland (Wieczorek *et al*., 2013); Tanzania (Kashoma *et al*., 2015); South Africa (Karama *et al*., 2020), and USA (Hailu *et al*., 2021). The low resistance to nalidixic acid may be as a result of a decrease in the use of quinolones including nalidixic acid, over most sought-after fluoroquinolones (such as ciprofloxacin) for curative or prophylactic purposes.

The overall resistance for gentamicin was low (11.7%); with *C. jejuni* isolates portraying slightly higher (12.2%) resistance compared to *C. coli* (10.3%). The findings concord with reports from other African and European states. For instance, in Tanzania 11.8% of the *Campylobacter* isolates from dressed beef carcasses and raw milk were resistant to gentamicin (Kashoma *et al*. 2016). In North African countries such as Morocco; 7.1% of the isolates from poultry were gentamycin-resistant (Asmai *et al*., 2020). Low resistance to gentamicin has also been observed in Spain; where 12.1% and 14.7% of *C. coli* strains from cattle and broilers were resistant (Lopez-Chavarrias *et al*., 2021). The relatively low resistance maybe associated with low restricted use for systemic infections (Elhadidy *et al*., 2020), and also owing to the fact that there are no oral formulations to be administered in drinking water or feeds for use in poultry.

However, the results of phenotypic and genotypic assays of resistance to various antimicrobials were partially concordant; moderate level of agreement being observed only in tetracycline. A similar observation was also reported by Kashoma *et al*. (2016). This shows that factors other than those tested for in this study could be involved.

The *tet*(O) gene is the most common ribosomal protection mechanism mediating *Campylobacter* resistance to tetracycline. However, other genes such as *tet*(A), *tet*(K), *tet*(B) and multi-drug efflux, have also been reported. Nearly all the tetracycline-resistant phenotypes were shown to harbour the gene *tet*(O) at 93.1%. This is higher in this study than the detected percentage of the same gene in chicken samples in a report by Nguyen *et al*. (2016); yet similar results to this study have been reported in China (Han *et al*., 2016).

The *gyrA* gene was confirmed in 61.2% of the isolates (62.1% *C. coli* and 60.8% *C. jejuni*) in this study. The substitution of threonine to isoleucine (Thr86Ile region) in the *gyrA* genome

confers cross-resistance to both quinolones (nalidixic acid) and fluoroquinolones (ciprofloxacin). However, Ge *et al*. (2005) reported upper-level resistance to ciprofloxacin linked to mutation in the Thr86Ile region of the *gyrA* genome. The results of this study, further revealed that presence of low nalidixic acid-resistant phenotypes possessing gyrA genome compared to the ciprofloxacin-resistant phenotypes possessing gyrA genome. The discrepancies in *gyrA* detection rate for ciprofloxacin and nalidixic acid resistance could further be explained by the fact that occurrence of point mutation in the Thr86Ala region of *gyrA* gene (by substitution of threonine to alanine) have been linked with high nalidixic acid-resistant and low ciprofloxacin-resistant *C. jejuni* (Ge *et al*., 2005). Indeed, further molecular studies need to be carried out to explore *gyrA* gene sequences and other antimicrobial resistance genes incriminated in *Campylobacter* spp resistance to nalidixic acid and ciprofloxacin.

The *cme*B gene, conferring resistance to multiple antimicrobials including macrolides (erythromycin), β-lactams (ampicillin), tetracyclines and fluoroquinolones (ciprofloxacin) was detected in over 54% of the isolates (69% *C. coli* and 48.6% *C. jejuni*). However, the findings of this study are much lower than those reported in previous studies in Tunisia (Gharbi *et al*., 2022). The discrepancy in these two studies may be attributed to; PCR protocols, primer specificity and type of production system (for the source sample).

Despite the high resistance to ampicillin reported in this study, β-lactam conferring gene (bla_{OXA-61}) was detected in only 36.9% of all *Campylobacter* isolates (44.8% in *C. coli* and 33.8% in *C. jejuni*), suggesting that other means of acquired ampicillin resistance could be involved. Comparable findings were reported by Kashoma *et al.* (2016), where 52.6% and 28.1% of *C. coli* and *C. jejuni* strains, respectively, were found to harbour bla_{OXA-61} gene. Undeniably, other genetic determinants including modifications in outer membrane porins and/or decreased affinity

of penicillin-binding protein (PBP) and efflux pump are most likely involved (Iovine 2013; Kashoma *et al*., 2016; De-Vries *et al*., 2018).

Over 22% of strains were found to possess the *aph*-3-1 gene. Obviously, gentamicin-resistant phenotypes cannot be elucidated by *aph-3-1* gene. However, this study's findings were much higher than previous reports in Africa (Kashoma *et al*., 2016); yet Hailu *et al*. (2021), reported 100% detection rate among *Campylobacter* isolates from dairy cattle and chicken manure in USA.

Multi-drug resistance (MDR) presents a public health threat by limiting the number of antibacterial agents to choose from for curative therapy. Nearly all the *Campylobacter* isolates (> 96%) in this study were resistant to three or more of the six tested antibacterial agents; with *C. coli* and *C. jejuni* posting 100% and 94.6% MDR, respectively. Drug combinations; Ampicillin-tetracycline-erythromycin-ciprofloxacin (AX-TE-E-CIP) and ampicillin-tetracycline-nalidixic acid-ciprofloxacin (AX-TE-NA-CIP) were the most common MDR patterns in both *C. coli* and *C. jejuni*. The MDR rate reported in this study is much higher than what has been reported in some European nations; for instance, in Poland, where MDR for *Campylobacter* isolates from raw chicken meat was 7% (Maćkiw *et al*., 2012). However, the findings of this study are concordant with some studies in other African countries: 95% of the *Campylobacter* isolates from broiler in Morocco displayed drug resistance to ≥3 drugs (Asmai *et al*., 2022); 95.5% of isolates from livestock (cattle and shoat), poultry, human and water in Ethiopia (Chala *et al*., 2021); 94.7% of the strains from poultry in Ghana (Karikari *et al*., 2021) and, 32.5% of *Campylobacter* isolates from beef cattle in South Africa (Karama *et al*., 2020). The observed discrepancies in MDR in *Campylobacter* may possibly be explained by: (1) level of intensification and type of production system, and (2) introduction and implementation of

legislation to minimize antimicrobial use in livestock in European countries. In underdeveloped nations including Kenya, there are legislations and rules in regard to antimicrobials use in food animals (Hosain *et al*., 2021); however, enforcement is done to a limited extent or practically non-existent. Consequently, higher resistances may be due to relatively unrestricted use of the same antimicrobial agents in animal treatment that is practiced in most of the developing countries (Cavaco and Aarestrup, 2013). Extensive use of antimicrobial drugs was observed in this study; with tetracyclines, aminoglycosides and β-lactams being the commonly used. Excessive use of these antimicrobials in livestock has also been reported in other studies (Omwenga *et al*., 2021; Makau *et al*., 2022). More-over, antimicrobial usage was positively correlated with the high level of resistance to tetracyclines and erythromycin among *Campylobacter* isolates in this study.

Extensive misuse of antimicrobials was observed in this study; where 56.4% of the farmers treated their animals themselves without prescription or advice from a qualified veterinarian. This finding agrees with what was reported by Chepkwony (2016); she reported that 67.5% of livestock owners admitted injecting drugs to their animals themselves without professional consultation. Even though the self-reported use of antimicrobials among farmers in this study precluded establishment of diagnosis and dosage regime; there is a possibility that they are often administered in absence of a confirmatory diagnosis. Moreover, lack of susceptibility testing may further compound the situation; where some antimicrobials end up being used in diseases caused by non-bacterial pathogens such as foot and mouth disease, lumpy skin disease or tick-borne diseases. Inadequate veterinary skills and accessibility is therefore of great concern and could accelerate antimicrobials overuse in livestock thus, they may be linked with evolution of MDR *Campylobacter* strains in the county.

Finally, where the use of fluoroquinolones among other antimicrobials in food production is banned, the frequency of campylobacter-resistant isolates is relatively low. This is exemplified in Australia, where administration of fluoroquinolones in food animals was prohibited; it recorded ciprofloxacin-susceptibility in *Campylobacter* organisms recovered from pigs in 2004 (Hart *et al*., 2004). However, years later, fluoroquinolone-resistant *Campylobacter* strains emerged and were detected among Australian chickens, even in the absence of fluoroquinolone application (Abraham *et al*., 2020). These fluoroquinolone-resistant *Campylobacter* isolates might have emerged from outside and brought into Australian chicken by people, vectors, or wild birds (Abraham *et al*., 2020). These findings dramatically underline the critical role of biosecurity in the overall fight against antimicrobial resistance. Consequently, even as nations call for a policy on minimizing application of antimicrobials in livestock; stringent farm biosecurity measures come handy in the overall fight against antimicrobial resistance.

5.5 Conclusions

- Extensive use of antimicrobial drugs was observed in this study; with tetracyclines, aminoglycosides and β-lactams being commonly used; with 54.5% of the farms generally reporting using antimicrobials in chicken production systems than in cattle
- Application of these antimicrobials in cattle and chicken production systems was positively correlated with the high level of resistance to tetracyclines and erythromycin. This highlights the significance of warranted application of antibacterial agents in the said production systems in the county
- With regard to antimicrobial resistance, nearly all the isolates (96.1%) displayed MDR, *C. coli* expressed greater resistance to three or more of the antimicrobials assayed. This might further limit treatment options for *Campylobacter* infections

- A high level of resistance to ampicillin, tetracycline, erythromycin and ciprofloxacin was found among the *Campylobacter* strains. As such, none of priority drugs in *Campylobacter* infections therapy can be prescribed with certainty in the county
- Chicken derived *Campylobacter* strains showed greater resistance; this could be due to widespread use of antimicrobials in the poultry production system compared to cattle production systems
- The *tet*(O), *gyrA* and *cmeB* were the most frequently detected genes, while the occurrence of $bla_{OXA\text{-}61}$ and *aph*-3-1 was significantly lower ($p < 0.05$)

5.6 Recommendations

- Further molecular studies should include all the cryptic antimicrobial resistance genes and plasmids in *C. jejuni* and *C. coli* strains to give insights on their transmission and possible transfer to other *Campylobacter* strains
- The existing national action plan on AMR spearheaded by the ministries of health and agriculture, livestock and fisheries in Kenya must strengthen the surveillance programs and policies advocating for a reduction in unwarranted use of antimicrobials
- More-over, the veterinary directorate at the county and national governments ought to be on the fore-front in managing and implementing appropriate biosecurity measures aimed at fighting antimicrobial resistance
- Screening of alternative treatment, e.g. use of medicinal plant extracts (*Aloe vera*, *Tithonia diversifolia* and chilli pepper) need to be encouraged, in effort to reduce usage of antimicrobials

- Other alternative treatments such as phages, and vaccination should be advocated-for so as to minimize antimicrobial resistance, improve food quality, and minimize negative impacts to humans and environment.

CHAPTER SIX: VIRULENCE FACTORS AND GENETIC RELATEDNESS OF *CAMPYLOBACTER* SPECIES ISOLATED FROM WATER, CATTLE AND CHICKEN FAECAL SAMPLES

6.1 Introduction

Campylobacters emanating from different sources including livestock and their environment may be transferred to humans causing gastroenteritis among other clinical syndromes. However, the precise role of campylobacters in causation of all these clinical syndromes is not clear and further research is therefore required (Kaakoush *et al*., 2015). The pathogenesis of campylobacteriosis is multifaceted and still not well comprehended (Wieczorek *et al*., 2019). Therefore, it is imperative to trace the source of these infections and also establish whether *Campylobacter* strains recovered from the said sources possess virulence properties. Furthermore, virutyping of *Campylobacter* isolates could be an important step in elaborating pathogenesis of associated infections in humans. Therefore, surveillance of virulence determinants in *Campylobacter* is highly applicable to public health. Additionally, lack of genetic information on *Campylobacter* strains emanating from livestock and environmental sources in Kajiado and Kenya at large warrants the need to screen their genotypes and probable phyletic-relationships. Against this background, the objective of this study was to establish the occurrence of virulence genes responsible for flagellin A protein (*flaA*), *Campylobacter* adhesion to fibronectin F (*cadF*), *Campylobacter* invasion antigen B (*ciaB*) and cytolethal distending toxin A (*cdtA*) in *Campylobacter* species isolated from chicken, cattle and water samples obtained in Kajiado County, Kenya, as described in Chapter 3; subsection 3.2.1. The study also assessed the genetic relatedness among these isolates.

6.2 Materials and Methods

6.2.1 Study design and origin of *Campylobacter* isolates

Campylobacter isolates used in virulotyping and phylogenetic assays were obtained from a cross-sectional study on seasonal prevalence on thermophilic campylobacters in cattle, chicken and water samples conducted in Kajiado County (South-western Kenya) between October 2020 and May 2022, as described in Chapter three. The study utilized cryopreserved genomic DNA of respective *Campylobacter* isolates.

6.2.2 Molecular detection of virulence genes

A total of 103 PCR-confirmed *Campylobacter* species including: 29 *C. coli* (16 isolates from cattle rectal swabs, 9 isolates from chicken cloacal swabs and 4 isolates from cattle-trough water samples) and 74 *C. jejuni* (38 isolates from cattle, 30 isolates from chicken and 6 isolates from cattle-trough water samples) were subjected to virutyping assays.

Polymerase chain reaction was used to screen *flaA*, *ciaB*, *cadF* and *cdtA* genes that are associated with virulence in the genomic DNA of *Campylobacter* isolates. The oligonucleotide primers were devised based on gene sequence data from previous published reports (Table 6.1). The primers used were sourced from Inqaba Biotechnologies (Pretoria, South Africa). The primer sequences were blasted against National Centre for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov) to assess their specificity.

Table 6.1: Primers used for virulence genes typing in this study

Virulent gene	Primer	Primers Sequence (5'- 3')	Amplicon size (bp)	Annealing Temperature	Reference
cadF	cadF-R	R-TTG AAG GTA ATT TAG ATA TG	400	48 °C	Konkel *et al*. (1999)
	cadF-F	F-CTA ATA CCT AAA GTT GAA AC			
cdtA	cdtA-F	F-CCT TGT GAT GCA AGC AAT C	370	57°C	Hickey et al., 2000
	cdtA-R	R-ACA CTC CAT TTG CTT TCT G			
flaA	flaA-F	F-AAT AAA AAT GCT GAT AAA ACA GGT G	855	57 °C	Datta et al., 2003
	flaA-R	R-TAC CGA ACC AAT GTC TGC TCT GAT T			
ciaB	ciaB-F	F-TGC GAG ATT TTT CGA GAA TG	527	57 °C	Zheng et al. (2006)
	ciaB-R	R-TGC CCG CCT TAG AAC TTA CA			

bp: base pair; R: Reverse primer; F: Forward primer

The cryopreserved genomic DNA samples were thawed and then amplified using primer specific for each of the virulence markers in a BIO-RAD, T100™ Thermal Cycler (Singapore). The final PCR mix of 25 µL reaction volume consisted of; 12.5 µl of OneTaq® 2x PCR Master Mix (New England Biolabs), 0.2 µl of each forward and reverse primer, 5 µl of template DNA, and 7.1 µl of nuclease free water. Optimized thermal cycler program entailed: initial denaturation at 95 °C for 5 minutes; further denaturation at 94 °C for 1 minute for 30 cycles; annealing at specific temperature for each primer; extension at 72 °C for 1 minute and terminal extension at 72 °C for 5 minutes.

