

الجمهورية الجزائرية الديمقراطية الشعبية  
Democratic and Popular Republic of Algeria/République Algérienne Démocratique et Populaire  
وزارة التعليم العالي والبحث العلمي  
Ministry of Higher Education and Scientific Research  
Ministère de l'Enseignement Supérieur et de la Recherche Scientifique  
المدرسة الوطنية العليا للبيطرة ربيع بوشامة  
Higher National Veterinary School Rabie Bouchama  
École Nationale Supérieure Vétérinaire Rabie Bouchama



N° d'ordre : 06/Master/2025

**FINAL YEAR PROJECT**  
In Partial Fulfillment of the Requirements for **Master** degree  
**Field:** Natural and Life Sciences  
**Major:** Veterinary Sciences

---

# INVESTIGATING CAMPYLOBACTER IN CAMEL FE- CES: ISOLATION, PREVALENCE AND ANTIMICRO- BIAL RESISTANCE IN SOUTHERN ALGERIA

---

Presented by:

**BOUDOUH Ala Roua**  
**KERAIS Lynda Hayat**

Publicly defended on 25/06/2025 before the jury composed of:

**President:** BAROUDI Djamel – Professor (ENSV)

**Supervisor:** GUESSOUM Meryem – Senior Lecturer (ENSV)

**Examiner 1 :** BAAZIZI Ratiba – Professor (ENSV)

Année universitaire : 2024 /2025

**Honor Statement**

I, the undersigned, **KERAIS Lynda Hayat**, hereby declare that I am fully aware that plagiarism of documents, or any part thereof, in any form or medium, including the internet, constitutes a violation of copyright and a clear act of fraud.

Accordingly, I commit to citing all the sources I have used in writing this thesis.

**Signature:**

**Honor Statement**

I, the undersigned, **BOUDOUH Ala Roua**, hereby declare that I am fully aware that plagiarism of documents, or any part thereof, in any form or medium, including the internet, constitutes a violation of copyright and a clear act of fraud.

Accordingly, I commit to citing all the sources I have used in writing this thesis

**Signature:**

# *Acknowledgment*

We would like to extend our sincere thanks to our supervisor, **Dr. Meryem GUESSOUM**, Senior Lecturer at the National Higher School of Veterinary Medicine of Algiers. We truly appreciate her guidance, availability, and continuous support throughout this work. Her positive attitude and vibrant energy made a significant difference in our interactions, and for that, we are incredibly grateful.

Our heartfelt thanks go to our professors and jury members. We are especially honored by **Djamel BAROUDI** acceptance to preside over our thesis jury. We also warmly thank **Ratiba BAAZIZI** for her commitment, expertise, and the valuable time they devoted to evaluating our work.

I would like to express my gratitude to my project partner. Your unwavering cooperation, calm support during challenging times, and genuine companionship have meant the world to me. This accomplishment reflects our shared efforts, and I feel truly fortunate to have experienced this journey with you. Our success reflects not only our dedication but also the bond we have built along the way.

We sincerely thank **Dr Hakima MESSAOUDENE** for her support, expertise and guidance in the laboratory work

We are deeply thankful to everyone who, in one way or another, contributed to the successful completion of this work.

Finally, our sincere appreciation goes to all the teachers who accompanied and guided us throughout our veterinary journey.



**Ala and Lynda**

*To my beloved **mother** and **father**, my role models and my number one supporters, your encouragement and prayers have been a treasured source of strength throughout this journey. Your unwavering belief in me gave me the courage to persevere during the toughest moments. Your wisdom and guidance have been a constant beacon, lighting my path even when the way seemed uncertain. This achievement is as much yours as it is mine, and I am forever grateful for your patience, your sacrifices, and the unconditional love that has carried me forward. I hope to make you proud and honor the values you have instilled in me every day of my life*

*To my siblings **Ayoub**, **Iyed**, **Assil**, my pride and joy*

*To all my family **BOUDOUEH**, **KALLA** and to every person who believed in me.*

*To my dear friends and classmates who shared this journey with me*

*To those few who always showed up (**Rania**, **Marwa**, **Ghada**) thank you for being real*

*May Allah SWT accept this humble effort, forgive my shortcomings, and make this journey a means of benefit for myself and others.*

**Alaa**

*This research is lovingly dedicated to the angel in my life, my Mother, and to the solid foundation of my life, my Father, who both supported and believed in me every step of the way. Their love, endless wishes, and prayers for my success gave me the strength to handle every task with discipline and enthusiasm. They are my constant source of inspiration.*

*To my joy in life my dear brothers, Mustapha Riad and Mouhamed Imad-Alddin.*

*To my loving family, the Keraïs and Rekkad.*

*To all the wonderful supporters and friends who have stood by me.*

**LYNDA**

## List of figures

<b>Figure 1:</b> Campylobacter colonies on mCCDA plate after 48 hours of microaerobic incubation at 42°C. Medium off-white glistening/shiny and spreading colonies (plate (a)) and the small gray colonies on the media (plate (b)) (Wanja et al., 2022).	21
<b>Figure 2:</b> Small Gram-negative short or curved rods to coccobacilli of Campylobacter isolate (×1000) with characteristic “seagull” shaped curved rods (Wanja et al., 2022).	22
<b>Figure 3:</b> Overview of pathways of Campylobacter transmission in both Human and animals (Amin et al., 2022).	28
<b>Figure 4:</b> Environmental reservoirs, routes of transmission, and clinical manifestations associated with Campylobacter species. Abbreviations: IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome. Question marks indicate conditions for which a role for Campylobacter is implicated but not certain (Kaakoush et al., 2015).	33
<b>Figure 5:</b> Photo micrograph of <i>Campylobacter jejuni</i> in the process of dividing. (Parker, Institute of Food Research, UK).	35
<b>Figure 6:</b> (A) Microaerophilic container, (B) Preston enrichment broth tubes	41
<b>Figure 7:</b> Preparation and isolation of Campylobacter spp. Using Karmali Selective Medium: (A) Media Weighing, (B) Autoclaving, (C) Plate Pouring, (D) Plate Storage (E) Incubator Setup, (F) Sample Inoculation, (G) Gas Pack (Microaerophilic), (K) Incubation in Anaerobic Jar. (Images documented during the study)	42
<b>Figure 8:</b> Colony appearance of Campylobacter jejuni strains in karmali medium	43
<b>Figure 9 :</b> Microscopic Morphology of Campylobacter on stained Smears.	43
<b>Figure 10 :</b> Positive Oxydase test showing the characteristic dark purple coloration in Campylobacter (Images documented during the study).	44
<b>Figure 11:</b> Application of API Campy system for campylobacter Identification	45
<b>Figure 12:</b> Steps of Antibiotics test: (A) Preparation of the bacterial; (B) Placement of antibiotic disks; (C) Incubation under microaerophilic conditions; (D) Reading of results (Images documented during the study)	47
<b>Figure 13:</b> Graph demonstrating the positive cases of Campylobacter depending on the clinical status in camels.	48
<b>Figure 14 :</b> Graph demonstrating the cases of Campylobacter jejuni and Campylobacter coli depending on the clinical status in camels.	48
<b>Figure 15:</b> Graph demonstrating the difference between the species of <i>C.jejuni</i> and <i>C.coli</i> depending on the clinical status in camels.	49

**Figure 16 :** Graph demonstrating the positive cases of *Campylobacter* depending on the geographic distribution of the samples collected from camels. .... 49

**Figure 17:** Graph demonstrating the difference between *Campylobacter jejuni* and *Campylobacter coli* depending the geographic distribution of the samples collected from camels..... 51

**Figure 18:** Graph demonstrating the antimicrobial resistance of the 12 *Campylobacter* isolates collected from camels..... 52

**Figure 19 :** Graph demonstrating the antimicrobial resistance rate in 12 *Campylobacter* isolates collected from camels..... 53

**Figure 20 :** Graph demonstrating the antimicrobial resistance rate the multidrug and the susceptible in the 12 *Campylobacter* isolates collected from camels. .... 53



**List of tables**

<b>Tableau 1:</b> Attributes of <i>Campylobacter</i> genus bacteria allowing them to infect and survive in a host organism (Chlebicz and Śliżewska, 2018). .....	29
<b>Tableau 2:</b> Overview of <i>Campylobacter</i> Species: Reservoirs, human Impact, and key research focus (Igwaran and Okoh., 2020). .....	32
<b>Tableau 3:</b> Distribution of <i>Campylobacter</i> species according to the clinical condition of dromedaries and the Wilayas. ....	50
<b>Tableau 4:</b> Antimicrobial resistance profile of the 12 <i>Campylobacter</i> isolates. ....	52
<b>Tableau 5:</b> Antimicrobial resistance profile of the 12 <i>Campylobacter</i> isolates (n = 12). .....	54

**List of annexes**

<b>Annex 1: Gram staining Technique .....</b>	<b>58</b>
<b>Annex 2: Technique for fresh preparation examination .....</b>	<b>58</b>

**Abstract**

*Campylobacter jejuni*, *Campylobacter coli* are recognized as leading causes of campylobacteriosis a major foodborne zoonotic disease and a growing public health concern, these pathogens are commonly found in intestinal tracts of animals and transmitted to humans through contaminated food and water or through direct contact with infected animals.

This study aimed to detect and identify these pathogens in camels by analyzing **120** fecal samples collected from four regions in southern Algeria: Oued souf, Ouagla, Biskra, and Boussada. Overall, **10%** of the samples tested positive for *Campylobacter*, with *C.jejuni* accounting for **6.7%** and *C.coli* for **3,3%** . Notably, **83%** of the positive samples were obtained from animals exhibiting digestive symptoms.

Isolation was performed on selective Karmali medium under microaerophilic conditions, followed by disk diffusion testing to evaluate antimicrobial resistance patterns. Among the antibiotics tested, chloramphenicol exhibited by the highest resistance rate **83,3%** followed by Amoxicillin-clavulanic acid **58,3%**, Ciprofloxacin **41,7%**, Nalidixin acid **33,3%**, Ceftriaxone **8,3%**. The isolates demonstrated alarming levels of resistance to several commonly used antibiotics, highlighting potential challenges in treatment and the need for continuous antimicrobial surveillance.

**Key words:** *Campylobacter coli*, *Campylobacter jejuni*, Camels, Prevalence, Antibiotic resistance, Foodborne disease.