PCR amplicons were separated by electrophoresis on a 1.5% agarose gel stained with ethidium bromide in 1X Tris-Borate-EDTA (TBE) buffer, and then visualised under ultraviolet light using gel document system (GelMax® 125 UVP imager, Cambridge UK). The size of the PCR amplicons was compared to that of the 100 bp DNA ladder.

6.2.3 Virulence encoding amplicon sequencing

A representative of positive amplicons encoding virulence genes generated with each primer was purified using QIAquick PCR Purification Kit (Qiagen) and then shipped to Inqaba Biotechnologies laboratories, Pretoria, South Africa for sequencing, using forward and reverse primers. The forward and reverse sequences were edited, aligned and assembled into a

complementary sequence using BioEdit software. Nucleotide sequences were subjected to BLASTn search tool (www.ncbi.nlm.nih.gov/BLASTn), for confirmation of genes detected. They were then submitted to GenBank for accession.

6.2.4 Amplification and sequencing of 16S rRNA gene, *hipO* gene for *Campylobacter jejuni* and *ceuE* gene for *Campylobacter coli* in isolates from Kajiado County

A total of 36 PCR-positive isolates based on the three primers (20 isolates based on 16S rRNA genus *Campylobacter*-specific primer and 8 isolates for each of the *ceuE* primer for *C. coli* and *hipO* primer for *C. jejuni*) were selected for the phylogenetic assay. To avoid selection bias, *Campylobacter* strains were selected on the basis of isolation source: sample type, season, farm and herd/flock.

Singleplex amplification of the 16S rRNA gene (genus), *hipO* gene (*C. jejuni*) and *ceuE* gene (*C. coli*) were done as described in Chapter 3 using: 16SrRNA-F and 16SrRNA-R primers (Denis *et al*., 1999), *hipO*-F and *hipO*-R primers (Han *et al*., 2016) and ceuE-F and ceuE-R primers (Denis *et al*., 1999), respectively. Thermocycler programming conditions were as described by Han *et al*. (2016).

After amplification of the 16S rRNA, *ceuE* and *hipO* genes, the positive PCR amplicons were purified using QIAquick PCR Purification Kit, following manufacturer's instructions (Qiagen). The purified PCR products were shipped to Inqaba Biotechnologies laboratories (Pretoria, South Africa) for forward and reverse primer sequencing through standard Sanger's sequencing method and further subjected to phylogenetic characterization.

6.2.5 Data handling and analysis

Data on occurrence of virulence encoding gene was entered and stored in Microsoft excel and then validated prior to descriptive and inferential statistical analyses on EPI INFO software (https://www.cdc.gov/epiinfo/). Pearson's correlation coefficient (R) was used to establish the associations between specific virulence-associated genes in order to find out whether presence of one virulence gene was interconnected with the presence of the other. Fisher's exact test and chi-square test were used to test significance of whether the presence of virulence genes detected was influenced by the source of isolates (cattle, chickens, and water). Statistical significance was measured at *P* value less than 0.05.

6.2.6 Bioinformatics analyses of the sequences

The forward and reverse sequences were edited, aligned and assembled in consensus sequences using BioEdit software. The sequences were blasted against BLASTn database (www.ncbi.nlm.nih.gov/BLASTn), for homologous search and comparison with highly similar *Campylobacter* sequences in GenBank. The sequences were then deposited in GenBank, and accession numbers were obtained against each sequence. Phylogenetic characterization for 16S rRNA, *ceuE* and *hipO* gene sequences was carried out using molecular evolutionary genetic analysis version 11 (MEGA11) to ascertain the homogeneity among cattle, chicken and water isolates.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. All positions with less than 90% site coverage were

eliminated. That is, fewer than 10% alignment gaps, missing data, and ambiguous bases were allowed at any position.

6.3 Results

6.3.1 Detection of virulent genes among *Campylobacter* isolates

Four virulence genes were detected in this study are shown in Figure 6.1 in relation to a 100-bp DNA marker as follows; 370 bp *cdtA* gene (Fig 6.1 A), 400 bp *cadF* (Fig 6.1 B); 527 bp *ciaB* (Fig 6.1 C) and 855bp *flaA* (Fig 6.1 D).

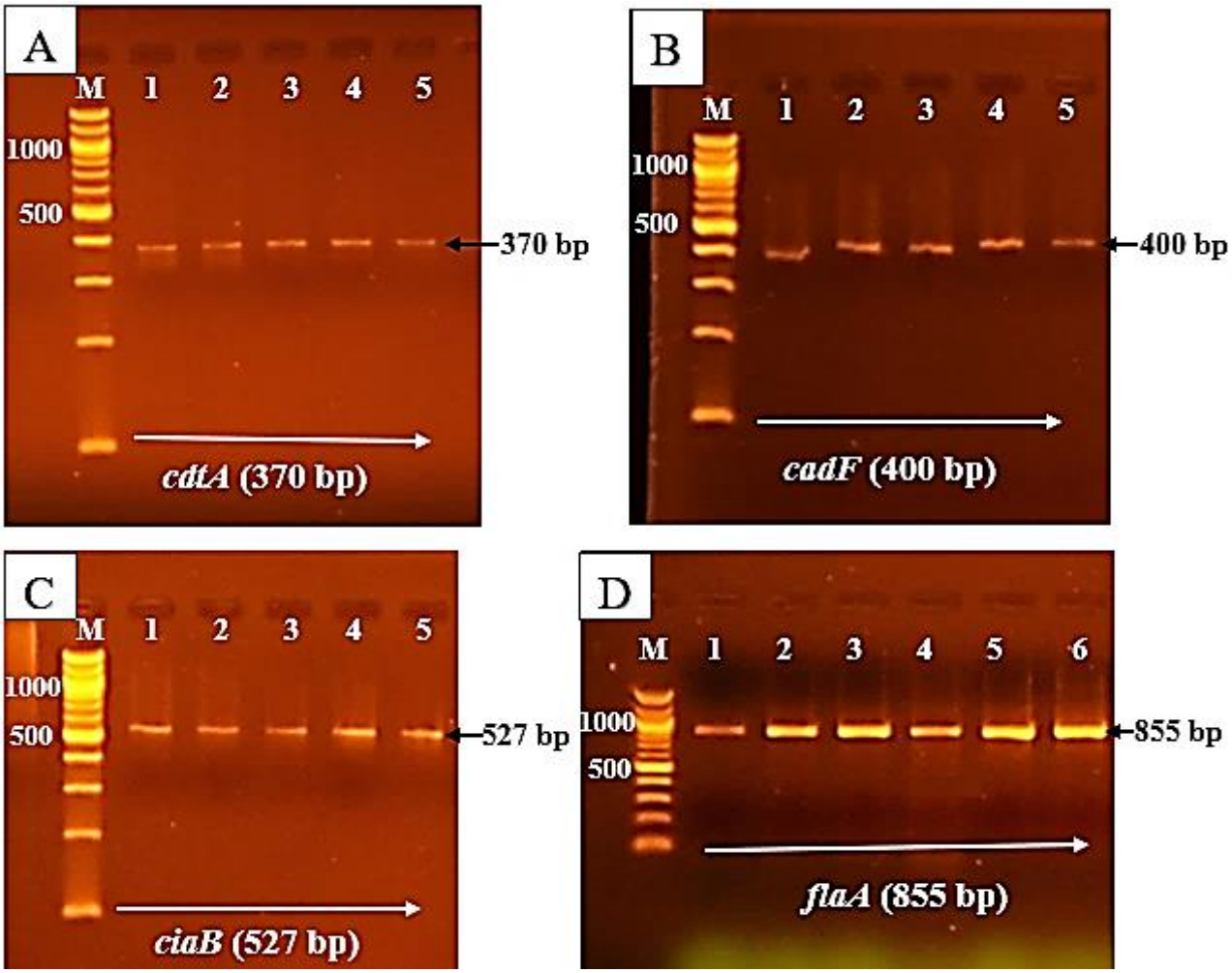


Figure 6.1: Agarose gel electrophoresis of amplicons for virulence-associated genes investigated in *Campylobacter* isolates. 100-bp marker (Lane M); 370 bp *cdtA* (A), 400 bp *cadF* (B); 527 bp *ciaB* (C) and 855bp *flaA* (D)

The frequency of detection of virulence-encoding genes among *Campylobacter* isolates irrespective of the source/sample type in this study are presented in Figure 6.2. The results show that both *C. jejuni* and *C. coli* are equally responsible for most of infections (no statistical difference in frequencies of virulence genes; P>0.05). The *ciaB* gene was the most detected virulence gene in both *C. jejuni* at 81.1% (60/74) and *C. coli* at 62.1% (18/29). The frequency of detection of *flaA* gene in *C. jejuni* and in *C. coli* isolates was at 62.2% (46/74) and 62.1% (18/29), respectively. A higher rate of *cadF* gene was found in *C. jejuni* isolates at 51.4% (38/74), as compared with *C. coli* at 44.8% (13/29). On the other hand, the *cdtA* gene was detected in only 27.6% (8/29) *C. coli* and in 43.2% (32/74) *C. jejuni* isolates.

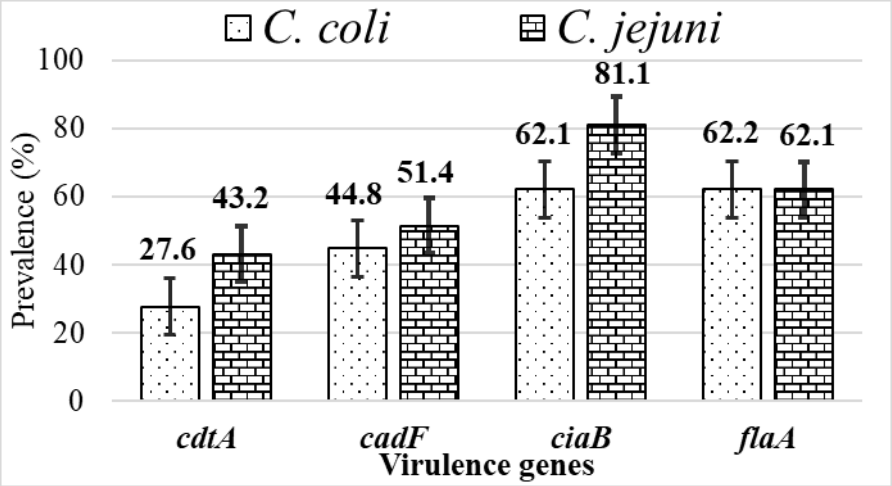


Figure 6.2: **Percentage occurrence of virulence-encoding genes in *Campylobacter coli* and *Campylobacter jejuni* isolates from all the sample types. Data are the percentage prevalence ± standard error [*Campylobacter coli* (N = 29), *C. jejuni* (N =74)]**

The proportions of *Campylobacter* isolates that were positive for each virulence gene in different samples are represented in Figure 6.3. There were statistically significant differences (*P<0.05*) observed between the prevalence of virulence-encoding genes found in cattle, chicken and water. *Campylobacter* isolates from chicken harboured the most virulence encoding genes.

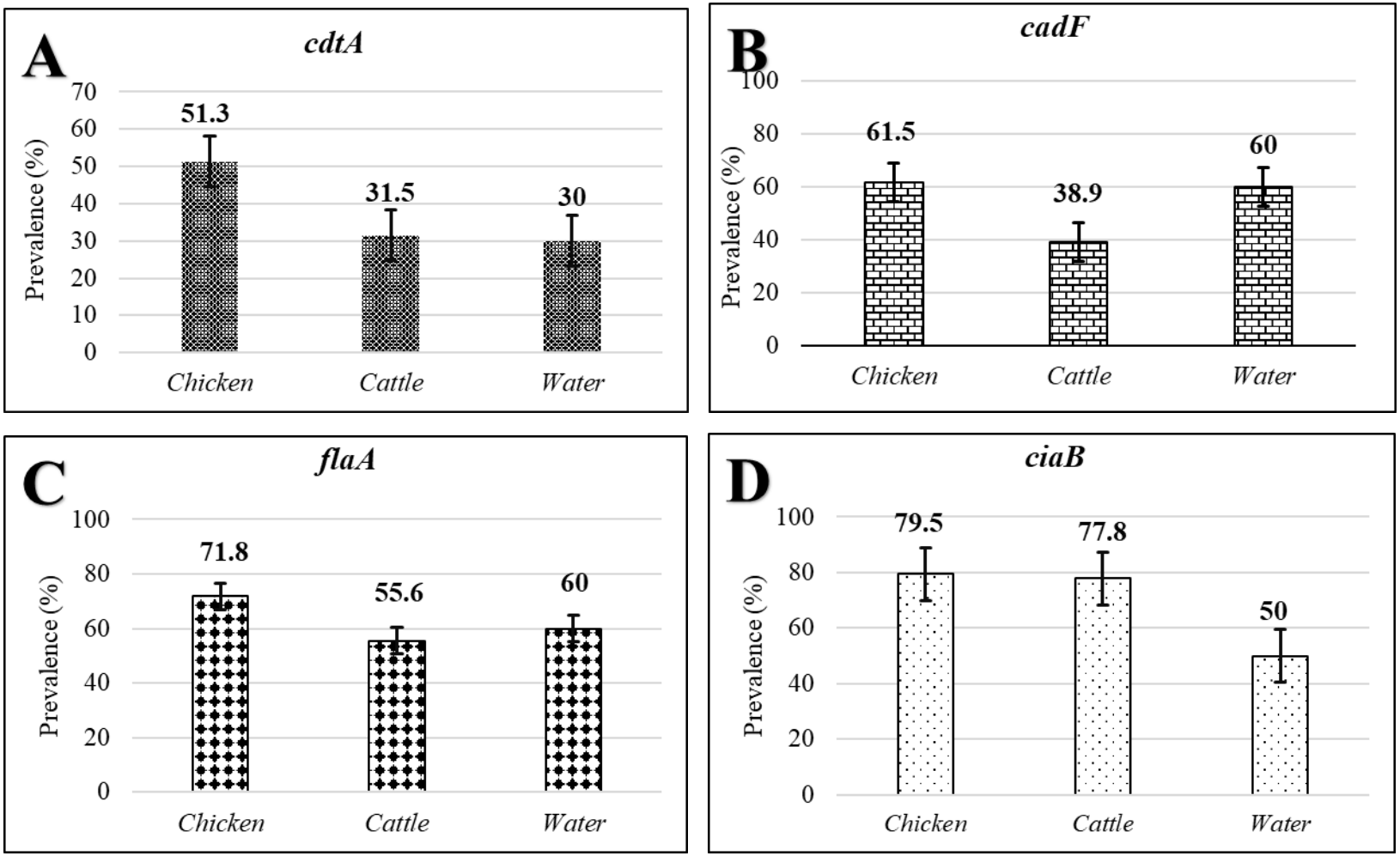


Figure 6.3: Percentage prevalence of virulence-encoding genes [*cdtA* (A), *cadF* (B), *flaA* (C) and *ciaB* genes (D)] in *Campylobacter* isolates from cattle, chicken and water samples. Data are the percentage prevalence ± standard error [chicken isolates (N = 51), cattle isolates (N= 58) and water isolates (N = 10)]

The results further showed variability in prevalence of virulence gene profiles among *Campylobacter* species from different sources (Figure 6.4). Highest detection rate of *cdtA* was encountered among *Campylobacter jejuni* isolates from chicken at 56.7% (17/30) as compared to *C. jejuni* isolates from cattle at 34.2% (13/38) and from water at 33.3% (2/6). Similarly, for *C. coli* isolates from chicken harboured *cdtA* at 33.3% (3/9), from cattle at 25% (4/16) and from water at 25% (1/4) as shown in Figure 6.4A. All the four isolates of *C. coli* isolates from water (100%, 4/4) harboured *cadF* gene; compared to *C. coli* isolates from chicken faecal samples (4/9, 44.4%) and cattle faecal samples (5/16, 31.3%), and *C. jejuni* isolates [66.7% (20/30) chicken, 42.1% (16/38) cattle and 33.3% (2/6) water, see Figure 6.4B. Water-derived *C. coli* and chicken-derived *C. jejuni* showed higher frequency of *flaA* (100% (4/4) and 76.7% (23/30), respectively), as compared to *C. coli* isolates from cattle at 56.3% (9/16) and chicken at 55.6% (5/9) and *C. jejuni* strains from cattle at 55.3% (21/38) and water at 33.3% (2/6), see Figure 6.4C. Furthermore, the detection rate for *ciaB* gene among isolates from water, chicken and cattle was 75% (3/4), 66.7% (6/9) and 56.3% (9/16), and 33.3% (2/6), 83.3% (25/30) and 86.8% (33/38) for *C. coli* and *C. jejuni*, respectively (Figure 6.4D).