## Résumé

*Campylobacter jejuni* et *Campylobacter coli* sont reconnus comme les principales causes de campylobactériose, une zoonose alimentaire majeure et un problème de santé publique croissant. Ces agents pathogènes sont fréquemment présents dans le tractus intestinal des animaux et transmis à l'homme par l'eau et les aliments contaminés, ou par contact direct avec des animaux infectés.

Cette étude visait à détecter et à identifier ces agents pathogènes chez les dromadaires en analysant 120 échantillons fécaux prélevés dans quatre régions du sud algérien : Oued Souf, Ouagla, Biskra et Boussada. Au total, 10 % des échantillons se sont révélés positifs à *Campylobacter*, *C. jejuni* représentant 6,7 % et *C. coli* 3,3 %. Il est à noter que 83 % des échantillons positifs provenaient d'animaux présentant des symptômes digestifs.

L'isolement a été réalisé sur milieu sélectif de Karmali en conditions microaérophiles, suivi d'un test de diffusion sur disque pour évaluer les profils de résistance aux antimicrobiens. Parmi les antibiotiques testés, le chloramphénicol présentait le taux de résistance le plus élevé (83,3 %), suivi de l'amoxicilline-acide clavulanique (58,3 %), de la ciprofloxacine (41,7 %), de la nalidixine acide (33,3 %) et de la ceftriaxone (8,3 %). Les isolats ont montré des niveaux de résistance alarmants à plusieurs antibiotiques couramment utilisés, soulignant les difficultés potentielles de traitement et la nécessité d'une surveillance antimicrobienne continue.

**Mots clés :** *Campylobacter coli*, *Campylobacter jejuni*, Chameaux, Prévalence, résistance aux antibiotiques, Maladie d'origine alimentaire.

## ملخص

تُعتبر بكتيريا العطيفة الصائمية (*Campylobacter jejuni*) والعطيفة القولونية (*Campylobacter coli*) من الأسباب الرئيسية لداء العطيفة، وهو مرض حيواني المنشأ منقول بالغذاء، ويُشكل مصدر قلق متزايد على الصحة العامة. توجد هذه البكتيريا الممرضة بشكل شائع في أمعاء الحيوانات، وتنتقل إلى البشر من خلال الطعام والماء الملوثين أو من خلال الاتصال المباشر بالحيوانات المصابة.

هدفت هذه الدراسة إلى الكشف عن هذه البكتيريا وتحديدتها في الإبل من خلال تحليل 120 عينة براز جُمعت من أربع مناطق في جنوب الجزائر: وادي سوف، ووقلة، وبسكرة، وبوسادة. بشكل عام، أظهرت نتائج فحوصات العطيفة الصائمية (*Campylobacter jejuni*) وجود 6.7% منها، بينما بلغت نسبة 3.3% منها بكتيريا العطيفة القولونية. والجدير بالذكر أن 83% من العينات الإيجابية تم الحصول عليها من حيوانات تظهر عليها أعراض هضمية.

تم عزل البكتيريا باستخدام وسط كرملي انتقائي في ظروف هوائية دقيقة، تلاه اختبار انتشار القرص لتقييم أنماط مقاومة مضادات الميكروبات. من بين المضادات الحيوية المختبرة، أظهر الكلورامفينيكول أعلى معدل مقاومة بنسبة 83.3%، يليه حمض الأموكسيسيلين-كلافولانيك بنسبة 58.3%، ثم سيبروفلوكساسين بنسبة 41.7%، ثم حمض الناليديكسين بنسبة 33.3%، ثم سيفترياكسون بنسبة 8.3%. أظهرت العزلات مستويات مقاومة مثيرة للقلق للعديد من المضادات الحيوية شائعة الاستخدام، مما يُبرز التحديات المحتملة في العلاج والحاجة إلى مراقبة مستمرة للمضادات الحيوية.

**الكلمات المفتاحية:** كامبيلوباكتر كولاي، كامبيلوباكتر جيجوني، الإبل، الانتشار، مقاومة المضادات الحيوية، الأمراض المنقولة بالغذاء.

**TABLE OF CONTENTS****TABLE OF CONTENTS****INTRODUCTION**

TABLE OF CONTENTS .....	14
INTRODUCTION .....	14
<b>I. BACKGROUND.....</b>	<b>18</b>
<b>II. THE GENUS OF <i>CAMPYLOBACTER</i> .....</b>	<b>19</b>
<b>III. TAXONOMY .....</b>	<b>20</b>
<b>IV. MORPHOLOGY AND STRUCTURE.....</b>	<b>20</b>
IV.1. MACROSCOPIC .....	21
IV.2. MICROSCOPIC.....	22
<b>V. GROWTH AND SURVIVAL CHARACTERISTICS.....</b>	<b>22</b>
V.1. PHYSIOLOGY AND SURVIVAL MECHANISMS.....	23
<b>I. EPIDEMIOLOGY AND TRANSMISSION .....</b>	<b>25</b>
I.1. PREVALENCE IN DIFFERENT REGIONS .....	25
I.2. TRANSMISSION ROUTES .....	26
<b>II. PATHOGENICITY OF <i>CAMPYLOBACTER</i> .....</b>	<b>28</b>
II.1. OTHER VIRULENCE MECHANISM IN <i>CAMPYLOBACTER</i> SPECIES .....	30
<b>III. CLINICAL MANIFESTATIONS .....</b>	<b>30</b>
III.1. IN ANIMALS .....	30
III.2. IN HUMANS .....	31
<b>IV. DIAGNOSIS OF <i>CAMPYLOBACTER</i> .....</b>	<b>33</b>
IV.1. DETECTION ISOLATION AND CONFIRMATION .....	34
IV.2. LABORATORY TESTING FOR <i>CAMPYLOBACTER</i> INFECTION .....	35
<b>V. PREVENTION AND TREATMENT OF <i>CAMPYLOBACTER</i>.....</b>	<b>36</b>
<b>I. MATERIALS AND METHODS .....</b>	<b>38</b>
I.1. MATERIALS .....	38
I.1.1. <i>Workplace and study period</i> .....	38

I.1.2. Sample Collection .....	38
I.1.3. Sampling Materials .....	38
I.1.4. Laboratory Equipment and Reagents.....	39
I.1.5. Sample Transport Conditions.....	39
I.2. METHODS .....	40
I.2.1. Isolation of Thermotolerant <i>Campylobacter jejuni</i> and <i>Campylobacter coli</i> .....	40
<b>II. RESULTS AND DISCUSSION .....</b>	<b>48</b>
II.1. OVERALL PREVALENCE AND DISTRIBUTION OF CAMPYLOBACTER SPECIES ....	48
II.2. ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF CAMPYLOBACTER ISOLATES ..	51
<b>III. DISCUSSION .....</b>	<b>54</b>
<b>IV. CONCLUSION AND RECOMMENDATIONS.....</b>	<b>56</b>
<b>REFERENCES .....</b>	<b>59</b>

## Introduction

The prevalence of thermotolerant *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, constitutes a major public health concern, as these organisms rank among the leading causes of foodborne illnesses worldwide (**Rawat et al., 2018**). These bacteria predominantly colonize the intestinal tracts of a wide range of domestic and wild animals, with poultry identified as the principal reservoir. Notably, these animals often carry *Campylobacter* asymptomatically, contributing to the silent spread of infection through the food chain (**Silvia et al., 2011**).

Transmission to humans typically occurs via the consumption of contaminated animal-derived products, especially undercooked poultry meat, unpasteurized milk, or untreated water (**Zanetti et al., 1996**). Campylobacteriosis, the disease caused by these pathogens, is characterized by acute gastrointestinal symptoms such as bloody diarrhea, fever, intense abdominal cramps, and sometimes headache. The disease can be particularly severe in vulnerable populations, including infants, the elderly, and immunocompromised individuals (**El Baaboua et al., 2017**).

In recent years, growing attention has been paid to the emergence of antibiotic resistance among thermotolerant *Campylobacter* strains, raising critical concerns regarding the effectiveness of conventional therapeutic approaches (**Bouhamed et al., 2018**). Multidrug resistance has been increasingly documented, especially among isolates from food-producing animals such as poultry and turkeys. These resistance patterns complicate not only the clinical management of human infections but also pose a challenge to animal health control strategies.

Investigations involving *Campylobacter* strains isolated from poultry ceca and feces have revealed a substantial proportion of multidrug-resistant strains, emphasizing the need for sustained epidemiological surveillance (**Bolton, 2016**). Furthermore, the environmental and clinical implications of these resistant strains call for a comprehensive understanding of their reservoirs and transmission pathways including lesser-studied sources such as camels (**Igwaran and Okoh., 2019**).

Although camels are widely consumed in North and East Africa and the Middle East, their role in the *Campylobacter* epidemiological cycle remains poorly understood. In Algeria, particularly in southern regions where camel breeding and consumption are common, limited data are available regarding the prevalence of *Campylobacter* species and the potential risk of zoonotic transmission via camel meat, milk, or feces.



The present study aims to isolate and identify *Campylobacter jejuni* and *Campylobacter coli* from camels, evaluate the antibiotic resistance profiles of the isolated strains, and determine the prevalence of thermophilic *Campylobacter* species in camels from the southern regions of Algeria.

### Chapter 1: Overview in general of Thermotolerant *Campylobacter*

#### I. Background

*Campylobacter* is a bacterium that has been responsible for a significant number of infections in both animals and humans for centuries. Despite its long history, it has often gone unrecognized as a major health threat.

*Campylobacter* was first identified as a non-culturable bacterium in 1886 by Escherich, from the colons of children who died from what he termed *Cholera infantum*. (Butzler, 2004).

In 1909, McFadyean and Stockman isolated a *Vibrio-like* bacterium, which is the agent responsible for epizootic abortion in sheep, from aborted fetuses (Butzler, 2004).

In 1919 Smith and Taylor discovered a spiral bacterium in aborted bovine fetuses. This bacterium was named *Vibrio fetus*. Its microaerophilic nature was so recognized, as researchers found it difficult to obtain growth on agar plates that were exposed to air (Smith and Taylor, 1919).

In 1931, linked episodes of (winter diarrhea) in cattle to an infection caused by a microaerophilic *Vibrio*, which they named *Vibrio jejuni*, based on its morphology and the site of isolation, the jejunum. (Jones *et al.*, 1931).

In 1938, the first recorded incident of foodborne illness caused by *Campylobacter* infection occurred. This outbreak of gastroenteritis affected 350 inmates across several American penitentiary institutions. The outbreak was believed to be caused by the accidental distribution of raw milk instead of the usual pasteurized milk (Levy, 1946).

In 1944, Doyle in the United States isolated and described a *Vibrio* species similar to *Vibrio jejuni* from the feces of prisoners suffering from diarrhea, classifying it as *Vibrio coli* (Doyle, 1944).

In 1947, Vinzent *et al.* isolated *Vibrio fetus* from the blood of three pregnant women who were admitted for hyperthermia of unknown origin. The illness persisted for approximately four weeks, and two of the women experienced miscarriages (Vinzent *et al.*, 1947; Butzler, 2004).

In 1949, Stegenga and Terpstra demonstrated the pathogenic role of *Vibrio fetus* in causing enzootic sterility in cattle (Butzler, 2004).

In 1954, King identified a relationship between the *Vibrio* species described by Jones and *al.* in 1931 and the occurrence of diarrhea in humans. He found that the *Vibrio fetus* is capable of growing at temperatures of 25°C and 37°C, but not at 42°C. Furthermore, King documented several human cases of enteritis that were associated with thermotolerant

*Campylobacter*, identifying it as a *Vibrio* species that shares similarities with the one described by Vinzent but possesses different biochemical and antigenic properties. King later named this new organism (Related *Vibrio*) (**King, 1957; King, 1962**).

In 1963, Sébald and Véron demonstrated that microaerophilic vibrios differ from true vibrios by lacking sugar fermentation metabolism. They proposed the genus *Campylobacter*, with *C. fetus* as the species type (**Sebald and Veron, 1963**).

In 1968, A significant advancement in the isolation of *Campylobacter* species was achieved at the National Institute for Veterinary Research in Belgium, thanks to a selective medium developed by Dekeyser and Butzler. Their method involved the differential filtration of fecal suspensions. *Campylobacter* passed through the filter, and the resulting filtrate was inoculated onto a selective medium. No other pathogenic microbes were detected in the sample (**Dekeyser et al., 1972; Butzler, 2004**). Since that time, bacteria of the genus *Campylobacter* have been recognized as pathogenic to humans.

In 1978, The first certified case of *Campylobacter* contamination in poultry occurred when a barbecued chicken caused an outbreak of enteritis in five people in the USA (**Doyle, 1981**).

In 1984, The name *Campylobacter pyloridis* was proposed for a group of *Campylobacter* isolated from the human stomach, which later became *Helicobacter pyloridis*, ushering in a new era in gastroenterology (**Marshall, 1986**). Additionally, an OMS report summarizes the veterinary public health aspects of *Campylobacter* infections.

In the 1980s, Numerous new strains were isolated from humans and animals, including *C. concisus* from the human oral cavity and *C. cryaerophila*, now known as *Acrobacter*, among others (**Vandamme, 2000**).

### II. The genus of *Campylobacter*

*Campylobacter* has a circular chromosome that spans 1.64 megabases (Mb), making its genome very small. Interestingly, 94% of its genome codes for proteins, which at the time made it the densest known genome at that time (**Parkhill et al., 2000**). The compact nature of the *Campylobacter* genome is likely related to the microorganism's delicate characteristics and specific nutritional requirements. However, this limitation is offset by its remarkable capacity for genomic rearrangements. Furthermore, analysis of the *Campylobacter* genome has shown that it lacks most of the DNA repair mechanisms found in other bacterial genera, which may account for the high mutation rates observed in this bacterium (**Peyret, 2008**).

Recently, four new strains of *Campylobacter* have been sequenced, providing a more comprehensive understanding of its genomes. These strains include:

1. *C. jejuni* strain RM1221: Isolated from a chicken carcass, this strain is capable of invading epithelial cells in vitro and colonizing chicken ceca and epidermis (**Miller *et al.*, 2000**).
2. *C. coli* strain RM2228: Also isolated from a chicken carcass, this strain was selected for its multidrug-resistant properties. It has a high prevalence in birds (**Miller *et al.*, 2000**).
3. *C. lari* strain RM2100: This clinical isolate is prevalent in birds and can be found in spring water, seawater, and crustaceans (**Endtz *et al.*, 1997**).
4. *C. upsaliensis* strain RM3195: This clinical isolate was obtained from a 4-year-old child diagnosed with Guillain-Barré syndrome. Strains of *C. upsaliensis* are often found associated with dogs and cats (**Hald and Madsen, 1997**).

### III. Taxonomy

The name *Campylobacter* is derived from the bacterium's characteristic curved shape, which can be observed through Gram staining. The term comes from Latin, where "*kampulos*" means curved (**Sebald and Véron, 1963**). The genus *Campylobacter* is the type genus of the family *Campylobacteraceae*, which also includes the genera *Arcobacter*, *Dehalospirillum*, and *Sulfurospirillum*. These genera are classified under the class *Epsilonproteobacteria*, within the phylum *Proteobacteria*, and belong to the domain *Bacteria* (or *Eubacteria*). The type species of this genus are *Campylobacter jejuni* and *Campylobacter coli* (**Euzeby, 2010**).

Currently, the genus *Campylobacter* consists of 22 species that have been isolated from humans and animals, most of which are of clinical and/or economic importance (**Euzeby, 2010**).

Within the genus *Campylobacter*, species can be categorized into three groups: the thermophilic group, the fetal group, and the anaerobic group. The thermophilic (or thermotolerant) group is the most clinically significant, comprising the species *C. jejuni* and *C. coli*. The fetal group includes *C. fetus* (**Megraud, 2004**). *C. jejuni* itself has two subspecies that differ significantly in their distribution and, to a lesser extent, in their ecology. *C. jejuni* subsp. *jejuni* is often simply referred to as *C. jejuni* and corresponds to the taxon isolated 1931 (**Jones *et al.*, 1931**).

Thermotolerant *Campylobacter* strains are those that can grow at 42°C but not at 25°C; this classification emerged in the 1980s (**Costas *et al.*, 1987**) and has since been adopted in standardized methods.

### IV. Morphology and structure

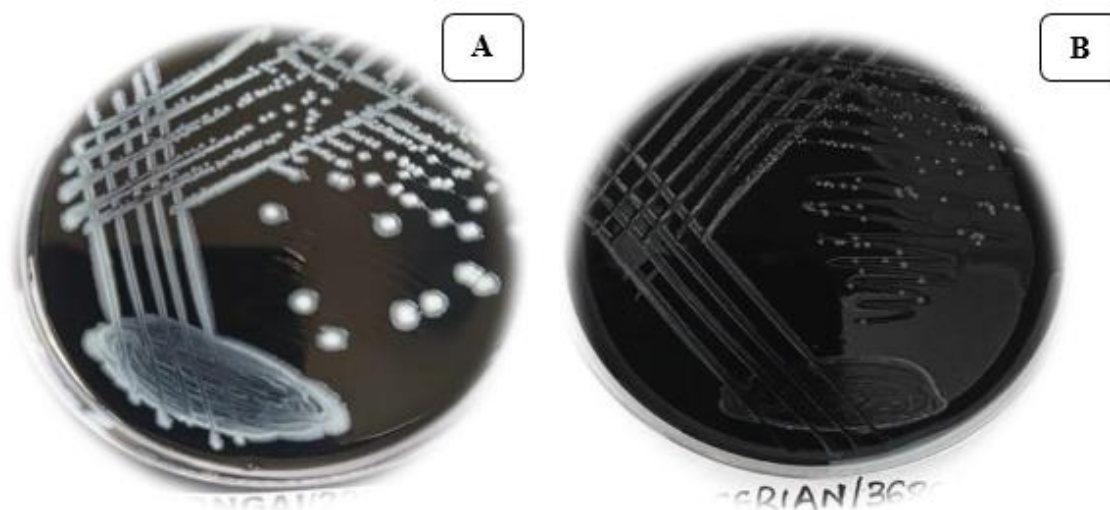
*Campylobacter* is a Gram-negative, non-spore-forming bacterium that may sometimes have a capsule. These bacteria are thin bacilli, measuring between 0.2 to 0.5 microns in diameter and up to 8 microns in length. They exhibit a vibrioid shape, meaning they can appear curved like a comma, spiraled into an "S," or helical in form. In older cultures, rounded coccoid forms can emerge, which cannot be cultivated (Smibert, 1978).

*Campylobacter* features a wavy outer membrane and complex cytoplasmic membranes. It typically has a polar flagellum at one or both ends, which contributes to its characteristic high mobility when observed in a fresh state (Freney, 2007).

Within a colony, bacterial cells display heterogeneity in age and physiological states. Spiral forms are predominantly located at the periphery, while coccoid cells are more commonly found at the center of the colony. This pattern suggests that the spiral forms represent active bacteria, while the coccoid forms indicate older, aging cells (NG *et al.*, 1985).

### IV.1. Macroscopic

Typical colonies usually become visible after 48 to 72 hours. They are small, flat, and rounded, and may appear grayish or translucent. These colonies tend to spread along the trails left by the platinum wire used for inoculation. When properly spaced, they resemble droplets that have splashed onto the agar (Dromigny, 2007).



**Figure 1:** *Campylobacter* colonies on mCCDA plate after 48 hours of microaerobic incubation at 42°C. Medium off-white glistening/shiny and spreading colonies (plate (a)) and the small gray colonies on the media (plate (b)) (Wanja *et al.*, 2022).

### IV.2. Microscopic

Microscopic examinations can be useful when samples are fresh, revealing a swarm of midges that resemble certain bacteria. A stained smear can identify the presence of small, curved bacteria (**Megraud, 2004**). Gram staining of stool samples and dark-field microscopy can be employed to detect *Campylobacter* spp. in patients' stools. This method shows their characteristic appearance and motility, with a sensitivity of 76% and a specificity of 99.5% for Gram staining compared to culture. This approach can be a viable alternative in regions where specialized laboratory tests are expensive or unavailable (**Mentor Aliber, 2012**).

Species identification of *Campylobacter* growing at 42°C reveals that the most commonly encountered species in animal and food samples are *C. jejuni* and *C. coli*.

*C. jejuni* can be distinguished from other species through hippurate hydrolysis, as it is the only species that tests positive for this characteristic; however, about 5% of strains may yield negative results (**OIE, 2008**).

Nalidixic acid sensitivity has been a widely used characteristic for confirming the *Campylobacter* genus and identifying its species. However, interpreting this sensitivity can be challenging due to acquired resistance, which is now very common in *C. jejuni* and even more prevalent in *C. coli* (**Megraud, 2004; WHO, 2003; OIE, 2008**). Biochemical identification methods can be supplemented or even replaced by molecular techniques, which are capable of detecting and differentiating all thermophilic species (**Fermer and Engvall, 1999**). PCR targeting the hippuricase gene has been shown to identify *C. jejuni* with greater sensitivity than the traditional hippurate hydrolysis test (**Bonjoch et al., 2010**). Several primers that are specific to the genus *Campylobacter* and specifically to the species *C. jejuni* and *C. coli* have been defined (**Peyerat, 2008**).