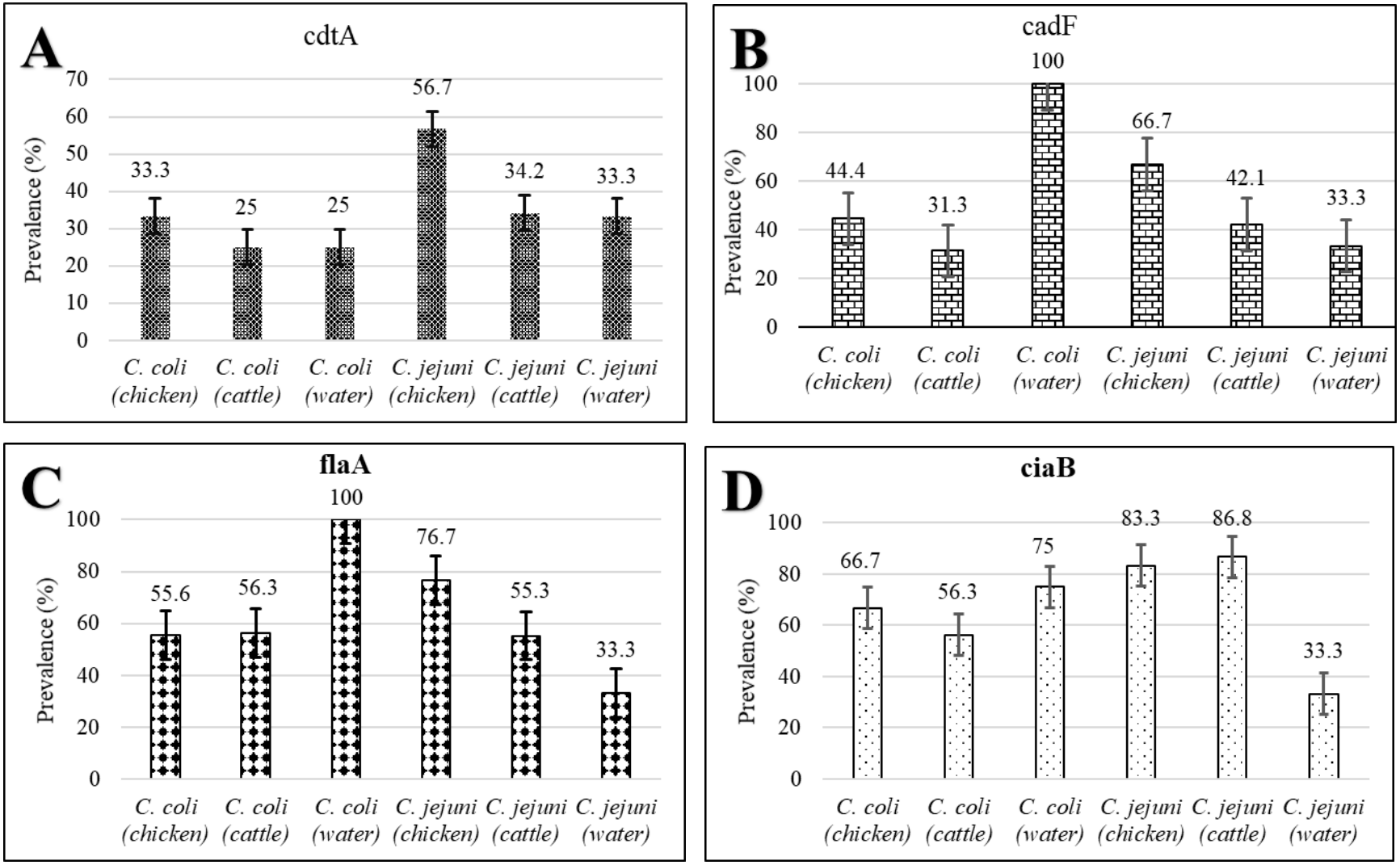


Figure 6.4: Proportion of *Campylobacter jejuni* and *Campylobacter coli* isolates from cattle, chicken and water, possessing *cdtA* (A), *cadF* (B), *flaA* (C) and *ciaB* genes (D). Data are the percentage prevalence ± standard error; *Campylobacter coli* isolate, N =29 [chicken (n = 9) cattle (n =16) water (n=4)] and *C. jejuni* isolates, N =74 [chicken (n = 30) cattle (n =38) water (n=6)]

Statistically significant ($P < 0.05$) positive correlations were observed between some virulence genes assayed in this study (Table 6.2). The *ciaB* gene that encodes *Campylobacter* invasion antigen B was the only gene which was not statistically significantly ($P > 0.05$) correlated with *cdtA* gene. The presence of *cadF* gene was strongly correlated with the presence of *flaA* gene (R = 0.733). The occurrence of the *cdtA* (one of the tripartite cytolethal distending toxin) that causes unwinding of DNA strands, was moderately correlated with the presence of both *cadF* (R = 0.645) and *flaA* (R = 0.544) genes ($P < 0.05$).

Table 6.2: Comparison of Pearson's correlations for virulence-encoding genes detected in *Campylobacter* isolates from cattle, chickens and water feces isolates

Virulence encoding gene	**Statistical test**	*cdtA*	***cadF***	***ciaB***	***flaA***
cdtA	Pearson Correlation (R)	1	.645**	.172	.540**
	Sig. (2-tailed)		.000	.082	.000
cadF	Pearson Correlation (R)	.645**	1	.198*	.733**
	Sig. (2-tailed)	.000		.045	.000
ciaB	Pearson Correlation (R)	.172	.198*	1	.212*
	Sig. (2-tailed)	.082	.045		.032
flaA	Pearson Correlation (R)	.540**	.733**	.212*	1
	Sig. (2-tailed)	.000	.000	.032	

Sig.: Significance; **: Correlation is significant at the 0.01 level (2-tailed); *: Correlation is significant at the 0.05 level (2-tailed)

6.3.2 Results of partial sequences for 16S rRNA gene (*Campylobacter* genus), *hipO* gene (*C. jejuni*) and *ceuE* gene (*C. coli*) for the isolates from Kajiado County

The nucleotide sequences of 16S rRNA genes amplicons were blasted, and the results showed that the sequences identities were between 99.65% and 100% for the 20 *Campylobacter* spp. isolated from cattle, chicken and water in Kajiado County and *C. jejuni* in the GenBank (CP054848.1 and CP047481.1) (Table 6.3). The BLASTn analysis of the sequenced amplicons for *C. coli-ceuE* genes revealed that all the 8 (100%) sequences were similar to *C. coli*, with

sequence identity of between 99.57% and 100% to annotated sequences in bank (Table 6.3). Seven (87.5%) of the *C. jejuni-hipO* genes were similar to *C. jejuni* with a sequence identity of between 99.65% and 100%. One *hipO* gene sequence matched an unidentified *Campylobacter* spp. with sequence identity of 99.83% (Table 6.3).

The partial sequences for 16S rRNA, *ceuE* and *hipO* genes of *Campylobacter* species of isolates from cattle, chicken and water sources from Kajiado County are available in the NCBI database (www.ncbi.nlm.nih.gov/), under the given accession numbers are shown in Table 6.3; Appendices 5, 6, 7.

Table 6.3: GenBank accession numbers and BLASTn analysis of 16S rRNA, *ceuE* and *hipO* gene partial sequences for *Campylobacter* isolates from Kajiado County, Kenya

16S rRNA gene partial sequence	Sample source (for this study)	Accession number (for this study)	Corresponding sequence in GenBank	Accession no. with the of highest BLASTn match	Expect (E) value	% Identity
1. 299C	Chicken	OQ363834	*C. jejuni*	CP054848.1	0.0	100
2. 176B	Cattle	OQ363835	*C. jejuni*	CP054848.1	0.0	99.3
3. 360B	Cattle	OQ363836	*C. jejuni*	CP047481.1	0.0	99.65
4. 362B1	Cattle	OQ363837	*C. jejuni*	CP047481.1	0.0	99.76
5. 310B	Cattle	OQ363838	*C. jejuni*	CP047481.1	0.0	99.65
6. 41W	Water	OQ363839	*C. jejuni*	CP047481.1	0.0	99.88
7. 312B	Chicken	OQ363840	*C. jejuni*	CP047481.1	0.0	99.88
8. 248C	Chicken	OQ363841	*C. jejuni*	CP054848.1	0.0	99.76
9. 354B1	Chicken	OQ363842	*C. jejuni*	CP054848.1	0.0	99.88
10. 254B	Cattle	OQ363843	*C. jejuni*	CP054848.1	0.0	99.76
11. 210B	Cattle	OQ363844	*C. jejuni*	CP054848.1	0.0	99.65
12. 323C	Chicken	OQ363845	*C. jejuni*	CP047481.1	0.0	99.88
13. 307C	Chicken	OQ363846	*C. jejuni*	CP054848.1	0.0	99.76
14. 343B2	Cattle	OQ363847	*C. jejuni*	CP054848.1	0.0	99.88
15. 262C	Chicken	OQ363848	*C. jejuni*	CP054848.1	0.0	99.53
16. 15W	Water	OQ363849	*C. jejuni*	CP054848.1	0.0	99.88
17. 319B	Cattle	OQ363850	*C. jejuni*	CP054848.1	0.0	99.65
18. 382C	Chicken	OQ363851	*C. jejuni*	CP054848.1	0.0	100
19. 217B	Cattle	OQ363852	*C. jejuni*	CP054848.1	0.0	100
20. 39W	Water	OQ363853	*C. jejuni*	CP054848.1	0.0	99.76
***ceuE* gene partial sequence**						
1. 342B	Cattle	OQ389474	*C. coli*	OM810313.1	0.0	99.57
2. 284C	Chicken	OQ389475	*C. coli*	OM810313.1	0.0	99.78
3. 55W2	Water	OQ389476	*C. coli*	CP007181.1	0.0	100
4. 33C	Chicken	OQ389477	*C. coli*	CP042463.1	0.0	99.78
5. 406C2	Chicken	OQ389478	*C. coli*	KC954627.1	0.0	99.78
6. 398B	Cattle	OQ389479	*C. coli*	KC954627.1	0.0	99.57
7. 61Bs	Cattle	OQ389480	*C. coli*	OM810313.1	0.0	100
8. 263C2	Chicken	OQ389481	*C. coli*	CP042463.1	0.0	99.78
***hipO* gene partial sequences**						
1. 376B	Cattle	OQ390087	*Campylobacter* spp.	CP040607.1	0.0	99.83
2. 368C1	Chicken	OQ390088	*C. jejuni*	CP012216.1	0.0	100
3. 397C1	Chicken	OQ390089	*C. jejuni*	CP047482.1	0.0	100
4. 386B	Cattle	OQ390090	*C. jejuni*	CP047480.1	0.0	99.65
5. 346B	Cattle	OQ390091	*C. jejuni*	CP053854.1	0.0	99.83
6. 399B	Cattle	OQ390092	*C. jejuni*	CP035891.1	0.0	100
7. 33W	Water	OQ390093	*C. jejuni*	CP047480.1	0.0	99.65
8. 402Cs	Chicken	OQ390094	*C. jejuni*	CP047482.1	0.0	99.83

6.3.3 Phylogenetic relationship of partial sequences of 16S rRNA for *Campylobacter* isolates from Kajiado County, Kenya

Ten (10) GenBank reference strains of the members of the *Campylobacter* genus (Table 6.4), together with nine cattle strains, eight chicken strains and three water strains (designated under GenBank accession numbers: OQ363834 to OQ363853 under Table 6.3 above) from this study, based on 16S rRNA, were included in this phylogenetic analysis. The 16S rRNA gene sequence of *Helicobacter pylori* (GenBank Accession No. NR_114587.1) was used as outgroup (Table 6.4).

Table 6.4: GenBank accession numbers of 16S ribosomal RNA gene partial sequences from different sources included in the phylogenetic analysis

Bacterial organism	Sources/sample	Country	Accession numbers
C. jejuni	Chicken thigh from market	Italy	MN736607
C. jejuni	Bovine faeces	Belgium	CP019838
C. coli	NS	USA	NR041835
C. coli	NS	United Kingdom	AH000014
C. fetus	Sheat wash	South Africa	MT138661
C. hyointestinalis subsp. hyointestinalis	Cattle feces	Japan	AB310964
C. hyointestinalis subsp. hyointestinalis	NS	United Kingdom	AF097689
C. lari	Chicken faeces	NS	NR_043034
C. lari	Chicken faeces	Italy	MN736591
C. upsaliensis	NS	Switzerland	NR_043602
C. coli	Soil near paddock	India	MK156113
C. coli	Poultry farm	Uganda	MW159724
C. coli	Chick caecal swabs	Iraq	OP263120
C. coli	Human	Bangladesh	MT774557
***Helicobacter pylori* (outgroup)**	Culture collection type	Canada	NR_114587

NS: Not stated

The tree with the highest log likelihood (-2067.01) and the percentage of trees (adjacent to the branches) in which the associated taxa clustered together is illustrated in Figure 6.5. The phylogenetic construction revealed two diverse clades (monophyletic groups). These clades were placed into two polyphyletic groups (Groups I and II) based on how the strains were genetically close. Both groups (I and II) contained sequences that were intermixed with *Campylobacter jejuni* strains from cattle, water and chicken samples (Figure 6.5). Twelve *Campylobacter jejuni*

strains from this study (OQ363834, OQ363841-43 and OQ363846-53) appeared closely related and clustered with *C. coli* strain from GenBank (MT774557) in one of the clades.