**Figure 2:** Small Gram-negative short or curved rods to coccobacilli of *Campylobacter* isolate ( $\times 1000$ ) with characteristic “seagull” shaped curved rods (**Wanja et al., 2022**).

### V. Growth and survival characteristics

### V.1. Physiology and Survival Mechanisms

#### 1. Temperature

All species of the genus *Campylobacter* can grow at 37°C, classifying them as mesophilic organisms. However, species of particular interest in food hygiene namely *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* are thermotolerant and grow better at 42°C, but not at 25°C. *Campylobacter*'s ability to thrive at different temperatures is an important characteristic that distinguishes the species, especially at 25°C and 42°C (**Fosse and Magra, 2004**).

These bacteria can survive in a humid environment at +4°C for up to 21 days in vacuum packaging. Although freezing causes some reduction in their numbers, it does not eliminate them entirely, as they can survive for up to 85 weeks at -18°C (**Bourgeois et al., 1996**).

#### 2. Atmosphere

*Campylobacter* relies on respiratory metabolism and requires an atmosphere enriched in CO<sub>2</sub> (making them capnophilic) and low in oxygen. They thrive in an oxygen concentration between 3% and 15% (which makes them microaerophiles) and typically use oxygen as the final electron acceptor. Some strains can grow anaerobically or tolerate oxygen concentrations as high as 20%. The ideal gas mixture for *Campylobacter* appears to be 5% oxygen, 10% carbon dioxide, and 85% nitrogen (**Fosse, 2004; Ghafir and Daube, 2007**).

Furthermore, thermotolerant species of *Campylobacter* predominantly use an oxygen-dependent pathway to synthesize DNA, which enables them to preferentially colonize the mucosa of the deep crypts of the ceca and large intestine near the surface of epithelial cells, where oxygen is available at levels compatible with microaerophilia (**Mentor Ali ber, 2012**).

#### 3. Sensitivity

*Campylobacter* bacteria are sensitive to heat; cooking and pasteurization effectively destroy them. They are more susceptible to adverse conditions, such as desiccation, heat, pH levels below 5, disinfectants, and irradiation than most other intestinal pathogenic bacteria (**Ghafir and Daube, 2007**). They do not grow in the presence of 3.5% NaCl (**Bourgeois et al., 1996**).

#### 4. Viable Non-Culturable Forms (VNC)

The first reported description of the VNC form of *Campylobacter* was by Rollins and Colwell (1986). Over the past twenty years, microbiologists have recognized the concept of viable non-culturable forms of bacteria. It was previously believed that a significant discrepancy existed between the number of bacteria observed under a microscope (which was significant) and the Colony Forming Unit (CFU) counts (which were low or even zero),



indicating the presence of dead enteric bacteria. This view changed when it was demonstrated that a portion of these bacteria retained metabolic activity, leading to the recognition of VNC forms as a public health concern (**Murphy *et al.*, 2006**)

### **5. Biofilm Formation**

*Campylobacter* can form biofilms in aquatic environments. The conditions within a biofilm can protect the bacteria from atmospheric oxygen. Within these biofilms, *Campylobacter* can transition into the VNC form (Murphy and al., 2006). Biofilms likely play a crucial role in the persistence of *Campylobacter* outside its host, particularly *C. jejuni* and *C. fetus*, thus enabling survival in agri-food environments (**Gunther and Chen, 2009**).

### **6. Biochemical Characteristics**

*Campylobacter* is unable to ferment either sugars or nitrogenous compounds, clearly distinguishing it from the genus *Vibrio*. They are positive in the oxidase test (**Leminor and Veron, 1989**) and possess a strictly oxidative energy metabolism. The primary energy substrates for *Campylobacter* are primary metabolites of the Krebs cycle or amino acids (**Fauchere and Avril, 2002**).



---

**Chapter 2: Campylobacteriosis****I. Epidemiology and transmission**

Understanding the epidemiology and transmission dynamics of thermotolerant *Campylobacter* strains, particularly from camel meat and feces, is integral to assessing the public health risks associated with these pathogens. *Campylobacter jejuni*, a predominant species in this context, is significantly implicated in foodborne illnesses worldwide and often emerges from livestock reservoirs. Studies conducted in various regions, particularly in Kenya, indicate that cattle are notable carriers of *Campylobacter*, presenting potential sources for human Campylobacteriosis (Wanja *et al.*, 2022).

However, there is a marked lack of research on the epidemiology of thermophilic *Campylobacter* in camel populations, especially in environments where camels and cattle coexist. Variables such as herd size, breed, and seasonal climatic variations can significantly influence prevalence rates. In regions with limited infrastructure, environmental exposure from contaminated water remains a crucial factor, underscoring the interconnectedness of livestock management and public health.

Moreover, the transmission route of *Campylobacter* involves complex interactions among various factors, including animal husbandry practices and environmental conditions. For instance, studies have shown that the proximity of livestock to contaminated water sources plays a vital role in the spread of *Campylobacter* species. This is particularly relevant in low- and middle-income countries where sanitation practices may not sufficiently mitigate risks. In South Africa, there are documented cases that highlight the prevalence of *Campylobacter* in meat carcasses and its potential transmission to humans via contaminated food products (Igwaran and Okoh., 2020). With investigations revealing significant levels of *Campylobacter* in retail meat, there is an urgent need for broader surveillance efforts in camel products to comprehensively understand the epidemiology of this pathogen in diverse settings. Establishing effective monitoring and control strategies will necessitate collaborative approaches integrating knowledge from both epidemiological research and food safety systems.

**I.1. Prevalence in different regions**

The prevalence of thermotolerant *Campylobacter* in camel meat raises significant concerns regarding food safety and public health, particularly due to the bacteria's link to enteric infections. A review of various studies indicates that thermophilic *Campylobacter* species, primarily *Campylobacter jejuni* and *Campylobacter coli*, are commonly found in the gastrointestinal tracts of a variety of animals, including camels, as these animals often serve as

asymptomatic carriers (**Rawat et al., 2018**). Similar to poultry and other livestock, these bacteria can be transmitted through fecal contamination, making meat products potential vehicles for infection.

Recent investigations have highlighted the prevalence rates of thermophilic *Campylobacter* in animal populations, suggesting that the isolation frequency in camel meat might be comparable to that found in turkeys and other meats. For example, in turkey farming environments, nearly half of the fecal samples tested positive for these bacteria, indicating their ability for horizontal transmission within herds (**Bouhamed et al., 2018**). While comprehensive documentation of prevalence rates specifically in camel meat is still lacking, patterns observed in other species suggest that camel meat could act as a significant reservoir for these pathogens.

The risk associated with consuming uncooked or undercooked camel meat is evident, as highlighted by studies showing that improper cooking practices contribute to the transmission of *Campylobacter*. Diagnostics focused on the prevalence of *Campylobacter* in camel meat should consider various epidemiological factors, including the incidence of contamination during handling, preparation, and cooking processes.

Importantly, the threat extends beyond the mere presence of bacteria in the meat; the potential for cross-contamination and the processing conditions of camel meat are critical elements that necessitate stricter regulatory oversight. To mitigate the risks associated with consuming contaminated camel meat, enhanced surveillance, and public awareness campaigns are essential, addressing broader public health concerns linked to *Campylobacter* infections.

### **I.2. Transmission routes**

Two primary modes of *Campylobacter* transmission are identified: direct and indirect.

#### **1. Direct Transmission**

*Campylobacter* can be transmitted to humans through direct contact with animals or contaminated animal carcasses. This is rare and mainly affects farmers, veterinarians, and slaughterhouse workers. Contact with pets and contaminated water can also pose a risk (**FAO/WHO, 2002**). While person-to-person transmission is uncommon in developed countries, it may be more significant in developing regions (**Hartnett et al., 2009**).

#### **2. Indirect Transmission**

Indirect transmission is observed in both epidemic and sporadic cases of campylobacteriosis, primarily through the consumption of contaminated foods. Poultry products, along with raw or undercooked meat from various animals, are the main sources (**Puterflam et al., 2007**). Unpasteurized cow's milk and tap water contaminated with animal

feces are also significant contributors to human infections (**Federighi, 2005**). Contamination can occur at multiple stages in the food chain, from farming to kitchen preparation (**Laberge, 2003**).

Overall, *Campylobacter* infections are mainly associated with raw milk, undercooked meat, and contaminated beverages (**WHO, 2014**).

### II. Zoonotic Potentials

Contamination source it can be with direct contact of cattle, also puppies with diarrhea (**Hermans *et al.*, 2012**)

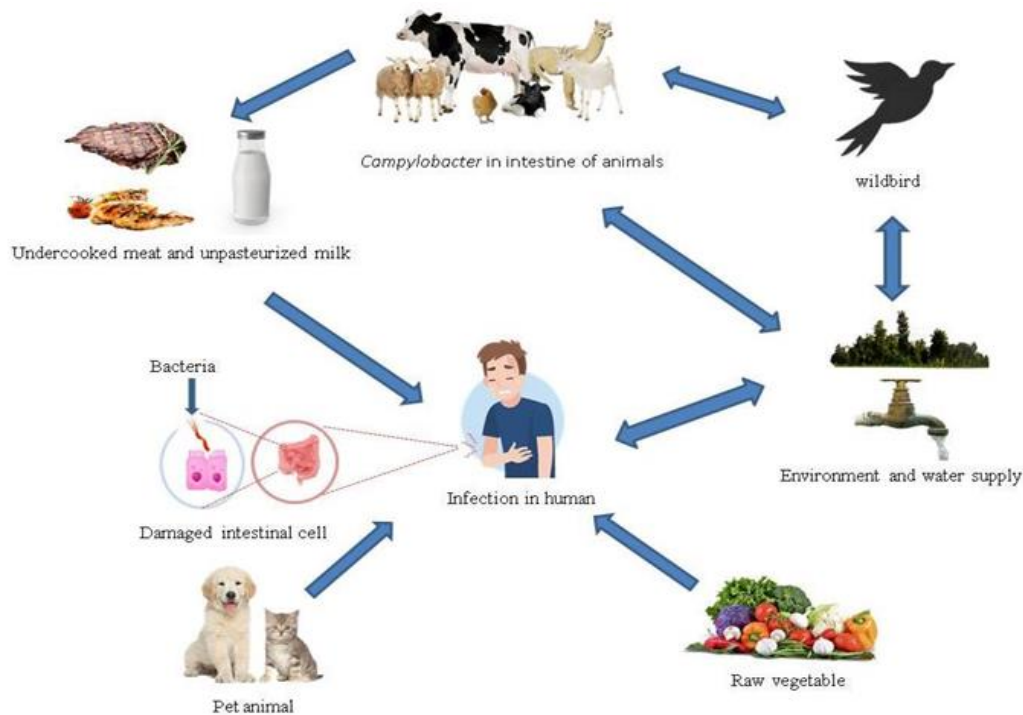
Diseases can spread to people through a number of routes, such as eating undercooked or contaminated meat particularly poultry, dairy products unpasteurized or contaminated milk is the cause of outbreaks, contaminated milk pathways might be during the milking process or through unsanitary handling practices (**Hermans *et al.*, 2012**).

Direct contact with cattle, as well as pets—particularly puppies suffering from diarrhea—can also serve as potential routes of *Campylobacter* contamination (**Igwaran and Okoh, 2019**).

Paradoxically, and despite its delicate nature can frequently be found in surface water, drinking water has been implicated as a possible source for human illness, although in the developed world waterborne infection of *Campylobacter* in humans is not very likely (**Young *et al.*, 2007**).

Finally, also raw vegetables, which can become contaminated by cross-contamination by other contaminated food products during preparation, but also directly at the farm, are an important source (**Kwan *et al.*, 2008**).

These results indicate that contaminated food products are the principal source for disease in humans (**Hermans *et al.*, 2012**)



**Figure 3:** Overview of pathways of *Campylobacter* transmission in both Human and animals (Amin *et al.*, 2022).

## II. Pathogenicity of *Campylobacter*

The pathogenicity of *Campylobacter* species, particularly *C. jejuni* and *C. coli*, is primarily associated with their ability to colonize, invade, and cause inflammation in the intestinal mucosa of the host. Upon ingestion of contaminated food or water, *Campylobacter* organisms adhere to the epithelial cells of the intestinal tract using surface structures such as flagella and adhesins. Following adhesion, they can penetrate the mucus layer and induce cellular damage through several virulence factors, including cytolethal distending toxin (CDT), which interferes with host cell division, leading to cell cycle arrest and apoptosis (Young *et al.*, 2007).

Additionally, lipooligosaccharides (LOS) present on the bacterial surface can mimic human neuronal gangliosides, triggering autoimmune responses such as those observed in Guillain-Barré syndrome (Nachamkin *et al.*, 1998). The host immune response contributes significantly to the clinical symptoms, resulting in acute gastroenteritis marked by diarrhea (often bloody), abdominal pain, fever, and malaise. The severity of disease varies depending on the infecting strain, host immune status, and bacterial load. Furthermore, some *Campylobacter* strains possess enhanced invasive capabilities and resistance to antimicrobial peptides, increasing their persistence in the host and environmental reservoirs.

These combined pathogenic mechanisms underline the importance of understanding *Campylobacter* virulence for both clinical management and public health interventions.

**Tableau 1:** Attributes of *Campylobacter* genus bacteria allowing them to infect and survive in a host organism (Chlebicz and Śliżewska, 2018).

The Mechanism of Survival	Virulence Description	Reference
<b>Mobility</b>	<ul style="list-style-type: none"> <li>– moving against the peristalsis, reaching target sites in the intestine <sup>1</sup>;</li> <li>– adhesion to host's cells, formation of a biofilm, secretion of invasive proteins <sup>1</sup>;</li> <li>– required flagella and a chemosensory system (regulation of the flagellar movement depending on environmental conditions) <sup>2</sup>.</li> </ul>	<b>(Bolton,2015)</b>
<b>Drug resistance</b>	<ul style="list-style-type: none"> <li>– increasing antibiotic resistance resulting from the misuse of antibiotics in medicine, veterinary medicine, and agriculture <sup>2</sup>;</li> <li>– acquiring antibiotic resistance while dwelling in the alimentary tract of livestock and humans <sup>2</sup>;</li> <li>– resistance to fluoroquinolones (e.g., ciprofloxacin), macrolides (e.g., erythromycin), aminoglycosides (e.g., gentamycin, canamycin and streptomycin), tetracyclines, and-lactams (e.g., penicillins and cephalosporins) <sup>2</sup>.</li> </ul>	<b>(Kaakoush <i>et al.</i>, 2015)</b>
<b>Adherence to host's epithelial cells</b>	<ul style="list-style-type: none"> <li>– initial colonisation of intestinal epithelium <sup>1</sup>;</li> <li>– mediation of the adhesins on the surface of bacterial cells, including: CadF (an external membrane protein), PEB1 (periplasmic binding protein), JlpA (lipoproteins engaged in adhesion to Hep-2 cells), and CapA (Campylobacter A adhesion protein) <sup>2</sup>.</li> </ul>	<b>(Epps <i>et al.</i>, 2013)</b>
<b>Invasion of host's cells</b>	<ul style="list-style-type: none"> <li>– avoiding immunological response <sup>2</sup>;</li> <li>– significant role played by the external lipopolysaccharide bacterial core <sup>2</sup>.</li> </ul>	<b>(Percival and Williams,2014)</b>

<b>Production of toxins— cytolethal distending toxin (CDT)</b>	<ul style="list-style-type: none"> <li>– a protein composed of the subunits coded by genes <i>cdtA</i>, <i>cdtB</i>, and <i>cdtC</i> 2; <i>cdtB</i> encodes the enzymatic part of the toxin 2;</li> <li>– <i>cdtA</i> and <i>cdtC</i> encode subunits responsible for binding the toxin to the membrane of an eukaryotic cell 2;</li> <li>– subunits <i>CdtA</i>, <i>CdtB</i>, and <i>CdtC</i> necessary for correct function of the toxin 2;</li> <li>– halting the eukaryotic cell during the G2/M phase of the cellular cycle, stopping from transition into the phase of mitosis—cellular death 2;</li> <li>– -not all strains produce CDT 2</li> </ul>	<p>(Silva <i>et al.</i>, 2011)</p>
--	---	------------------------------------

<sup>(1)</sup> Attributes common for *C. jejuni*; <sup>(2)</sup> Attributes common for *Campylobacter* genus.

### II.1. Other virulence mechanism in *Campylobacter* species

Lipooligosaccharide can be provided by a defensive barricade that helps campylobacter pathogenicity in disrupting epithelial cells that mimic the action of human ganglioside in causing diarrhea by Sialyltransferases (*cstII*) activity.

Kat A is a gene that encode catalase which is responsible to convert H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> to protect the bacteria from oxidative stress (Igwaran and Okoh., 2019)

The genes for the proteins *cj0012c* and *cj1371*, which are linked to defense against reactive oxygen species. Another *Campylobacter* virulence mechanism that is important for exporting proteins to the outer membrane is the *Peb4* chaperone. The *cosR*, *cj1556*, *spoT*, *ppk1*, *csrA*, *nuoK*, and *cprS* genes, as well as the cell surface modification genes *waaF*, *pgp1*, and *peb4*, are the genes in charge of the stress response (Igwaran and Okoh., 2019).

## III. Clinical Manifestations

### III.1. In animals

*Campylobacter* species can be found in a wide variety of animal species asymptotically and its main amplification site, is the intestinal tract (Komba *et al.*, 2013).

#### 1. Poultry

Poultry is the main, natural reservoirs for these thermotolerant bacteria (*campylobacter jejuni* and *campylobacter coli*) and is commonly found in their gut, infected poultry show no visible signs of illness, making it difficult to detect colonization in flocks, On average, 60% to 80% of broiler flocks analyzed worldwide test positive for *Campylobacter* species by the time

they reach slaughter age, carrier flocks leads to contamination of the environment and eventually other farms and humans (**Baali et al., 2023**)

Studies have demonstrated that experimental inoculation of young poultry, such as chicks and turkey poults, with *Campylobacter* strains can lead to the development of clinical symptoms. These include bloody or mucoid diarrhea, significant weight loss, and in severe cases, mortality the severity depends both on on both the pathogenicity of the strain and the host's immune system (**Humphrey et al., 2014**)

**Wilson et al., (2008)** estimated that 97% of sporadic human campylobacteriosis cases in England are linked to animals raised for meat production, with chickens and cattle being the primary sources of *C. jejuni*.

### 2. Cattle and sheep

Cattle and sheep consider also one of the reservoirs of this zoonotic pathogen. *C. fetus subsp. venerealis* (CFV) causes bovine genital campylobacteriosis characterized by infertility, early embryonic deaths, and abortions, so the bacteria lives in the genital tract of cattle and is transmitted venereally to cows by carrier bulls (**Stanley and Jones., 2003**).

These animals are primarily colonized by *C. jejuni*, *C. coli*, *C. fetus* and *C. hyointestinalis*, *C. fetus* does not belong to the thermophilic species group (**Mendonca et al., 2015**).

### 3. Puppies

Studies have shown that dogs are reservoir for *C.upsaliensis* and *C.jejuni*, while cats are reservoirs for *C.helveticus*, *C. jejuni* (**Wieland et al., 2005**).

Dogs and cats with *Campylobacter* infections experience mild to watery diarrhea, dehydration, lethargy, anorexia, vomiting (**Sykes and Mark., 2013**).

### 4. Camels

Camels are typically asymptomatic carriers of campylobacter jejuni and *C. coli*, with no well documented clinical signs, making detection reliant on laboratory testing rather than visible illness (**Mohammadpour et al., 2020**)

## III.2. In humans

It's one of the four key global bacterial causes of Gastrointestinal infections and major of diarrhea disease (**WHO, 2015**) infecting the lining of the intestines and causing inflammation in the stomach and intestines, traveler's diarrhea (**Bullman et al., 2011**) and children's diarrhea (**liu et al., 2016**).

**Tableau 2:** Overview of *Campylobacter* Species: Reservoirs, human Impact, and key research focus (Igwaran and Okoh., 2020).