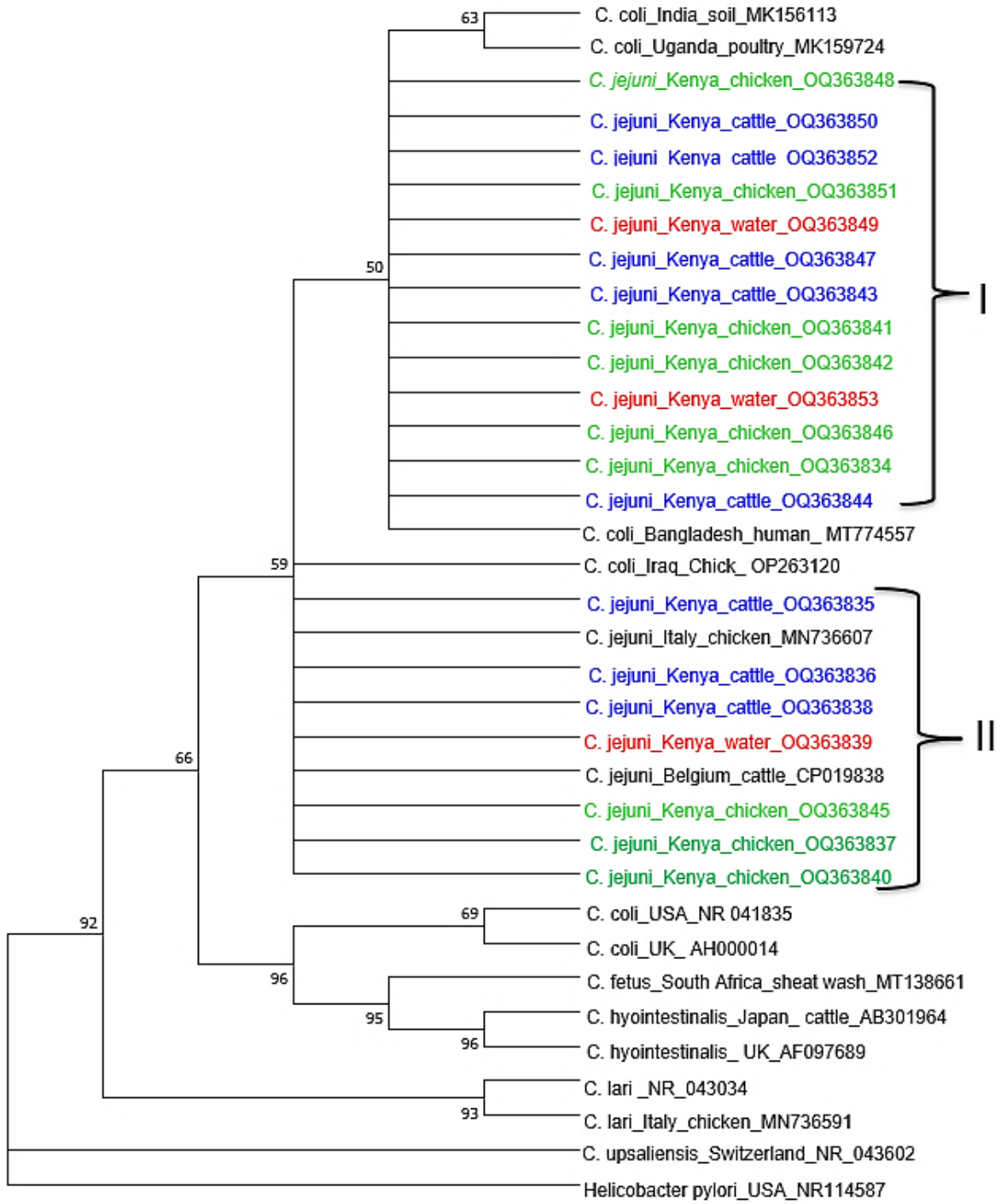


Figure 6.5: Phylogenetic tree based on the 16S rRNA gene partial sequences of 20 *C. jejuni* strains from this study, with *Helicobacter pylori* used as an outgroup

6.3.4 Phylogenetic relationship of *Campylobacter coli* isolates from Kajiado County, Kenya

Six GenBank reference strains of *Campylobacter coli* (Table 6.5), together with one water strain, three cattle strains and four chicken strains (designated GenBank accession numbers: OQ389474 to OQ389481 under Table 6.3) from this study, based on lipoprotein (*ceuE*) gene, were included in this phylogenetic analysis. Two GenBank strains of *C. jejuni*, based on *ceuE* gene (FJ946068 and FJ946073), were also included in phyloanalysis. *Helicobacter hepaticus* (GenBank Accession No. NR_114584.1) was used as outgroup (Table 6.5).

Table 6.5: Gen bank accession numbers of lipoprotein (*ceuE*) gene partial sequences from different sources included in the phylogenetic analysis

Bacterial organism	Sources/sample	Country	Accession numbers
C. coli	Piglet rectal swab	India	MK156107
C. coli	Diarrhoeic calf	India	MK156104
C. coli	Soil near paddock	India	MK156108
C. coli	Human	Egypt	KY435369
C. coli	Culture collection	USA	KF541298
C. coli	*Gallus gallus*	China	KF541297
C. jejuni	NS	Switzerland	FJ946068
C. jejuni	NS	Switzerland	FJ946073
***Helicobacter hepaticus* strain ATCC 51448 (outgroup)**	culture collection type	Canada	NR_114584

Key: NS: Not stated

The *ceuE* phylogenetic tree with the highest log likelihood (-1519.85) and the percentage of trees (next to the branches) in which the associated group clustered together is shown in Figure 6.6. The phylotree construction revealed four diverse clades (monophyletic groups). These clades were placed into six polyphyletic groups (Groups I-VI) based on how the strains were genetically homogenous. Groups IV and VI, contained sequences that were intermixed with *C. coli* strains isolated from both cattle and chicken samples (Figure 6.6). Groups I and III consisted of *C. coli* strains that only existed in chicken. Groups II and V comprised of *C. coli* strains that were circulating in cattle and water, respectively.

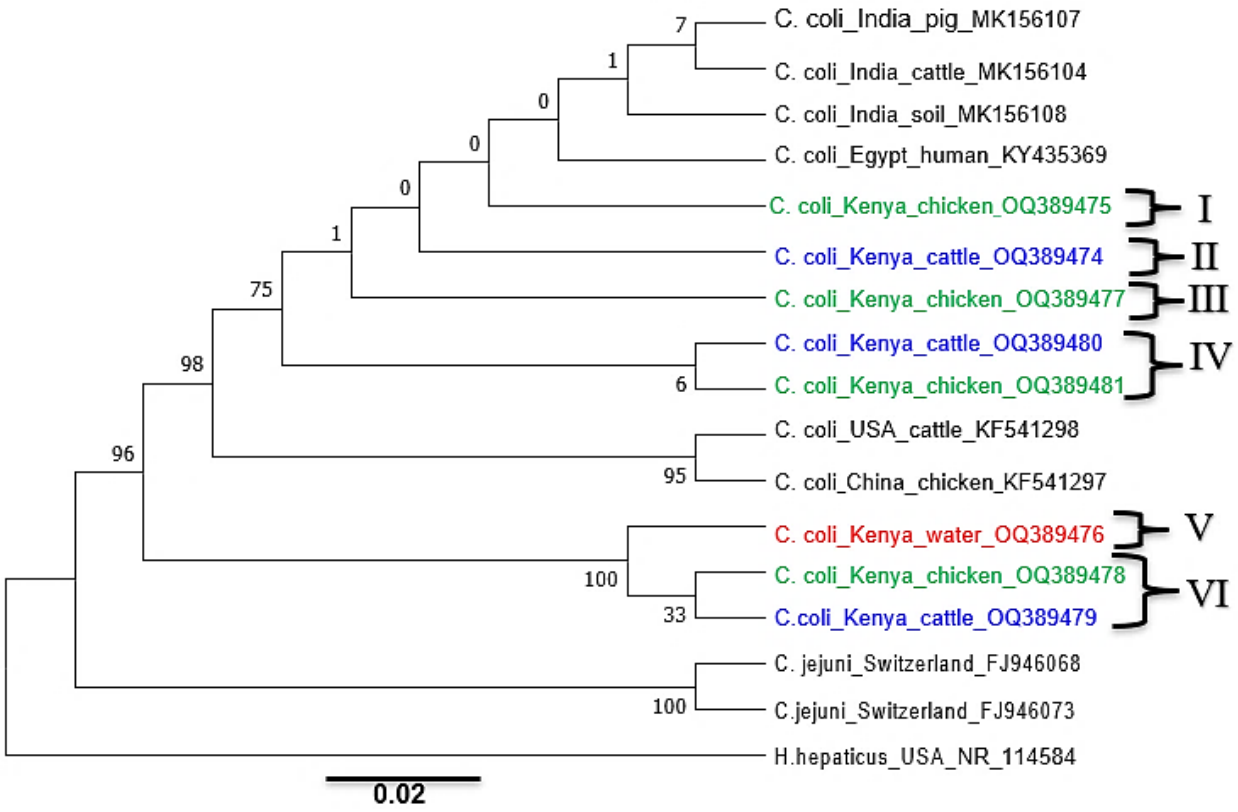


Figure 6.6: Phylogenetic tree based on the lipoprotein (*ceuE*) gene partial sequences of 8 *C. coli* strains from this study, with *Helicobacter hepaticus* used as an outgroup

6.3.5 Phylogenetic relationship of *Campylobacter jejuni* isolates from Kajiado County, Kenya

Six GenBank reference strains of *Campylobacter jejuni* (Table 6.6), together with one water strain, three chicken strains and four cattle strains (designated under GenBank accession numbers: OQ390087 to OQ390094 under Table 6.3 above) from this study, based on hippurate hydrolase (*hipO*) gene, were included in this phylogenetic analysis. *Campylobacter lari* (GenBank Accession No. JF747609.1) was used as outgroup (Table 6.6).

Table 6.6: Gen bank accession numbers of hippurate (*hipO*) gene partial sequences from different sources included in the phylogenetic analysis

Bacterial organism	Sources/sample	Country	Accession numbers
C. jejuni	Environmental sample	Australia	KP164636
C. jejuni	Human stool	Australia	KP164641
C. jejuni	Chicken	China	GQ249183
C. jejuni	Bovine faeces	USA	GQ249180
***Campylobacter* sp.**	Yellowhammer	United Kingdom	MW139886
Campylobacter jejuni* subsp. *doylei	Child diarrhoeic stool	Switzerland	DQ174144
***Campylobacter lari* strain ATCC 43675 (outgroup)**	Poultry	USA	JF747609.1

The tree with the highest log likelihood (-1387.08) alongside the percentage of trees (next to the branches) in which the associated group clustered together is shown in Figure 6.7. Phylotree revealed four diverse monophyletic groups (clades). These monophyletic groups/clades were placed into five polyphyletic groups (Groups I-V) according to how close the strains were. Group I, contained sequences that were intermixed with *Campylobacter jejuni* isolated from both cattle and chicken samples (Figure 6.7). Groups I and III and Groups II and IV comprised *Campylobacter jejuni* strains that only existed in chicken and cattle, respectively. Group V comprised *Campylobacter jejuni* strain that were circulating in cattle and water.

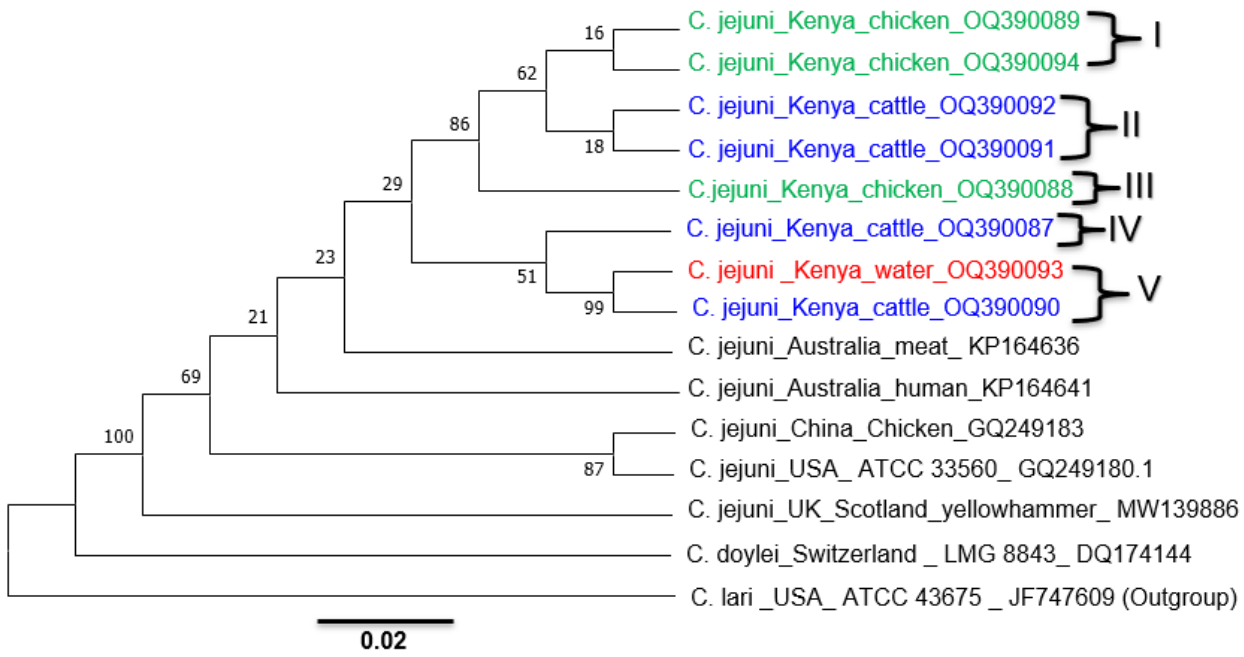


Figure 6.7: Phylogenetic tree based on the hippurate hydrolase (*hipO*) gene partial sequences of 8 *Campylobacter jejuni* strains from this study, with *Campylobacter lari* used as an outgroup

6.4 Discussion

Virulence-encoding genes are responsible for *Campylobacter*'s pathogenicity, and therefore the virulence-associated factors in livestock (cattle and chicken) and non-livestock (water) reservoirs warrant studies for the sake of human safety. There are limited studies that investigated virulence genes in *Campylobacter* strains from the environmental sources such as water; most of them having focused on occurrence of virulence markers in *Campylobacter* strains in humans and domestic animals, particularly poultry. Subsequently, this study investigated genes encoding virulence markers including *cdtA*, *flaA*, *ciaB* and *cadF* in cattle, chicken and water samples.

Overall, the *ciaB* gene which is responsible for the attack of host epithelial cells was the most detected virulence-encoding gene in this study, at 79.5%, 77.8% and 50% of chicken, cattle and water isolates, respectively. Thus, chicken isolates showed the highest prevalence of *ciaB* gene. The detection rate of ciaB gene in *C. jejuni* (83.3%) and *C. coli* (66.7%) isolates from chicken was comparable with the findings from previous studies (Ngobese *et al*., 2020). However, the percentage prevalence of *ciaB* gene among chicken isolates reported in this study was higher than in other studies: 47% and 10% in *C. jejuni* and *C. coli* isolates respectively (Reddy and Zishiri, 2018); and 23.1% among *Campylobacter* isolates (Ramatla *et al*., 2022). The *ciaB* gene was detected in 56.3% and 86.8% of *C. coli* and *C. jejuni* isolates recovered from cattle and this finding is akin with the study of Raeisi *et al*. (2017). The current study further showed high prevalence (75% of *C. jejuni* and 33.3% of *C. coli*) of *ciaB* gene in water isolates and the results are comparable with results of the study by Chukwu *et al*. (2019). On the contrary, Igwaran and Okoh (2020) reported zero prevalence of the same gene in *Campylobacter* isolates from water samples from both pond/dams and rivers. The *ciaB* gene is necessary for the early phases of *Campylobacter* colonization (Guerry, 2007), therefore, the high detection of the latter in cattle, chicken and water samples means that the recovered strains were able to overcome stressful conditions during the passage through the gut and induce the disease (Guerry, 2007). Additionally, this gene had a significant low positive correlation of 19.7% and 21.2% with the *cadF* and *flaA* genes ($p < 0.05$). The significance of this finding follows the fact the first stage in pathogenesis of invasive versus toxigenic pathogens, is attachment to the host cell. Thus this gene very essential for the pathogenic strains which, despite having other virulence genes, could result in infections.

The flagellin protein FlaA, a major protein encoding *flaA* gene, was the second most detected putative virulence gene. The *flaA* gene is necessary for bacterial motility and establishment in epithelial cells of ileum. Furthermore, *flaA* gene is also responsible for the expression of attachment, attacking and establishment in the host epithelial cells, thereby halting the immune response (Ngobese *et al*., 2020). Presence of either *flaA* and *cadF* gene results in attachment and hence more likely success in disease development. Studies have reported presence of *flaA* gene in *C. jejuni* and *C. coli* strains recovered from chicken (Ngobese *et al*., 2020; Andrzejewska *et al*., 2022), from cattle (Ngobese *et al*., 2020) and from water samples (Andrzejewska *et al*., 2022); and the findings of this study are also in line with these studies. However, higher prevalence of up to 100% has been reported in *Campylobacter* species from diverse sources (Wieczorek and Osek, (2013b); Gahamanyi *et al*., 2021); with the higher detection of *flaA* gene being associated with high conservation of the FlaA region among *Campylobacter* strains.

From the findings of this study, 61.5%%, 60% and 38.9% of *Campylobacter* isolates from chicken, water and cattle samples harboured the *cadF* gene, respectively. In chicken, 66.7% of *C. jejuni* and 44.4% of *C. coli* possessed *cadF* gene and this is concordant with the findings by Ngobese *et al*. (2020). *Campylobacter coli-cadF* gene-possessing strains of cattle origin were also reported by Wieczorek and Osek, (2013b); however, much higher frequency (90-100%) of *cadF* gene in both *C. jejuni* and *C. coli* isolates have also been reported (Wieczorek *et al*., 2013; Gahamanyi *et al*., 2021). All the *C. coli* isolates from water were found to harbour *cadF* markers. Similarly, higher prevalence of *cadF* gene (100%) was demonstrated in *C. coli* isolates recovered from water samples from rivers, freshwater beaches, lakes and ponds in Northern Poland (Andrzejewska *et al*., 2022).

Campylobacters are known to produce cytolethal distending toxins (CDT) (encrypted by *cdtA*, *cdtB*, and *cdtC*) which cause DNA destruction, chromatin disintegration, cytoplasm distension and halts mitotic cell division, leading to progressive cellular distension and eventually, pathogen-induced host cell death (Ngobese *et al*., 2020). In this study, regardless of *Campylobacter* spp., *cdtA* gene was found more in chicken isolates at 51.3% including 33.3% in *C. coli* and 34.2% in *C. jejuni*. More or less similar findings were reported by Ramatla *et al*. (2022) who found that 26.9% of *C. jejuni* isolates broiler harboured *cdtA* gene. Ngobese *et al*. (2020) also reported high detection rate of *cdtA* from chicken samples; but at a much higher percentage (96% of *C. jejuni* and 83% of *C. coli*). Additionally, *C. jejuni* isolates from cattle in the current study demonstrated the highest (56.7%) detection rate of *cdtA* gene. Studies have reported discrepant detection rate [37% for *C. jejuni* and 50% of *C. coli* (Ngobese *et al*., 2020); 100% in both *C. jejuni* and *C. coli* (Wieczorek and Osek, 2013b) of this gene among *Campylobacter* strains of cattle origin. Limited data exist on the prevalence of virulence markers (including *cdtA*) among *Campylobacter* strains from the environmental water. Subsequently, these results can be compared with findings on other CDT encoding genes (*cdtB* or *cdtC*), where studies have detected varying prevalence of *cdtB* gene in *C. jejuni* and *C. coli* strains isolated from water samples (Igwaran and Okoh, 2020; Andrzejewska *et al*., 2022). Presence of cytotoxicity genes (*cdtA*) among livestock and environmental sources highlights a food safety alarm as well as a public health one.

The findings however, revealed relatively higher prevalence rates in occurrence of virulence associated genes, compared to other studies. This study evokes that the observed discrepancies may be due to primer specificities, PCR protocols, climate and/or environmental conditions, geographical factors and seasonality, freezing and thawing. Additionally, these virulence

encoding genes are carried on plasmids, which may influence their occurrence in different strains (Oh *et al*., 2017). Additionally, the virulence markers reported in this study have also been documented in *Campylobacter* strains of human origin (González-Hein *et al*., 2013; Oh *et al*., 2017; Reddy and Zishiri, 2018), highlighting the potential virulence of these strains in causing human campylobacteriosis. It is, however, crucial to highlight that occurrence of virulence markers is merely indicative/suggestive and may not predict exactly how deadly a *Campylobacter* strain might be. Subsequently, to rule out this possibility, comparative analysis of patient demographics and the interaction between virulence and presenting symptoms are needed to prove disease causation, noting that the severity of a disease relies upon the virulence of the strain and on the host's immune state (Younis *et al.,* 2018).

Phylogenetic analysis was carried out to establish the homogeneity among *Campylobacter* (*C. coli* and *C. jejuni*) sequences derived from cattle, chicken and water based on amplicon sequencing targeting 16S rRNA, *ceuE* and *hipO* genes. Phyletic analysis of *Campylobacter* strains from this study formed well-supported clades with other closely related *Campylobacter* strains from different hosts/reservoirs and environments from diverse geographical regions.

The 16S ribosomal RNA (16S rRNA) gene sequencing is commonly used in identification of bacteria and phylogenetic studies. In this study, the 16S rRNA tree revealed two diverse groups both of which were intermixed with *Campylobacter jejuni* strains circulating in cattle and chicken populations and environment (water). These findings show that these strains were genetically related and may belong to the same lineage, suggesting that there was a likelihood of multiple spread of *Campylobacter* from either chicken to cattle and *vice versa*; or environmental (water) contamination from either cattle and/or chicken, which resulted in *Campylobacter* infection of chicken and cattle within the study area. Both chicken and cattle can serve as

Campylobacter reservoirs (contributing to bidirectional infection pathways), and as potential sources for the contamination of environment (water) through faecal shedding.

The clustering of 16S rRNA sequences of *C. jejuni* strains from the current study with 16S rRNA sequences of *C. coli* strains from GenBank in one monophyletic group is concordance with the findings described in previous reports (Gunther *et al*., 2011; Ioannidou *et al*., 2013; Ramatla *et al*., 2022). These phyletic analysis findings demonstrate a closer relatedness than actual facts, due to the highly conserved sequence homogeneity among the 16S rRNA gene of *C. coli and C. jejuni*. Additionally, this emphasizes the need to use multigene target approach in discerning and elaborating ancestry history of *C. jejuni and C. coli* globally, as evoked by other authors (Kawasaki *et al*., 2008; Ramatla *et al*., 2022).

The *ceuE* and hipO gene derived trees revealed six and five different polyphyletic groups, respectively. Groups I and III, Group II, and Group V for the *ceuE* sequences comprised *Campylobacter coli* strains that were exclusively circulating in chicken, cattle and water respectively. Similarly, Groups I and III and Groups II and IV for the *hipO* sequences were *Campylobacter jejuni* strains that only existed in chicken and cattle, respectively. These polyphyletic groups (Groups I, II, III and V for *ceuE* tree and Groups I to IV for the *hipO* tree) were genetically diverse and/or of different lineage; suggesting unlikelihood of transmission of respective *Campylobacter* strains (*C. coli* or *C. jejuni*) from cattle/chicken to water or *vice versa*.

Groups IV and VI for the *ceuE* gene derived tree and Group I for the *hipO* gene derived tree, contained sequences that were intermixed with *C. coli* and *C. jejuni* strains respectively, isolated from both cattle and chicken samples. These results affirm that these strains were closely related

and of same ancestry, alluding that there was a possibility of spread of *C. coli* and *C. jejuni* infection from cattle to chicken and *vice versa* within the study area.

Group V for the *hipO* tree comprised *C. jejuni* strains that were circulating in both cattle and water. These findings show that these strains were homogeneous and could have originated from similar lineage, hinting a possibility of spread of *C. jejuni* infection from water to cattle within Kajiado County. These results support the notion that water is a significant reservoir of campylobacter infection in cattle (Ellis-Iversen *et al*., 2009a; Chala *et al*., 2021); i.e. faecal contaminated waters whether in water troughs or standing water bodies like dams are sources for persistent reinfection within a group of cattle upon drinking such waters. This highlights the significance of providing farm animals with clean drinking water. Clustering of cattle- and water- derived *C. jejuni* strains could alternatively imply that water had become contaminated from faecal shedding from cattle within the study population and duration. Contamination of water sources with *Campylobacter* strains grants an opportunity for transmission of environmentally adapted genotypes to food animals including chicken and cattle (Chala *et al*., 2021); probably becoming more virulent. Subsequently, the epidemiology of campylobacters in Kajiado County is likely to be a complex web of transmission between poultry, cattle, and the environment (water); with probable interaction with humans, other livestock species and wild reservoirs.

6.5 Conclusions

- *Campylobacter jejuni* and *C. coli* strains from cattle, chicken and water harbour virulence markers responsible for motility/colonization (*flaA*), invasiveness (*ciaB*), adherence (*cadF*), and toxin production (*cdtA*); evoking their important role in campylobacteriosis development among humans and livestock

- Detection rate of the virulence encoding genes was higher in faecal samples from chicken than from other sources
- Regardless of the sample source and/or type for *C. jejuni* strains, the study demonstrated higher detection of virulence markers
- Phylogenetic reconstruction revealed two (16S rRNA), six (*ceuE*) and five (*hipO*) intermixed clades within cattle-, chicken- and water-derived *Campylobacter* strains
- The genetic profiles analysis revealed that some *Campylobacter* strains originating from cattle, chicken and water sources appeared to be genetically homogenous; portraying that the studied sources are potential reservoirs responsible for either *Campylobacter* colonization and/or infection in cattle and chicken or for *Campylobacter* contamination of water samples in Kajiado County. Alternatively, the studied cattle and chicken might have acquired *Campylobacter* through the ingestion of contaminated water

6.6 Recommendations

- Further multilocus sequence typing (MLST) of whole genomic studies involving large number of environmental, humans and livestock isolates are recommended to ascertain the possible cycle of *Campylobacter* transmission pathways and interactions at household level in different localities in Kenya
- Additional studies on full-genomic sequencing to assess other virulence loci would further elucidate the potential role of chicken, cattle and environmental waters as a transport host of thermophilic *Campylobacter* in human disease epidemiology

CHAPTER SEVEN: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

Thermotolerant *Campylobacter* are significant targets for veterinary and public health studies owing to their zoonotic potential, wide variety of reservoirs/hosts, and environmental persistence, for instance survivability in water (Hannon *et al*., 2009). *Campylobacter jejuni* and *C. coli* are the most significant human and animal pathogens. Thermophilic *Campylobacter* are commonly isolated from chicken and livestock species including shoats, cattle and swine. Chicken, followed by cattle having the greatest impact as sources of infection for humans. The scarce information on this organism is compounded by its fastidious nature (requiring special culture conditions and nutrients) and therefore it is rarely tested-for in most laboratories. Consequently, effective treatment based on antimicrobial susceptibility tests (ASTs) and the prevention as well as control of the resultant disease (campylobacteriosis) have been hampered. Most *Campylobacter* infections in humans are food related, caused by antimicrobial resistant strains emanating from livestock and their byproducts (Tenhagen *et al*., 2021). Thus, control of cattle, chicken and water-linked infections in humans depends on the control of *Campylobacter* colonization in respective animals and the environment. Despite mounting evidence regarding the burden of *Campylobacter*-attributed infections (Snelling *et al*., 2005), the genotypic information and transmission dynamics regarding what sources, how, and where livestock (cattle and poultry) contract *Campylobacter* infection is poorly comprehended in Kajiado County and in Kenya at large as well as other low- and middle-income countries. Therefore, this study investigated the seasonal prevalence, phenotypic antimicrobial susceptibility profiles, presence of antimicrobial resistance and virulence gees, and risk factors

associated with thermotolerant campylobacters in cattle, chicken and water samples from Kajiado County, as well as their phylogenetic relationships; in order to inform on control measures, improve clinical treatment and control emergence and spread of AMR strains.

Singleplex PCR (sPCR) was initially used to screen 213 culture positive isolates (which is 46.6% prevalence by culture/biochemical characteristics). These positive isolates were initially subjected to sPCR assay using 16S rRNA primers specific for *Campylobacter* genus. A total of 162 isolates were confirmed as *Campylobacter* species (35.4% prevalence by PCR assay). Molecular techniques including PCR assays were found to be highly specific, sensitive, and of considerable interest in detection and confirmation of *Campylobacter* species over conventional culture as reported elsewhere (Abd-El-Aziz *et al*., 2017; Youseef *et al*., 2017).

All the 162 PCR (16S rRNA) confirmed positive isolates were further subjected to sPCR assays targeting *hipO* gene specific for *Campylobacter jejuni* and the *ceuE* gene of *Campylobacter coli*. The sPCR results confirmed 90 isolates as *Campylobacter jejuni* (55.6%), 29 (17.9%) as *Campylobacter coli,* and the rest could neither be identified by either probe and were thus recorded as other thermophilic *Campylobacter* species (OTCs). The differentiation of species using biochemical tests i.e. hippurate hydrolysis is difficult owing to the genetic relatedness of *C. jejuni* and *C. coli* species (Miller *et al*., 2010). Therefore, further verification using PCR is very useful. The OTCs could represent some of the "emerging *Campylobacter* species" including *C. concisus*, *C. lari*, *C. upsaliensis* and *C. ureolyticus* (Costa and Iraola, 2019; Soto-Beltrán *et al*., 2022).

As cited in Chapter 3; the prevalence of *Campylobacter* species in cattle, chicken and water reported in this study varied from different studies done elsewhere. A total percentage of 66.7%,

51.2%, and 35.3% of isolates from chicken, cattle and water samples were confirmed as *C. jejuni*; 14.3%, 19.5% and 23.5% of isolates from respective tested sources were confirmed as *C. coli*; while 19%, , 29.3% and 41.2% were identified as OTCs, respectively.The variations might be elucidated by differences in study design, sampling strategy, duration, season, animal production system and husbandry practices, biosecurity measures, agroecological differences, laboratory isolation techniques (Chatur *et al*., 2014; Osbjer *et al*., 2016; Chuma *et al*., 2016). This study was a seasonal-based cross-sectional study in a semi-arid study area, entailing sampling of water samples and unpooled rectal or cloacal swabs from apparently healthy cattle and chicken. Additionally, samples were enriched in Bolton broth before plating on selective media (mCCDA). In contrast, some studies conducted in Kenya utilized direct plating isolation with culture-based identification only (Chepkwony, 2016); this could potentially have contributed to reported variation in prevalence. This study utilized colony morphology, biochemical and molecular characterization for identification of *Campylobacter* species.

Even though seasonality effect in both humans and animals has been reported in most temperate European countries (where there are distinct climate) (Bouwknegt *et al*., 2004; Hansson *et al*., 2004; Hofshagen and Kruse, 2005; Taylor *et al*., 2013; Friedrich *et al*., 2016), there was negligible and insignificant climatic variation in this study. This study was conducted during rainy and dry seasons, however, there was no clear-cut difference between the two seasons because environmental temperature and rainfall were more or less the same (occasional showers occurred during dry season at the time of sampling). Additionally, the amount of rainfall during the rain season was reduced. This, therefore, hypothesizes that thermophilic *Campylobacter* can occur in animals and environmental sources independent of season in Kajiado County. However, environmental temperature across the two seasons during the study period was below 24℃,

which has been shown to affect presence and persistence of *C. jejuni* in farm environment (An *et al*., 2018). Consequently, other factors including husbandry and management practices or other unidentified factors could have been involved.