Species	Primary Reservoirs	Human Impact	Key Article Focus
<i>C. jejuni</i>	Poultry, cattle, sheep, pets, wild birds, camel.	Gastroenteritis	Poultry as a major reservoir
<i>C. coli</i>	Pigs (primary reservoir), poultry, cattle sheep, camels.	Gastroenteritis	Swine as a primary reservoir
<i>C. upsaliensis</i>	Dogs, cats.	Gastroenteritis	Pets as zoonotic sources
<i>C. lari</i>	Wild birds, shellfish.	Gastroenteritis	Wild birds and shellfish as reservoirs
<i>C. fetus</i>	Cattle, sheep.	Systemic infections	Cattle and sheep as reservoirs
<i>C. hyointestinalis</i>	Pigs, cattle.	Rare gastroenteritis	Swine as a reservoir
<i>C. concisus</i>	Humans	Gastroenteritis, IBD	Emerging human pathogen
<i>C. helveticus</i>	Cats, dogs	Rare infections	Pets as reservoirs
<i>C. ureolyticus</i>	Humans	Gastroenteritis, extraintestinal infections	Neglected human pathogen

In 2021 campylobacteriosis was the most reported food-borne zoonosis in the EU, with 127,840 cases – a 2.1% increase in EU notification rate compared with 2020 (EFSA and ECDC., 2022).

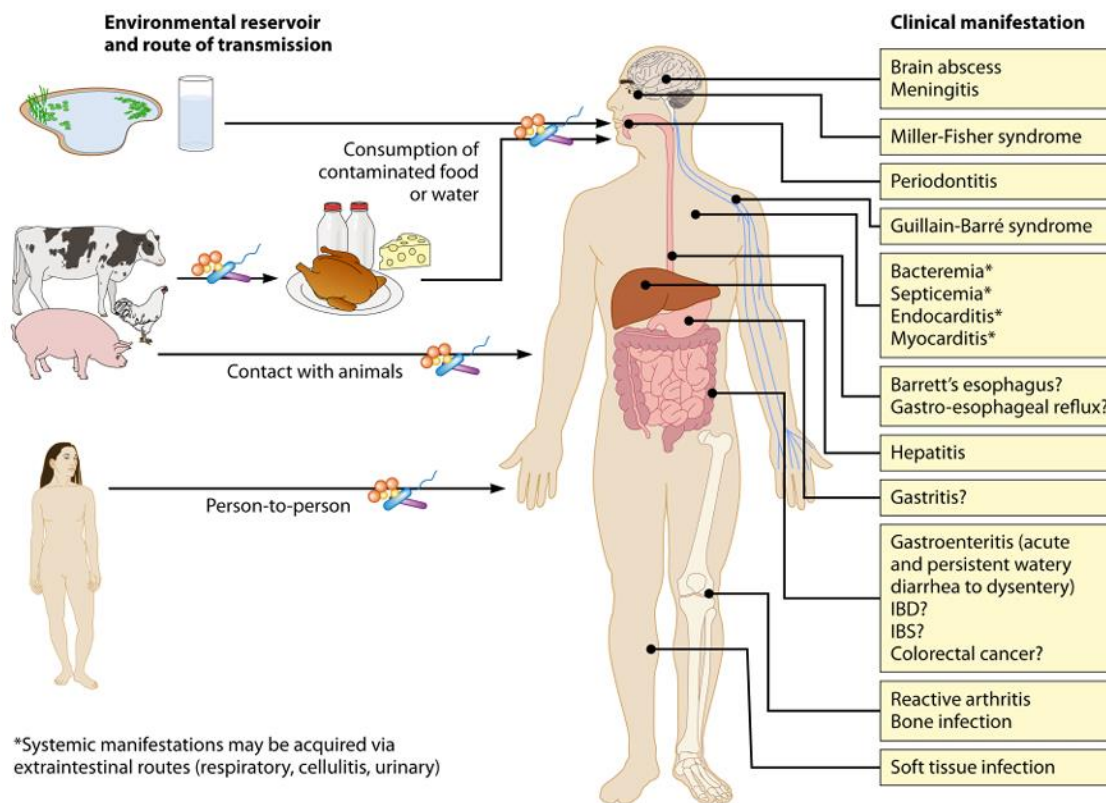
*C. jejuni* and *C. coli* are the two species that cause the majority of *Campylobacter* infections. Between 500 and 800 colonies of *C. jejuni* can infect immunocompromised individuals, the elderly, and children (El Baaboua *et al.*, 2017).



In the acute phase, campylobacteriosis is primarily characterized by gastrointestinal symptoms, such as watery (sometimes bloody) diarrhea, abdominal cramps, nausea, vomiting and fever. The disease is usually self-limiting, lasting a week or less.

Antimicrobial treatment is only indicated in severe cases (bloody diarrhea or systemic infection) (Wagenaar *et al.*, 2023).

Extra gastrointestinal infections have exhibit that reactive arthritis GBS (kuwabara and Yuki., 2013) bacteremia, septicemia (Man, 2011), septic arthritis, endocarditis, neonatal sepsis, osteomyelitis, and meningitis (Allos, 2011) been reported In a fraction of occurrences neurological dysfunction, neurological disorders and a polio-like form of paralysis (Igwaran and Okoh, 2019) includes one of the major complications Guillain-Barré Syndrome (GBS), irritable bowel syndrome, the Miller Fisher Syndrome (Facciola *et al.*, 2017).



**Figure 4:** Environmental reservoirs, routes of transmission, and clinical manifestations associated with *Campylobacter* species. Abbreviations: IBD, inflammatory bowel diseases; IBS, irritable bowel syndrome. Question marks indicate conditions for which a role for *Campylobacter* is implicated but not certain (Kaakoush *et al.*, 2015).

#### IV. Diagnosis of *Campylobacter*

**IV.1. Detection isolation and confirmation**

*Campylobacter* identification is challenging due to their biochemical inertness and fastidious growth requirement, For the laboratory diagnosis of *Campylobacter* in infected animals, fecal specimens are the preferred sample type for collection, transport, and storage (Girgis *et al.*, 2014).

During the acute phase of the diarrheal illness a stool sample should be collected and before the start of antibiotic treatment. Unpreserved stool samples stored at room temperature must be delivered to the laboratory within 2 hours. A transport medium like modified Cary-Blair or squart should be used at 4C when a delay of more than 2 hours is expected or for the transport of rectal swabs (CDC, 2024).

While *Campylobacter* is primarily isolated from stool samples, it can occasionally be detected in blood or wound cultures. Proper collection, transport, and incubation in a microaerobic atmosphere at 37°C are crucial for successful isolation and identification. This approach ensures accurate diagnosis and appropriate treatment, especially in cases of systemic or severe infections (Tresse *et al.*, 2017).

*C.jejuni* and *C.coli* are the most common species isolated from fecals clinical laboratories should prioritize using methods specifically designed for the isolation of these two species. *C. jejuni* and *C. coli* can be isolated from fecal specimens by direct plating of the specimen on selective media and incubation in a microaerobic atmosphere at 42C for 72 hours

*Campylobacter spp.* are microaerophilic bacteria, meaning they require reduced oxygen levels (around 5-10% O<sub>2</sub>) and increased carbon dioxide (around 5-10% CO<sub>2</sub>) for optimal growth. Their sensitivity to oxygen and reactive oxygen species (ROS) has driven the development of specialized selective media containing one or more oxygen scavengers, such as blood, ferrous iron pyruvate, etc., and selective agents, particularly antibiotics to support their growth while inhibiting competing microorganisms and some type of highly contaminated samples (Tresse *et al.*, 2017).

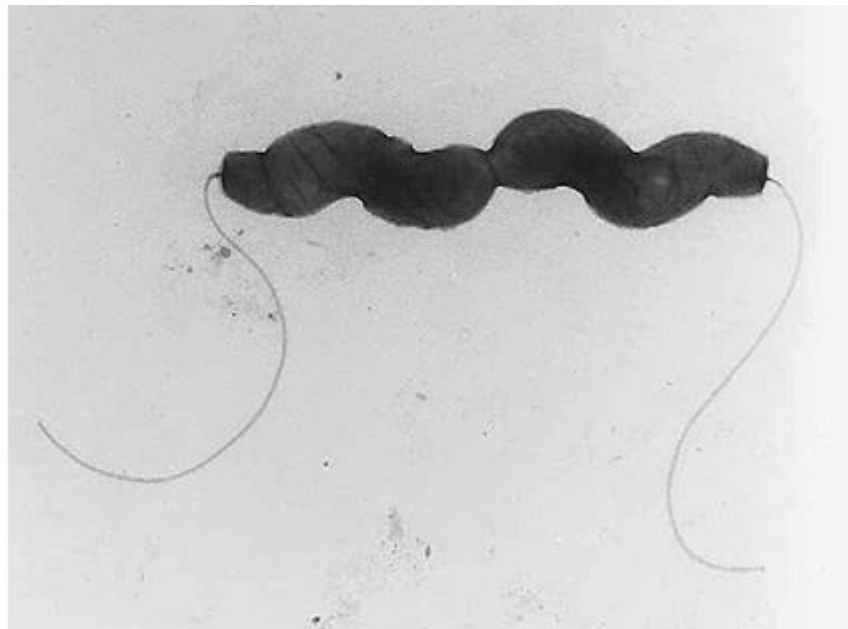
In some protocols, to reduce the inhibitory effects of selective agents on potentially damaged cells, samples are initially suspended in a basal broth without selective agents. The selective agents are then gradually introduced after a brief incubation period.

A number of the selective broths, including Preston broth (PB), Bolton broth (BB), and *Campylobacter* enrichment broth (CEB), have been evaluated for effectiveness (Baylis *et al.*, 2000).

Adding the enzyme Oxidase to selective broths is very useful for lowering oxygen levels and enhancing the separation of *Campylobacter* species from naturally contaminated samples, however, adding the enzyme oxidase nor special atmospheres to a blood free enrichment broth not required (Abeyta *et al.*, 1997).

A number of selective agars have been developed and evaluated for campylobacter isolation. For instance, it has been discovered that Butzler, Preston, and charcoal cefoperazone deoxycholate (CCDA) agars are similarly effective. The preferred technique is often to employ CCDA and incubate at 42°C instead of 37°C (Zanetti *et al.*, 1996).

After enriching a large number of diverse samples in either Preston or Park and Sanders broths, (Federighi *et al.*, (1999)) compared Karmali, Butzler, and Skirrow isolation agars and found that the more successful combination was Park and Sanders broth followed by isolation on Karmali agar.



**Figure 5:** Photo micrograph of *Campylobacter jejuni* in the process of dividing.  
(Parker, Institute of Food Research, UK).