A number of studies have incriminated poultry as the most critical reservoir in dissemination of *Campylobacter* to the environment and to other animals (the avian intestinal temperature of 42 ℃ being conducive for the replication of campylobacters). Subsequently, the role of chicken alongside other potential risk factors in epidemiology of *Campylobacter* in cattle was investigated in this study, analysed using multivariable logistic regression. The results output revealed high odds for acquisition of *Campylobacter* in farms which were rearing dairy cattle over those rearing beef. The odds were also high in farms raising/co-grazing cattle with multiple animal species like cats, dogs, donkey, and pigs; keeping chicken and farms with low levels of hygiene in the farm. These herd observations were in line with other studies (Ellis-Iversen *et al.*, 2009b; Thépault *et al*., 2018; Hoque *et al*., 2021). Without information on cattle herd-level risk factors connected with the *Campylobacter* dissemination or colonization, most farms are not able to design appropriate and working control programs. Consequently, incidences of livestock disease are rampant, necessitating use of antimicrobials.

This study reported a tremendously high level of antimicrobial resistance in *C. jejuni* and *C. coli* with worrying multi-drug resistance (MDR) patterns dominated by ampicillin-tetracycline-erythromycin-ciprofloxacin (AX-TE-E-CIP) combinations. *Campylobacter* isolates were highly resistant to β-lactam (ampicillin), tetracycline and quinolones (ciprofloxacin and nalidixic acid). The trend in AMR/MDR profiles in this study are comparable with trends reported in most African states and China; however, it is higher than what has been reported in studies conducted in European countries (Maćkiw *et al*., 2012). Discrepancies in the application of antimicrobials

in food animals, as well as differences in susceptibility test methods used may account for the observed variation in the results (Englen *et al*., 2007). Type of production system (extensive vs intensive) and existence of government legislation enforcing prudent application of antimicrobials probably contribute to variability in AMR and/or MDR profiles for the isolates. Animal health services-seeking behavior (where majority of farmers treated their sick animals by themselves), coupled with widespread use of existing antimicrobials particularly tetracyclines, macrolides and β-lactams, could explain the high AMR and/or MDR patterns among the isolates.

Antimicrobial resistance has devastating consequences. Currently, *Campylobacter* infections cannot be treated or are difficult to treat with commonly available first- and second-line antimicrobials as the organisms have developed resistance to antimicrobials, such as erythromycin and fluoroquinolones. This is happening in both animals and humans (Wieczorek and Osek, 2013a). The Government of Kenya has formulated a National Policy on Prevention and Containment of Antimicrobial Resistance and is implementing it through a National Action Plan (GOK, 2017). The effective implementation of this plan is of paramount importance as, in the future scenarios, the risk of large improper use of antimicrobials in animals will increase because farmers will have to deal with an increased risk of zoonotic diseases while at the same time attempting to fully tap into the growing business opportunities provided by the expanded market for animal source foods.

Studies indicate substantial use of antimicrobial agents including tetracycline and erythromycin in animal production to be an important factor driving the emergence of antimicrobial resistance in *Campylobacter* strains (Tenhagen *et al*., 2021). Even though further reduction in antimicrobial use remain necessary, as high levels of AMR are still reported, there is need to change the tact.

Such initiatives should minimize spread of AMR, while simultaneously ensuring food safety and food security. Such decision is informed by the fact that even in countries like Australia and South Korea, which years ago introduced a legislation banning use of antimicrobials in food animals', are lately reporting rise in AMR (Obeng *et al*., 2012; Abraham *et al*., 2020). Even though knowledge gap exists on the most significant risk factors for the presence of AMR and effective interventions, farm biosecurity has been touted as one intervention that can tremendously control AMR spread (Davies and Wales, 2019; Kumar *et al*., 2021). Kumar *et al*. (2021) demonstrated that improved farm biosecurity is interrelated with improved productivity and reduction of antimicrobial use; the latter minimizing the spread of AMR.

The isolates in this study harboured antimicrobial resistance-coding (*cmeB* gene, *aph-3-1*, *tet*(O), $bla_{\text{OXA-61}}$, and *gyrA*) and virulence (*flaA*, *cadF*, *ciaB* and *cdtA*) genes. Respectively, these genes contribute to the antimicrobial resistance and pathogenicity of *Campylobacter* strains. It is, however, not clear whether an increase in *Campylobacter* resistance to antimicrobials has enhanced its virulence potential or the contrary (Gharbi *et al*., 2022). To date there is no unanimity among researchers about the relationship between antimicrobial resistance and virulence and/or pathogenicity (Beceiro *et al*., 2013). The associations between virulence genes and phenotypic/genotypic AMR in *Campylobacter* isolates were not assessed; it therefore, remains unclear whether an upsurge in AMR leads to an upsurge in occurrence of genes conferring virulence or contributing to its pathogenicity. However, possession of genes conferring resistance to antimicrobials have been linked with a decrease in virulence/pathogenicity, while some findings show the contrary-that AMR may enhance or potentiate virulence (Roux *et al*., 2013).

The transmission dynamics regarding what sources, how, and where livestock (cattle and poultry) contract *Campylobacter* infection is complex because of: (1) wide host range for the bacterium, (2) varying interrelationship with the hosts from just commensals to pathogen in some, and (3) genetic diversity (Khoshbakht *et al.*, 2013). Therefore, research is essential to characterize this organism in terms of reservoirs and transmission dynamics (tracing sources of infection), pathogenicity markers and antimicrobial susceptibility profiles; so as to institute proper control and prevention.

Target (amplicon) sequencing uses amplification of conserved regions such as 16S ribosomal RNA genes in the analysis of bacterial community profiling. However, other conserved regions coded by specific genes have also been reported. Additionally, PCR amplification and sequencing, can enable easy, quick, and precise detection and address epidemiological inquiries (Vinueza-Burgos *et al*., 2017).

Molecular confirmation of *Campylobacter* organisms was affirmed by target sequencing of proportional samples of 16S rRNA, *ceuE* and *hipO* genes. The sequences were BLASTn searched with those in GenBank. Homology findings (99-100%) revealed that all the 16S rRNA and *hipO* sequences were *C. jejuni*, while the *ceuE* sequences were identified as *C. coli* and accessioned with the GenBank. Phylogenetic construction was performed separately for the 3 target genes. The neighbour-joining (NJ) phylogenetic analyses, based on the three genes, showed that this study isolates (Kajiado/Kenya) clustered with sequences of human origin, environmental sources and also with other animal hosts from different countries. Considering the overlap (similarity in lineage) between the *Campylobacter* strains in this study, either livestock sources (cattle or chicken) or environmental sources (water) could have acted as the primary source of infection and/or contamination. This spotlights the continuous and complex pathogen

loop from livestock sources to environment and *vice versa*. The finding is important in elucidating the transmission dynamics of *Campylobacter* in Kajiado and Kenya at large.

7.2 General conclusions

- Seasonal effects on *Campylobacter* carriage in livestock and water was negligible or small and therefore other factors particularly animal husbandry and management practices could have been involved
- *Campylobacter jejuni*, *C. coli*, and OTCs were identified and were widespread in Kajiado; the moderately high percentage of thermophilic campylobacters in both livestock (cattle and chicken) and non-livestock (water) sources pose a potential health for humans
- The risk factors significantly associated with bovine campylobacteriosis in this study were rearing dairy cattle over those rearing beef, raising/co-grazing cattle with multiple animal species cats, dogs, donkey, and pigs; keeping chicken and low levels of hygiene in the farm
- Tetracyclines, aminoglycosides and β-lactam-based antimicrobials were the most commonly used antimicrobials; with 54.5% of the farms generally reporting using antimicrobials in chicken production systems than in cattle
- High antimicrobial resistance to ampicillin, tetracycline, erythromycin and to ciprofloxacin, and a high prevalence of multidrug resistant *Campylobacter* strains was observed in this study. The AX-TE-E-CIP antimicrobial cluster was the most prevalent MDR pattern and it was more commonly observed among *C. coli* than in *C. jejuni* isolates

- Chicken isolates showed the greatest resistance; this could be due to widespread use of antimicrobials in the poultry production system compared to cattle production systems
- Virulence determinants (*ciaB* and *flaA* genes being the most prevalent) and antimicrobial-resistant (*tet*(O), *gyrA* and *cmeB* being the most prevalent) genotypes were detected among *Campylobacter* isolates posing an alarming threat to food safety and public health.
- A significant overlap/clustering exists between *Campylobacter* strains found in cattle and water and those from chicken isolates, indicating a common lineage and also suggesting a potential co-infection dynamic

7.3 General Recommendations

- Isolation of thermophilic *Campylobacter* species from water sources, alludes that water can be a transmission vehicle for *Campylobacter*; however, there is need for further research to establish the extent of survival of the organisms in different water sources under different environmental conditions. The high prevalence of *Campylobacter* species isolated from various water sources, highlights the need for further work to identify the extent of survival of the organisms in water sources, and to ascertain their dissemination within the environment
- Whole-genome sequencing/next generation sequencing of thermophilic *Campylobacter* strains is recommended; so as to give better insights and/or picture of their phylogenomic and genomic features
- Recognizing the devastating implications of AMR, it is important for the government of Kenya to utilize a One Health approach in its Action plan on AMR and, also broaden its interventions to: (1) accommodate bio-exclusion and biocontainment measures as part of farm biosecurity, (2) strengthen legislation on the rational application of antimicrobials,

(3) make performance of ASTs mandatory for curative therapy and, (4) introduce bans on use of selected antimicrobials for enhancing growth or prophylaxis in food animal production, so as to understand and control the AMR problem

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APPENDICES

Appendix 1:Ethical considerations

This study was approved by Biosafety, Animal use and Ethics committee, Faculty of Veterinary Medicine, University of Nairobi, prior to commencement of the project, annexed as FVM BAUEC/2020/274 (Appendix 1.1). Written and informed consent was obtained from the Kenya Meteorological Department to retrieve data from the local weather stations where sampling was done. Verbal consent was sought from farmers prior to interviewing; where the objectives of the study and their privileges were elaborated in local languages (Maa, Kikuyu) and Swahili.

Appendix 1.1: Ethical clearance letter by Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi

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REF: FVM BAUEC/2020/274

Dr. Daniel Wambua Wanja
University of Nairobi
Dept. Pathology, Microbiology & Parasitology
10th October 2020

Dear Dr. Wanja,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

Seasonal Prevalence, Molecular characterization, Antimicrobial Resistance and Factors associated with *Camplobacter* species in Cattle, Chicken and Water in Kajiado, County Kenya.

Dr. Daniel Wambua Wanja J80/55717/2019.

We refer to your PhD proposal submitted to our committee for review and your application letter dated 21st September 2020. We have reviewed your application for ethical clearance for the study.

Fecal sample collection from both poultry and cattle, bacteriological and molecular techniques (PCR) used to identify *Campylobacter* from the fecal sample. *Campylobacter* isolates subjected to antimicrobial resistance and virulence characterization using PCR techniques meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.
We also note that registered veterinary surgeons will supervise the work.
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Dr. Catherine Kaluwa, PhD
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix 2: List of the 55 farms surveyed and their geographical location in Kajiado County

S/NO.	Farm location	Farm site	Eastings	Southings	Altitude
KT/01	Kajiado East	Kitengela	36°57'32.4612E	S1°28'33.132	1565.2m
KT/02	Kajiado East	Kitengela	36°56'08.7972E	S1°32'44.3688	1611.4m
KT/02.1	Kajiado East	Kitengela	36°57'37.53E	S1°30'33.132	1572.0m
KT/03	Kajiado East	Kitengela	36°56'08.0988E	S1°34'00.7788	1612.4m
KT/04	Kajiado East	Kitengela	36°54'30.7152E	S1°35'31.4412	1561.9m
MA/05	Ngong	Ngong	Missing	Missing	1892.0m
NG/06	Ngong	Kerarapon	36°39'50.3568E	S1°19'35.0976	1892.0m
NG/07	Ngong	Ngong 46	36°40'13.062E	S1°22'20.3808	1891.0m
NG/08	Ngong	Ngong 46	36°40'27.876E	S1°22'26.5728	1947.1m
MA/09	Matasia	Olkeri	36°40'38.7372E	S1°23'21.1668	1865.0m
MA/010	Matasia	Silanga	36°40'39.66E	S1°23'17.6748	1867.1m
MA/011	Matasia	Silanga	36°40'37.4772E	S1°23'20.9652	1860.3m
NG/012	Ngong	Ole Kalaso Highlights Rd	36°39'3.3966E	S1°22'38.56512	1998.0m
NG/013	Ngong	Ole Kalaso Highlights Rd	36°39'05.2848E	S1°22'42.7008	1983.0m
NG/014	Ngong	Gichagi	36°39'06.894E	S1°22'41.9916	1987.0m
NG/015	Ngong	Upper Matasia Lekuriki	36°40'07.5144E	S1°21'22.3632	1905.0m
NG/016	Ngong	Ngong	36°43'51.693E	S1°15'12.615	1851.0m
IS/017	Isinya	Leto	36.850°E	Missing	Missing
IS/018	Isinya	Ol kinos	36°55'37.43796E	Missing	Missing
IS/019	Isinya	Kaptei-Reto	Missing	Missing	1632.0m
MA/020	Mashuru	Noompala	37°23'8.566E	S2°9'38.478"	1170.0m
MA/021	Mashuru	Noompala	37°23'8.566E	S2°9'38.478"	1170.0m
MA/022	Mashuru	Noompala	37°23'8.566E	S2°9'38.478"	1170.0m
MA/023	Mashuru	Noompala	37°23'8.566E	S2°9'38.478"	1170.0m
MA/024	Mashuru	Noompala	37°23'8.566E	S2°9'38.478"	1170.0m
MA/025	Mashuru	Ilaimiror	37°23'60"E	S2°12'36"	1191.0m
MA/026	Mashuru	Nembuya	37°27'20.988E	S1°13'29.442"	1173.0m
MA/027	Mashuru	Nembuya	37°27'20.988E	S1°13'29.442"	1173.0m
MA/028	Mashuru	Nembuya	37°27'20.988E	S1°13'29.442"	1173.0m
MA/029	Mashuru	Nembuya	37°27'20.988E	S1°13'29.442"	1173.0m
MA/030	Mashuru	Nembuya	37°27'20.988E	S1°13'29.442"	1173.0m
MA/031	Mashuru	Nembuya	37°27'20.988E	S1°13'29.442"	1173.0m
MA/032	Mashuru	Nembuya	37°27'20.988E	S1°13'29.442"	1173.0m
KI/033	Kiserian	Olkeri	36°40'21.473E	S1°25'37.273"	1882.3m
KI/034	Kiserian	Keekonyokie/Corner Baridi	36°39'16.9776E	S1°27'42.2208"	1979.0m
OR/035	Rongai	Olkiramatian	36°44'49.1784E	S1°25'26.5776"	1790.0m
OR/036	Rongai	Kadisi	36°44'25.9008E	S1°24'56.1492"	1771.9m
OR/037	Rongai	Rongai	36°44'02.6232E	S1°23'25.7208"	1754.0m
IS/038	Isinya	Rongai	36°45'04.554E	S1°23'41.982"	1719.0m
NG/039	Ngong	Vet Farm	36°40'3.67E	S1°22'25.852"	1896.0m
OR/040	Rongai	Exciting	36°43'51.4668E	S1°23'19.3668"	1749.0m
OR/041	Rongai	Latia Rd/VICODEC	36°44'14.784E	S1°23'19.3668"	1755.6m
OR/042	Rongai	Mayor Rd 16	36°45'33.9048E	S1°23'26.6928"	1722.9m
OR/043	Rongai	Nosim Rd	36°43'07.3848E	S1°23'47.2668"	1747.4m
NG/044	Ngong	Ngong	36°39'00.5328E	S1°21'40.1652"	2090.9m
KI/045	Kiserian	Kiserian	36°40'31.5768E	S1°24'11.9592"	1949.4m
KI/046	Kiserian	Kiserian	36°41'14.7252E	S1°24'52.182"	1909.2m
KI/047	Kiserian	Ol choronyoro	36°42'09.8388E	S1°28'23.8008"	1794.0m
NG/048	Ngong	EM-bulbul	36°40'20.0028E	S1°20'13.9272"	1844.8m
KI/049	Kiserian	Kiserian	36°40'21.6372E	S1°25'16.266"	1911.1m
NG/050	Ngong	Lower Matasia	36°41'32.316E	S1°23'35.6748"	1890.7m
KI/051	Kiserian	Kiserian	36°42'06.4872E	S1°25'42.4848"	1899.2m
NG/052	Ngong	Upper Matasia	36°39'21.8052E	S1°23'57.426"	2067.9m
NG/053	Ngong	Drug Efficacy Trial Farm	Missing	Missing	Missing
OR/054	Rongai	Nkaimurunya Gataka RD	Missing	Missing	Missing
OR/055	Rongai	Nkaimurunya Gataka RD	Missing	Missing	Missing

Appendix 3: A Questionnaire used to assess antimicrobial use and farm risk factors associated with the occurrence and transmission of thermotolerant *Campylobacter* species in small-holder cattle herds and chicken flocks in Kajiado County-Kenya

UNIVERSITY OF NAIROBI
COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES

Questionnaire to assess antimicrobial use and farm risk factors associated with the occurrence and transmission of thermotolerant *Campylobacter* species in small-holder cattle herds and chicken flocks in Kajiado County-Kenya

Research Title: Seasonal Prevalence, Molecular Characterization, Antimicrobial Resistance and Factors Associated with Thermophilic *Campylobacter* in Cattle, Chicken and Water in Kajiado County, Kenya

Dear farm owner,

I **Dr Daniel W Wanja**, the Principal Investigator (PI) and also a PhD student studying Applied Microbiology at the University of Nairobi; is currently undertaking a study to examine seasonal occurrence, molecular characteristics, antimicrobial resistance profiles and risk factors associated with thermophilic *Campylobacter* spp. in cattle, chicken and water in Kajiado County, Kenya. I sincerely invite you to participate in this survey by filling in the following questionnaire. Your contribution to insight is precious. Finally, I assure you that I will keep the information confidential and only use it for academic purposes. Glad to get your help! Thank you!

A. Farm identification

(1) County…….Sub-County.....Ward----------Village.....

(2) GPS reading: Eastings…….Southings……

B. Farmer's Biodata

(3) Name of the farm owner ……..Mobile….……

(4) Age of the respondent

[1] 10-15 years **[2]** 16-20 years **[3]** 21-30 years **[4]** 31years and above

(5) Gender of the owner? **[1]** Male **[2]** Female

(6) What is the education level of the respondent

[1] No Formal Education **[2]** Primary Level **[3]** Secondary Level **[4]** Tertiary Level

(7) What is your responsibility at the farm?

[1]Owner **[2]** Family member [3] Manager/Worker **[3]** Other (Specify)……

C. FARM CHARACTERISTICS

(1) Herd size [1] = ≥5 [2] = 6-10 [3] = 11-14 [4] = ≤15

(2) What is the type of cattle farming enterprise?

[1] = Dairy [2] = Beef [3] = Mixed

(3) Herd structure

[1] = Mature cows (lactating, in-calves, non-pg) [2] = Heifers [3] = Bulls [4] = Calves

(4) Type of production system

i. Intensive cattle movements and feeding are confined to the farm premises □

ii. Semi-intensive: Cattle are farm-fed □ Cattle are free-roaming □

(5) Which other animals are kept in the farm?

(i)	Poultry	[1] = Yes	[2] = No
(ii)	Pigs	[1] = Yes	[2] = No
(iii)	Shoats	[1] = Yes	[2] = No
(iv)	Donkey	[1] = Yes	[2] = No
(v)	Others (Specify)_____		

FARM MANAGEMENT PRACTICES

(6) What is the main type of animal housing? [1] = Indoor housing [2] = Outdoor housing

(7) Note the floor type

[1] = Earthen [2] = Concrete [3] = Others, Specify_____

(8) What are the main water sources present in cattle grazing fields;

[1] = Tap water [2] = Borehole [3] = Well [4] = River

[5] = Dams [6] = Others, Specify_____

(9) In case of outdoor housing; is the water source(s) named above shared with other cattle herds?

[1] = Yes [2] = No

(10) Where do you usually dispose wet manure/slurry on cattle grazing fields

[1] = Cattle grazing fields [2] = Allowed to dry before disposal [6] = Others, Specify_____

(11) Do you allow a rest period between spreading of manure, slurry or dirty water on grazing fields and resumption of grazing?

[1] = If yes, for how long? _____ [2] = No

(12) Do you co-graze cattle with other ruminants

[1] = If yes, which animals in particular_____ [2] = No

(13) Which feed do you give your cattle?

(i)	Silage	[1] = Yes	[2] = No
(ii)	Hay	[1] = Yes	[2] = No
(iii)	Straw	[1] = Yes	[2] = No

(14) Do you supplement your cattle feed with poultry litter and/or droppings?

[1] =Yes [2] = No

(15) Are there any biosecurity measures in place e.g. foot dip? [1] = Yes [2] = No

(16) Wetness and cleanliness of the enclosure **RATINGS**

[1] = very dry [2] = moderately dry [3] = Wet and dirty [4] = very wet, dirty

DISEASE HISTORY & ANTIMICROBIAL USE

(1) Which diseases and/or condition have you encountered in your farm in the last 6months? If yes, which ones?

……
……
……
……
……
……
……
……

(2) What actions do you take when your animals are sick? (*Please tick appropriately*)

[1] = Call a Vet/ Paravet [2] = Self-treat [3] = Do nothing

[4] = Others (Specify) _____

(3) If self-treat, what do you use? Or rather mention any drug used by a veterinarian in the last 6 months (Check from treatment records if any)

Treatment option	**Please specify the name (take photos of the drug/container/satchet)**
[1]	
[2]	
[3]	
[4]	
[5]	

(4) Where do you obtain information on the choice of antimicrobial to use?

[1] = Other farmers [2] = Agrovet owners [3] = Vet/paravets

[4] = Others (Specify) _____

(5) Have noticed or are you aware that drugs are failing in response upon treatment?

[1] = Yes [2] = No

.....Thank you for taking your time to fill this questionnaire.

Appendix 4: Features of selected sequences for genes encoding antimicrobial resistance and their assigned accession numbers [OQ389471 to OQ389473 for gyrA gene, OQ390085 and OQ390086 for tet (O), OQ421183 and OQ421184 for BlaOXA-61]

1) LOCUS OQ389471 233 bp DNA linear BCT 02-FEB-2023

DEFINITION Campylobacter jejuni strain 354B1 gyrase subunit A (gyrA) gene.
ACCESSION **OQ389471**
VERSION **OQ389471**
KEYWORDS .
SOURCE Campylobacter jejuni
ORGANISM Campylobacter jejuni
Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Campylobacter.
REFERENCE 1 (bases 1 to 233)
AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.
TITLE Antimicrobial usage, susceptibility profiles and resistance genes in Campylobacter isolated from cattle, chicken and water samples in Kajiado County, Kenya

##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##

FEATURES Location/Qualifiers
source 1..233
/organism="Campylobacter jejuni"
/mol_type="genomic DNA"
/strain="354B"
/isolation_source="Cattle faecal sample"
/specimen_voucher="354B"
/db_xref="taxon:197"
/country="Kenya"
/collection_date="29-Mar-22"
/collected_by="D. Wanja"
/note="[cultured bacterial source]"
gene <1..>233
/gene="gyrA"
CDS <1..>233
/gene="gyrA"
/note="[intronless gene]"
/codon_start=1
/transl_table=11
/product="Gyrase subunit A"
/translation="*RILYAMXNDEAKSRTXFVKSARIVGXVIGRYHPHGDTAVYDAL
VRMAQDFSMRYPSITGQGNFGXIDGDXAAAMRYT"
BASE COUNT 67 a 32 c 56 g 69 t 9 others
ORIGIN
1 tgaagaattt tatatgctat gcawaatgat gaggcaaaaa gtagaacagm wtttgtcaaa
61 tcagcccgta tagtgggkgm tgttataggt cgttatcatc cacatggaga tacagcagtt
121 tatgatgctt tggttagaat ggctcaagat ttttctatga gatatccaag tattacagga
181 caaggcaact ttggatwkat mgatggtgat rgcgctgctg cgatgcgtta tac

2) LOCUS OQ390085 561 bp DNA linear BCT 02-FEB-2023

DEFINITION Campylobacter jejuni Strain 376B tetracycline resistance ribosomal protection.

ACCESSION OQ390085
VERSION OQ390085
KEYWORDS .
SOURCE Campylobacter jejuni
ORGANISM Campylobacter jejuni
Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Campylobacter.
REFERENCE 1 (bases 1 to 561)
AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.
TITLE Antimicrobial usage, susceptibility profiles and resistance genes in Campylobacter isolated from cattle, chicken and water samples in Kajiado County, Kenya

##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##

FEATURES Location/Qualifiers
source 1..561
/organism="Campylobacter jejuni"
/mol_type="genomic DNA"
/strain="376B"
/isolation_source="Cattle faecal sample"
/specimen_voucher="376B"
/db_xref="taxon:197"
/country="Kenya"
/collection_date="04-Apr-22"
/collected_by="D. Wanja"
/note="[cultured bacterial source]"
gene <1..>561
/gene="tet(O)"
CDS <1..>561
/gene="tet(O)"
/note="[intronless gene]"
/codon_start=3
/transl_table=11
/product="tetracycline resistance ribosomal protection"
/translation="RFVYVRIYSGTLHLRDVIRISEKEKIKITEMCVPTNGELYSSDT
ACSGDIVILPNDVLQLNSILGNEILLPQRKFIENPLPMLQTTIAVKKSEQREILLGAL
KEISDGDPLLKYYVDTTTHEIILSFLGNVQMEVICAILEEKYHVEAEIKEPTIIYMER
PLRKAEYTIHIEVPPNPFWASVGLSI"

BASE COUNT 183 a 93 c 119 g 166 t
ORIGIN

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      1 ggcgttttgt ttatgtgcgt atatatagcg gaacattgca tttgagggat gttattagaa
     61 tatctgaaaa agagaaaata aaaatcacag agatgtgtgt tccgacaaac ggtgaattat
    121 attcatccga tacagcctgc tctggtgata ttgtaatttt accaaatgat gttttgcagc
    181 taaacagtat tttggggaac gaaatactgt tgccgcagag aaaatttatt gaaaatcctc
    241 tccctatgct ccaaacaacg attgcagtaa agaaatctga acagcgggaa atattgcttg
    301 gggcacttaa agaaatttca gatggcgacc ctcttttaaa atattatgtg gatactacaa
    361 cgcatgagat tatactttct tttttgggga atgtgcagat ggaagtcatt tgtgccatcc
    421 ttgaggaaaa atatcatgtg gaggcagaaa taaaagagcc tactattata tatatggaaa
    481 gaccgcttag aaaagcagaa tataccatcc acatagaagt cccgccaaat cctttctggg
    541 cttctgtcgg gttgtccata t
```

3) LOCUS OQ421183 377 bp DNA linear BCT 10-FEB-2023

DEFINITION Campylobacter jejuni strain 342B1 Beta-lactamase (BlaOXA-61)gene.

ACCESSION OQ421183

VERSION **OQ421183**

KEYWORDS .

SOURCE Campylobacter jejuni

ORGANISM Campylobacter jejuni

Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Campylobacter.

REFERENCE 1 (bases 1 to 377)

AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.

TITLE Antimicrobial usage, susceptibility profiles and resistance genes in Campylobacter isolated from cattle, chicken and water samples in Kajiado County, Kenya

.

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..377

/organism="Campylobacter jejuni"

/mol_type="genomic DNA"

/strain="342B1"

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/specimen_voucher="342B1"

/db_xref="taxon:197"

/country="Kenya"

/collection_date="29-Mar-22"

/collected_by="D. Wanja"

/note="[cultured bacterial source]"

gene <1..>377

/gene="blaOXA-61"

CDS <1..>377

/gene="blaOXA-61"

/note="[intronless gene]"

/codon_start=3

/transl_table=11

/product="Beta-lactamase"

/translation="EYNTSGTFVFYDGKTWASNDFSRAMETFSPASTFKIFNALIALD
SGVIKTKKEIFYHYRGEKVFLSSWAQDMNLSSAIKYSNVLAFKEVARRIGIKTMQEYL
NKLHYGNAKISKIDTFWLDNSLK"

BASE COUNT 129 a 53 c 65 g 130 t

ORIGIN

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      1 tagagtataa tacaagcggc acttttgttt tttatgatgg aaaaacttgg gcgagtaacg
     61 acttttcaag ggctatggag actttctctc ccgcttccac ttttaaaatt tttaatgctc
    121 taattgcact tgatagtggt gtgataaaaa ctaaaaaaga aatttttttat cactatagag
    181 gtgaaaaagt atttttatct tcttgggcgc aagatatgaa tttaagttca gctataaaat
    241 attctaatgt tcttgctttt aaagaagtgg caagaagaat tggtatcaaa actatgcaag
    301 aatatttaaa caagcttcat tatggtaatg ctaaaatttc caagatcgat acttttggc
    361 ttgacaactc actaaaa
```

Appendix 5: Features of selected sequences for 16S rRNA gene specific for identification of genus *Campylobacter* and their assigned accession numbers (OQ363834 to OQ363853)

1) LOCUS OQ363834 851 bp DNA linear BCT 02-FEB-2023

DEFINITION Campylobacter jejuni strain 299C 16S ribosomal RNA gene, partial sequence.

ACCESSION OQ363834
VERSION OQ363834
KEYWORDS .
SOURCE Campylobacter jejuni
ORGANISM Campylobacter jejuni
Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales;
Campylobacteraceae; Campylobacter.
REFERENCE 1 (bases 1 to 851)
AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.
TITLE Virulence factors and genetic relatedness of Campylobacter isolates from water, cattle and chicken samples

COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..851
/organism="Campylobacter jejuni"
/mol_type="genomic DNA"
/strain="299C"
/db_xref="taxon:197"
rRNA <1..>851
/product="16S ribosomal RNA"
ORIGIN
1 ttctaatggc ttaaccatta aactgcttgg gaaactgata gtctagagtg agggagaggc
61 agatggaatt ggtggtgtag gggtaaaatc cgtagatatc accaagaata cccattgcga
121 aggcgatctg ctggaactca actgacgcta aggcgcgaaa gcgtggggag caaacaggat
181 tagataccct ggtagtccac gccctaaacg atgtacacta gttgttgggg tgctagtcat
241 ctcagtaatg cagctaacgc attaagtgta ccgcctgggg agtacggtcg caagattaaa
301 actcaaagga atagacgggg acccgcacaa gcggtggagc atgtggttta attcgaagat
361 acgcgaagaa ccttacctgg gcttgatatc ctaagaacct tttagagata agagggtgct
421 agcttgctag aacttagaga caggtgctgc acggctgtcg tcagctcgtg tcgtgagatg
481 ttgggttaag tcccgcaacg agcgcaaccc acgtatttag ttgctaacgg ttcggccgag
541 cactctaaat agactgcctt cgtaaggagg aggaaggtgt ggacgacgtc aagtcatcat
601 ggcccttatg cccagggcga cacacgtgct acaatggcat atacaatgag acgcaatacc
661 gcgaggtgga gcaaatctat aaaatatgtc ccagttcgga ttgttctctg caactcgaga
721 gcatgaagcc ggaatcgcta gtaatcgtag atcagccatg ctacggtgaa tacgttcccg
781 ggtcttgtac tcaccgcccg tcacaccatg ggagttgatt tcactcgaag ccggaatact
841 aaactagttt a
//

2) LOCUS OQ363835 854 bp DNA linear BCT 02-FEB-2023

DEFINITION Campylobacter jejuni strain 176B 16S ribosomal RNA gene, partial sequence.