### IV.2. Laboratory testing for campylobacter infection

Various rapid methods have been proposed, for laboratory testing to detect and confirm campylobacter including a wide range culture, stool immunoassays for *Campylobacter*-specific antigen, and molecular tests, such as nucleic acid–amplified tests latex agglutination (commercially available; e.g., Wilma *et al.*, 1992; Microscreen® *Campylobacter* kit), and a physical enrichment method (filtration) that permits the separation of *Campylobacter* from other organisms present in the food matrix (Debretsion *et al.*, 2007)

Since the phenotypic reactions are often atypical and difficult to read, e.g., the hippurate hydrolysis test for differentiating *Campylobacter coli* from *C. jejuni*. The PCR reaction has been combined with immuno-separation with some success (e.g., Docherty *et al.*, 1996; Waller *et al.*, 2000) in detecting low numbers of the organism in only about 6 hours. However, some components of both food samples and selective broths can be inhibitory to the PCR reaction.

More recently real-time PCR methods have been developed that show the potential of detecting as few as 1 cfu in chicken samples, and in less than 2 h (Debretsion *et al.*, 2007).

Epidemiological studies (e.g., outbreak investigations) have been benefited from the use of molecular typing techniques such as PCR that increase the sensitivity and specificity of *Campylobacter* differentiation, random amplification of polymorphic DNA (RAPD) and pulsed field gel electrophoresis (PFGE) (Silvia *et al.*, 2011).

### V. Prevention and treatment of campylobacter

Biosecurity, water/ litter acidification, feed additives(probiotics) flock management in poultry can be implemented in as a prevention Strategies, till day no routine antibiotics, experimental phage, no licensed vaccines (Humphrey *et al.*, 2014).

Artificial insemination can control or prevent bovine genital campylobacteriosis (Cha *et al.*, 2017).

While in pets involves good hygiene, avoiding raw meat diets, and regular cleaning of living areas. Treatment is usually supportive, but severe cases may require antibiotics like erythromycin (Igwaran and okoh., 2019)

Patients are occasionally self-limiting however replacing lost fluid and electrolytes are a key supportive treatment, in severe cases antibiotics are administrated (Igwaran and Okoh., 2019)

Antibiotic resistance in *Campylobacter* is a growing global concern. **Fluoroquinolones**- such as **ciprofloxacin** were once commonly used, but resistance has become wide spread, especially in many countries, limiting their effectiveness. Although some *Campylobacter* isolates still show sensitivity to **ciprofloxacin**, this is no longer the norm in many settings (Xiao *et al.*, 2023).

**Macrolides**, especially **Azithromycin**, are now considered the first line treatment for *Campylobacteriosis*, as they remain more effective and show lower resistance rates in many regions. However, in several developing countries, studies have reported a high prevalence of **macrolide** resistant strains, which complicates treatment (Ramatla *et al.*, 2022)

In some cases, **amoxicillin-clavulanic acid** has shown effectiveness, but its reliability against *Campylobacter* is not consistent, and it's not generally considered a primary option. For systemic infections- such as *Campylobacter*- associated bacteremia or **sepsis-Carbapenems** (a class of broad-spectrum **B-lactam** antibiotics) have been suggested as an alternative. This is based on case reports and clinical success in treating severe *Campylobacter* infections where first line antibiotics failed (**Zhang et al., 2007**).

## Objective

*Campylobacter* is a major cause of foodborne disease, commonly linked to contaminated animal products. In Algeria's southern regions camels are an important food source, this lack of data raises concerns about potential public health risks.

This study aims to detect and isolate *Campylobacter coli* and *Campylobacter jejuni* species from feces samples of camels in Ouargla, Oued Souf, Boussada, and Biskra and to assess the antimicrobial resistance profiles of the isolate.

In addition, the study will estimate the prevalence of thermotolerant *C.coli* and *C.jejuni* species in camels from the southern regions of Algeria, this will help to evaluate the potential risk of transmission to humans through the consumption or handling of contaminated camel products, highlighting the public health implications of zoonotic *Campylobacter* infections.

## I. Materials and Methods

### I.1. Materials

#### I.1.1. Workplace and study period

Our work was carried out in the Clinical Microbiology Laboratories of the National Higher School of Veterinary Medicine (ENSV-Algiers). This study was conducted over a period extending from February 2024 to March 2025.

#### I.1.2. Sample Collection

A total of 120 fecal samples were collected from apparently healthy camels across several farms in southern Algeria during the period of May and June 2023. The samples were collected by practicing veterinarians and veterinary inspectors in the following regions:

- Ouargla : 40 samples (33%).
- Oued Souf : 40 samples (33%).
- Other areas (Boussada, Biskra): 40 samples (33%).

All samples were collected aseptically using sterile swabs and immediately placed into sterile containers containing Preston transport medium, suitable for maintaining *Campylobacter* viability.

#### I.1.3. Sampling Materials

The following sterile materials and equipment were used during sample collection and handling:

- Sterile swabs.
- Sterile scissors.
- Sterile scalpels.

- Sterile spatulas.
- Sterile sampling containers.
- Sterile sampling bags.
- Portable cooler for transport.
- Transport medium: Preston enrichment broth (selective for *Campylobacter spp.*).

### **I.1.4. Laboratory Equipment and Reagents**

For microbiological analyses and identification, the following materials and reagents were used:

- Anaerobic jars.
- Microaerophilic atmosphere-generating sachets (GENbox Microaer system).
- Culture media (e.g., Karmali agar) and associated reagents.
- API Campy strips for biochemical identification.
- Antibiotic discs for susceptibility testing:
  - ⇒ Amoxicillin-clavulanic acid.
  - ⇒ Chloramphenicol.
  - ⇒ Ceftriaxone.
  - ⇒ Ciprofloxacin.
  - ⇒ Nalidixic acid.
- Sterile physiological saline.
- Sterile distilled water.
- Gram staining reagents.
- Reagents for API Campy tests.
- Immersion oil.
- Oxidase reagent.

### **I.1.5. Sample Transport Conditions**

Samples were stored in cool boxes at 4°C immediately after collection and were transported to the Microbiology Laboratory of the National High School of Veterinary Medicine (ENSV), Algiers, within a maximum of 48 hours to ensure sample integrity and avoid microbial overgrowth or loss.

Upon arrival, fecal samples are labeled, registered, and inspected to ensure proper identification and transport conditions before processing.

## I.2. Methods

### I.2.1. Isolation of Thermotolerant *Campylobacter jejuni* and *Campylobacter coli*

#### 1. Preparation of Karmali Agar

- ⇒ 46 g of dehydrated Karmali base medium was suspended in 600 mL of distilled water and heated until fully dissolved.
- The medium was sterilized by autoclaving at 121°C for 15 minutes.
  - After cooling to 45–50°C, a selective supplement containing antibiotics was added aseptically.
  - The medium was poured into sterile Petri dishes, allowed to solidify, and stored at 2–8°C until further use.
- ⇒ The selective supplement consists of a mixture of antibiotics, typically including:
- Vancomycin (10 mg) – to inhibit Gram-positive bacteria.
  - Colistin (5 mg) – to suppress the growth of many Gram-negative bacteria.
  - Trimethoprim (5 mg) – to inhibit *Proteus* species.
  - Cycloheximide (50 mg) – to inhibit fungal growth.

These additives enhance the selectivity of Karmali agar for *Campylobacter spp.* by suppressing the growth of competing microbial flora.

- The supplemented medium was thoroughly mixed, poured into sterile Petri dishes under aseptic conditions, and allowed to solidify.
- Plates were stored at 2–8°C and used within a few days to ensure optimal performance.

#### 2. Enrichment step

The veterinarians carried out the enrichment step on site, directly after collecting the samples. They were also responsible for performing the sampling procedures under hygienic conditions. After the initial enrichment using Preston broth, which is specially formulated to favor the growth of *Campylobacter* while suppressing other bacteria. The broth contains peptone, yeast extract, mineral salts, and selective antibiotics such as cefoperazone, amphotericin B, trimethoprim, and cycloheximide.

After enrichment, the samples were transferred into microaerophilic transport containers, to preserve the viability of *Campylobacter* during transportation to the laboratory.





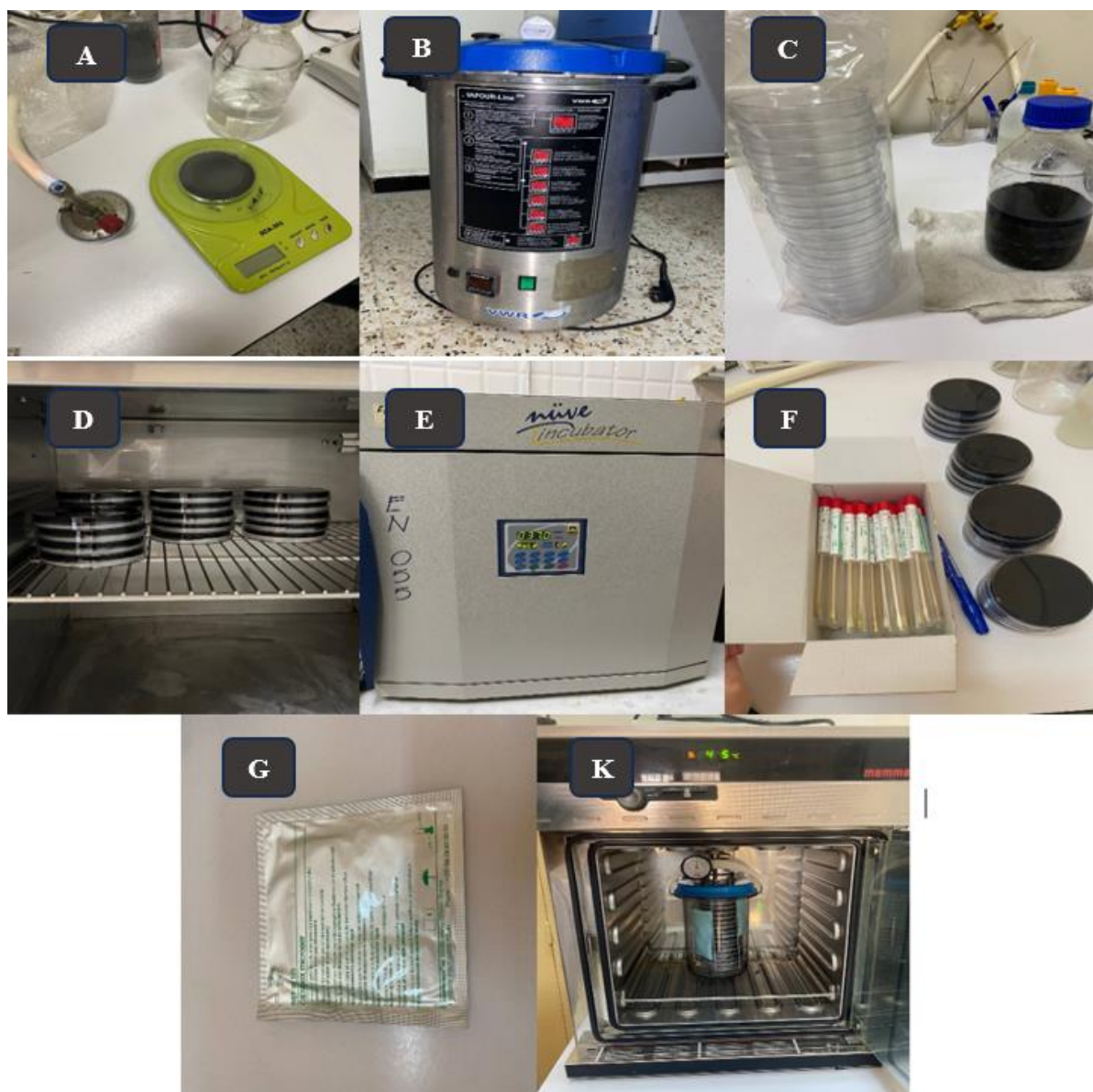
**Figure 6:** (A) Microaerophilic container, (B) Preston enrichment broth tubes  
(Images documented during the study)

### **3. Inoculation onto Karmali Agar**

Using a sterile cotton swab or inoculation loop, dip into the prepared suspension.