ACCESSION OQ363835
VERSION OQ363835
KEYWORDS .
SOURCE Campylobacter jejuni
ORGANISM Campylobacter jejuni
Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales;
Campylobacteraceae; Campylobacter.
REFERENCE 1 (bases 1 to 854)
AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.
TITLE Virulence factors and genetic relatedness of Campylobacter isolates from water, cattle and chicken samples

COMMENT ##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..854
/organism="Campylobacter jejuni"
/mol_type="genomic DNA"
/strain="176B"
/db_xref="taxon:197"
rRNA <1..>854
/product="16S ribosomal RNA"
ORIGIN

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  61 gaattggtgg tgtaggggta aaatccgtag atatcaccaa gaatacccat tgcgaaggcg
 121 atctgctgga actcaactga cgctaaggcg cgaaagcgtg gggagcaaac aggattagat
 181 accctggtag tccacgccct aaacgatgta cactagttgt tggggtgcta gtcatctcag
 241 taatgcagct aacgcattaa gtgtaccgcc tggggagtac ggtcgcaaga ttaaaactca
 301 aaggaataga cggggacccg cacaagcggt ggagcatgtg gtttaattcg argatacgcg
 361 aagaacctta cctgggcttg atatcctaag aaccttwtag agatawgagg gtgctagctt
 421 gctagaactt agagacaggt gctgcacggc tgtcgtcagc tcgtgtcgtg agatgttggg
 481 ttaagtcccg caacgagcgc aacccacgta tttagttgct aacggttcgg ccgagcactc
 541 taaatagact gccttcgtaa ggaggaggaa ggtgtggacg acgtcaagtc atcatggccc
 601 ttatgcccag ggcgacacac gtgctacaat ggcatataca atgagacgca ataccgcgag
 661 gtggagcaaa tctataaaat atgtcccagt tcggattgtt ctctgcaact cgagagcatg
 721 aagccggaat cgctagtaat cgtagatcag ccatgctacg gtgaatacgt tcccgggtct
 781 tgtactcacc gcccgtcaca ccatgggagt tgatttcact cgaagccgga atactaaact
 841 agtttacccg tcca
```

Appendix 6: Features of selected sequences for ceuE gene specific for *Campylobacter coli* identification and their assigned accession numbers (OQ389474 to OQ389481)

(1) LOCUS OQ389474 463 bp DNA linear BCT 02-FEB-2023

DEFINITION Campylobacter coli cattle strain 342B1 lipoprotein (ceuE) gene.
ACCESSION **OQ389474**
VERSION **OQ389474**
KEYWORDS .
SOURCE Campylobacter coli (Campylobacter hyoilei)
ORGANISM Campylobacter coli
Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Campylobacter.
REFERENCE 1 (bases 1 to 463)
AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.
TITLE Virulence factors and genetic relatedness of Campylobacter isolates from water, cattle and chicken samples

##Assembly-Data-START##
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##

FEATURES Location/Qualifiers
source 1..463
/organism="Campylobacter coli"
/mol_type="genomic DNA"
/strain="342B1"
/isolation_source="Cattle faecal sample"
/specimen_voucher="342B1"
/db_xref="taxon:195"
/country="Kenya"
/collection_date="29-Mar-22"
/collected_by="D. Wanja"
/note="[cultured bacterial source]"
gene <1..>463
/gene="ceuE"
CDS <1..>463
/gene="ceuE"
/note="[intronless gene]"
/codon_start=1
/transl_table=11
/product="Lipoprotein"
/translation="XLKIAPTMFVGLDNANFLSSFENNVLSVAKLYGLEKEASEKIAD
IKNEIEQAKSIVDEDKKALIVLTNSNKISAFGPQSRFGIIHDVLGINAVDENVKVGTH
GKSINSEFILEKNPDYLFVVDRNIIVGNKERAQGILDNALVTKTNAATNNKI"
BASE COUNT 180 a 64 c 76 g 140 t 3 others
ORIGIN
1 ttwttgaaaa ttgctccaac tatgtttgta ggacttgata atgcaaattt cttaagctct
61 tttgaaaaca atgttttaag tgttgcaaaa ctttatggyt tagaaaaaga agcttctgaa
121 aaaattgcag atattaaaaa tgagatagaa caagcaaaaa gcatagtaga tgaagataaa
181 aaagctctta ttgttctaac caattctaac aaaatttccg cttttggacc tcaatctcgc
241 tttggaatca ttcatgatgt tttaggaatc aatgctgtgg atgaaaatgt aaaagtaggc
301 acacatggaa aaagcattaa ttctgaattt atactagaaa aaaatcctga ttatctattt
361 gtagttgata gaaatatcat tgtgggyaat aaagaacgcg cacaaggcat acttgataat
421 gcacttgtaa ctaaaaccaa cgctgctaca aataataaaa tca
//

(2) LOCUS OQ389475 459 bp DNA linear BCT 02-FEB-2023

DEFINITION Campylobacter coli chicken strain 284Cm lipoprotein (ceuE)gene.

ACCESSION **OQ389475**

VERSION **OQ389475**

KEYWORDS .

SOURCE Campylobacter coli (Campylobacter hyoilei)

ORGANISM Campylobacter coli

Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Campylobacter.

REFERENCE 1 (bases 1 to 459)

AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.

TITLE Virulence factors and genetic relatedness of Campylobacter isolates from water, cattle and chicken samples

##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..459

/organism="Campylobacter coli"

/mol_type="genomic DNA"

/strain="284Cm"

/isolation_source="Chicken faecal sample"

/specimen_voucher="284Cm"

/db_xref="taxon:195"

/country="Kenya"

/collection_date="10-Mar-22"

/collected_by="D. Wanja"

/note="[cultured bacterial source]"

gene <1..>459

/gene="ceuE"

CDS <1..>459

/gene="ceuE"

/note="[intronless gene]"

/codon_start=3

/transl_table=11

/product="Lipoprotein"

/translation="KIAPTMFVGLDNANFLSSFENNVLSVAKLYGLEKEASEKIADIK
NEIEQAKSIVDEDKKALIVLTNSNKISAFGPQSRFGIIHDVLGINAVDENVKVGTHGK
SINSEFILEKNPDYLFVVDRNIIVGNKERAQGILDNALVTKTNAATNNKI"

BASE COUNT 180 a 65 c 76 g 137 t 1 others

ORIGIN

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      1 tgaaaattgc tccaactatg tttgtaggac ttgataatgc aaatttytta agctcttttg
     61 aaaacaatgt tttaagtgtt gcaaaacttt atggcttaga aaaagaagct tctgaaaaaa
    121 ttgcagatat taaaaatgag atagaacaag caaaaagcat agtagatgaa gataaaaaag
    181 ctcttattgt tctaaccaat tctaacaaaa tttccgcttt tggacctcaa tctcgctttg
    241 gaatcattca tgatgtttta ggaatcaatg ctgtggatga aaatgtaaaa gtaggcacac
    301 atggaaaaag cattaattct gaatttatac tagaaaaaaa tcctgattat ctatttgtag
    361 ttgatagaaa tatcattgtg ggcaataaag aacgcgcaca aggcatactt gataatgcac
    421 ttgtaactaa aaccaacgct gctacaaata ataaaatca
```

//

Appendix 7: Features of selected sequences for *hipO* gene specific for *C. jejuni* identification and their assigned accession numbers (OQ390087 to OQ390094)

(1) LOCUS OQ390087 578 bp DNA linear BCT 02-FEB-2023

DEFINITION Campylobacter jejuni cattle strain 376B hippurate hydrolase (hipO) gene.
ACCESSION **OQ390087**
VERSION **OQ390087**
KEYWORDS .
SOURCE Campylobacter jejuni
ORGANISM Campylobacter jejuni
Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales; Campylobacteraceae; Campylobacter.
REFERENCE 1 (bases 1 to 578)
AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.
TITLE Virulence factors and genetic relatedness of Campylobacter isolates from water, cattle and chicken samples
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
source 1..578
/organism="Campylobacter jejuni"
/mol_type="genomic DNA"
/strain="376B"
/isolation_source="Cattle faecal sample"
/specimen_voucher="376B"
/db_xref="taxon:197"
/country="Kenya"
/collection_date="04-Apr-22"
/collected_by="D. Wanja"
/note="[cultured bacterial source]"
gene <1..>578
/gene="hipO"
CDS <1..>578
/gene="hipO"
/note="[intronless gene]"
/codon_start=2
/transl_table=11
/product="Hippurate hydrolase"
/translation="SDSYSIEVIGRGGHGSAPEKAKDPIYAASLLVVALQSIVSRNVD
PQNSAVVSIGAFNAGHAFNIIPDIATIKMSVRALDNETRKLTEEKIYKICKGLAQAND
IEIKINKNVVAPVTMNNDEAVDFTSEVAKELFGEKNCEFNHRPLMASEDFGFFCEMKK
CAYAFLENENDIYLHNSSYVFNDKLLARAASY"
BASE COUNT 197 a 69 c 119 g 193 t
ORIGIN
1 ttcggatagt tatagcattg aagttattgg aagaggtggt catggaagtg ctccagaaaa
61 ggcaaaagat cctatttatg ctgcttcttt gcttgttgtg gctttgcaaa gtatagtatc
121 tcgcaatgtt gatccccaaa attcagcagt tgtaagcata ggagctttta atgcaggaca
181 tgcttttaat atcattccag atattgcaac gattaaaatg agtgttagag cattagataa
241 tgaaactaga aagctaactg aagaaaaaat ttataaaatt tgtaaaggtc ttgcacaggc
301 taatgatata gagattaaaa tcaataaaaa tgttgttgca ccagtgacta tgaataacga
361 tgaagctgtg gattttacta gtgaggttgc aaaagaatta tttggcgaaa aaaattgtga
421 atttaatcat cgtcctttaa tggcaagtga ggattttgga ttttttgcg aaatgaaaaa
481 atgtgcctat gctttttag aaaatgaaaa cgacatttat ttacataatt ctagttatgt
541 ttttaatgat aagcttttag ctagggctgc aagttatt
//

(2) LOCUS **OQ390088** 581 bp DNA linear BCT 02-FEB-2023
DEFINITION Campylobacter jejuni chicken strain 368C1 hippurate hydrolase (hipO) gene.
ACCESSION **OQ390088**
VERSION **OQ390088**
KEYWORDS .
SOURCE Campylobacter jejuni
ORGANISM Campylobacter jejuni
Bacteria; Pseudomonadota; Epsilonproteobacteria; Campylobacterales;
Campylobacteraceae; Campylobacter.
REFERENCE 1 (bases 1 to 581)
AUTHORS Wanja, D.W., Mbuthia, P.G., Bebora, L.C. and Aboge, G.O.
TITLE Virulence factors and genetic relatedness of Campylobacter isolates from water, cattle and chicken samples
Sequencing Technology :: Sanger dideoxy sequencing
##Assembly-Data-END##
FEATURES Location/Qualifiers
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/mol_type="genomic DNA"
/strain="368C1"
/isolation_source="Chicken faecal sample"
/specimen_voucher="368C1"
/db_xref="taxon:197"
/country="Kenya"
/collection_date="04-Apr-22"
/collected_by="D. Wanja"
/note="[cultured bacterial source]"
gene <1..>581
/gene="hipO"
CDS <1..>581
/gene="hipO"
/note="[intronless gene]"
/codon_start=3
/transl_table=11
/product="Hippurate hydrolase"
/translation="SDSYSIEVIGRGGHGSAPEKAKDPIYAASLLVVALQSIVSRNVD
PQNSAVVSIGTFNAGHAFNIIPDIATIKMSVRALDNETRKLTEEKIYKICKGLAQAND
IEIKINKNVVAPVTMNNDEAVDFASEVAKELFGEKNCEFNHRPLMASEDFGFFCEMKK
CAYAFLENENDIYLHNSSYVFNDKLLARAASYY"
BASE COUNT 200 a 71 c 117 g 193 t
ORIGIN
1 cttcggatag ttatagcatt gaagttattg gaagaggtgg tcatggaagt gctccagaaa
61 aggcaaaaga tcctatttat gctgcttctt tacttgttgt ggctttacaa agcatagtat
121 ctcgcaatgt tgatccccaa aattcagcag ttgtaagcat aggaactttt aatgcaggac
181 atgcttttaa tatcattcca gatattgcaa cgattaaaat gagtgttaga gcattagata
241 atgaaactag aaagctaact gaagaaaaaa tttataaaat ttgtaaaggt cttgcacagg
301 ctaatgatat agagattaaa atcaataaaa atgttgttgc accagtgact atgaataacg
361 atgaagctgt ggattttgct agtgaggttg caaaagaatt atttggcgaa aaaaattgtg
421 aatttaatca tcgtccttta atggcaagtg aggattttgg atttttttgc gaaatgaaaa
481 aatgtgccta tgctttttta gaaaatgaaa acgacattta tttacataat tctagttatg
541 tttttaatga taagctttta gctagggctg caagttatta t
//

Appendix 8: List of publications and conference paper from this research work

1) **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**. https://doi.org/10.1155/2022/1526641.
2) **Wanja, D. W., Mbuthia, P. G., Bebora, L. C., Aboge, G. O., & Ogoti, B. (2023):** Antimicrobial usage, susceptibility profiles and resistance genes in Campylobacter isolated from cattle, chicken and water samples in Kajiado County, Kenya. *International Journal of Microbiology*, **2023**. https://doi.org/10.1155/2023/8394605.
3) **Wanja, D. W., Mbuthia, P. G., Bebora, L. C., Aboge, G. O., Muasya, D.W., & Ofwete, R. (2023):** Risk Factors Associated with Occurrence of Thermophilic Campylobacter in Cattle Herds Raised on Integrated Small-Scale Farms in Kajiado County, Kenya. *International Journal of Veterinary Science,* **x(x):** xxxx. https://doi.org/10.47278/journal.ijvs/2023.040. *In press*
4) **Wanja, D. W., Mbuthia, P. G., Bebora, L. C., & Aboge, G. O. (2023).** Molecular detection of virulence genes in *Campylobacter* isolated from cattle, chicken and water. *Onderstepoort Journal of Veterinary Research*, Under Review
5) **Wanja D.W., Mbuthia P.G., Aboge G. O., and Bebora L.C. (2021):** Preliminary Findings on Effects of Seasonality and Climate on Thermophilic Campylobacters Occurrence in Cattle, Chicken and Water in Kajiado County. In: *Proc. of KCSAP 1st Scientific Conference* held in Lake Naivasha Resort, Naivasha, Kenya, November 22nd - 26th, ID no 12, p.65.