Streak the sample onto the karmali agar plate:

- Used the four quadrant method to isolate single colonies
- If screening multiple samples or for colony count, use spread plate technique



**Figure 7:** Preparation and isolation of *Campylobacter* spp. Using Karmali Selective Medium: (A) Media Weighing, (B) Autoclaving, (C) Plate Pouring, (D) Plate Storage (E) Incubator Setup, (F) Sample Inoculation, (G) Gas Pack (Microaerophilic), (K) Incubation in Anaerobic Jar. (Images documented during the study)

#### 4. Identification of Thermotolerant *Campylobacter* spp.

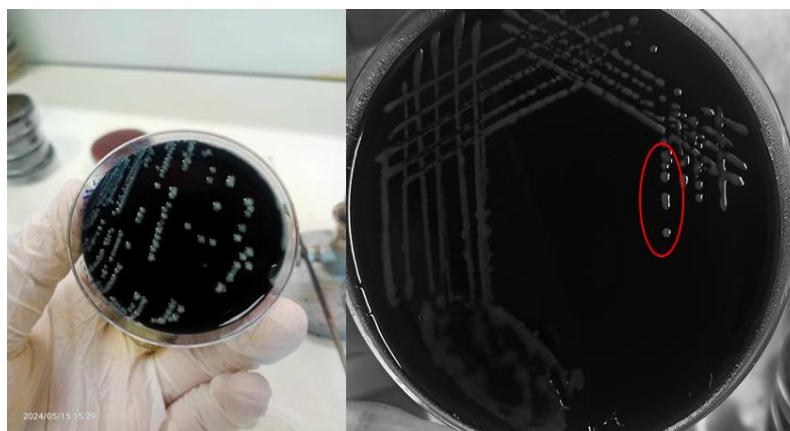
The initial identification of *Campylobacter* is based on their fresh smear examination, typical morphology in Gram stain, and oxidase test. This approach was used in this study.

##### 1. Colony appearance

*Campylobacter* colonies typically appear after 48 hours of incubation at 42°C under microaerophilic conditions (Figure 8)

The colonies are usually small, round, grayish or translucent, with a moist and slightly mucoid texture, *C. coli* and *C. jejuni* are not easily distinguishable based on colony morphology

alone, however, in some cases, *C.jejuni* may produce slightly more compact and raised colonies, whereas *C.coli* tends to form larger, flatter colonies, but the definitive identification requires biochemical tests (Figure 11)



**Figure 8:** Colony appearance of *Campylobacter jejuni* strains in karmali medium  
(Images documented during the study)

## 2. Motility test

Fresh preparation allows for the examination of bacterial motility and the detection of living organisms. To observe motility, care must be taken not to damage the fragile flagella during sampling and slide preparation it reveals *campylobacter* with their characteristic corkscrew-like (spiral) motion.

## 3. Morphological examination on stained smear

Under Gram staining, campylobacter exhibits distinctive morphology, appearing as thin, Gram negative bacilli with variable shapes often curved like a comma, spiral(S-shaped), or gull-winged (Figure 9)



**Figure 9 :** Microscopic Morphology of *Campylobacter* on stained Smears.  
(Images documented during the study)

#### 4. Oxidase test

The oxidase test detects the presence of the enzyme cytochrome c oxidase, which is involved in the bacterial respiratory chain. *C. coli* and *C. jejuni* possess this enzyme, so a positive reaction is expected.

##### Technique

- 1- Freshly isolated colony of suspected campylobacter (24-48hours old).
- 2-A drop of oxidase reagent(tetramethyl-p-phenylenediamine) onto filter paper.
- 3- Using a non-metallic tool, smear the colony onto the reagent.

##### Reading results

Positive: A dark purple or blue color appears within 10-30 seconds (indicative of Campylobacter) (Figure18)

Negative: no color change or color change after 30 seconds.



**Figure 10** : Positive Oxidase test showing the characteristic dark purple coloration in Campylobacter (Images documented during the study).

#### 5. Biochemical Identification Using API Campy Gallery

The biochemical identification of *Campylobacter* isolates was performed using the API Campy® system (bioMérieux, France), a standardized miniaturized identification gallery specifically designed for *Campylobacter* species. After subculturing the isolates on selective media under microaerophilic conditions at 42 °C for 48 hours, well-isolated colonies were selected and suspended in the API Campy inoculation medium, following the manufacturer's instructions.

The inoculated strips were incubated under microaerophilic conditions at 37 °C for 24 to 48 hours. The results were then interpreted by observing color changes in the wells corresponding to specific enzymatic activities or biochemical reactions. The obtained biochemical profiles were compared to the database provided by the manufacturer, allowing

presumptive identification of *Campylobacter jejuni*, *C. coli*, or other thermophilic *Campylobacter* species.

This method facilitated rapid and reliable species-level identification, which is crucial for understanding the epidemiology and antimicrobial resistance profiles of the isolates.



**Figure 11:** Application of API Campy system for campylobacter Identification  
(Image documented during the study).

## **6. Antibiotic Susceptibility Testing**

The antibiotic susceptibility of *Campylobacter* isolates was evaluated in accordance with the recommendations provided by the French Society for Microbiology (CA-SFM, 2013) and the World Health Organization (WHO, 2011) guidelines. This testing aimed to determine the resistance profile of isolated strains against a selected panel of commonly used antibiotics.

### **a. Antibiotics Test**

The following antibiotics were included in the susceptibility testing panel:

- Amoxicillin–clavulanic acid.
- Chloramphenicol.
- Ceftriaxone.
- Ciprofloxacin.
- Nalidixic acid.

### **b. Procedure**

#### 1. Preparation of the Bacterial Suspension

- A well-isolated colony from a fresh culture (18–24 hours old) on selective agar was carefully selected.
- The colony was suspended in sterile physiological saline solution (0.9% NaCl).
- The suspension was adjusted to match the turbidity of the 0.5 McFarland standard, ensuring a standardized inoculum.

#### 2. Inoculation of the Agar Plate

- A sterile cotton swab was dipped into the prepared suspension and gently pressed against the wall of the tube to eliminate excess liquid.

- The swab was then used to evenly inoculate the surface of a Mueller-Hinton agar plate (supplemented with 5% defibrinated sheep blood if required for *Campylobacter*), by streaking in three directions to ensure a uniform bacterial lawn.

### 3. Application of Antibiotic Discs

- After allowing the inoculated plate surface to dry for a few minutes, antibiotic discs were aseptically placed onto the agar surface using sterile forceps or an automatic disc dispenser.
- Discs were spaced adequately apart to avoid overlapping of inhibition zones.

### 4. Incubation Conditions

- Inoculated plates were incubated under microaerophilic conditions, at 42°C for *Campylobacter* species, for a period of 18–24 hours.
- Anaerobic jars and microaerophilic sachets (e.g., GENbox Microaer) were used to ensure appropriate atmospheric conditions.

### 5. Interpretation of Results

- After incubation, the diameter of the inhibition zones around each antibiotic disc was measured in millimeters using a calibrated ruler or a zone reader.
- The results were interpreted according to CA-SFM or WHO breakpoints, classifying isolates as susceptible, intermediate, or resistant.

This testing provided valuable data on the resistance patterns of *Campylobacter jejuni* and *Campylobacter coli* isolated from camels, contributing to the understanding of antibiotic resistance dissemination within the One Health framework.





**Figure 12:** Steps of Antibiotics test: **(A)** Preparation of the bacterial; **(B)** Placement of antibiotic disks; **(C)** Incubation under microaerophilic conditions; **(D)** Reading of results  
**(Images documented during the study)**

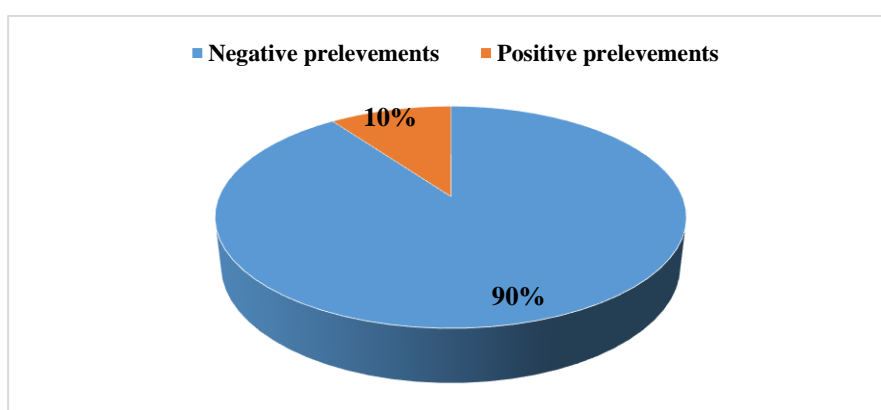
## II. Results and Discussion

### II.1. Overall prevalence and distribution of *Campylobacter* species

A total of 120 fecal samples were collected from dromedaries in different regions of southeastern Algeria. Among these, 70 samples came from healthy animals in appearance and 50 from dromedaries showing clinical gastrointestinal signs (diarrhea, tenesmus, etc.).

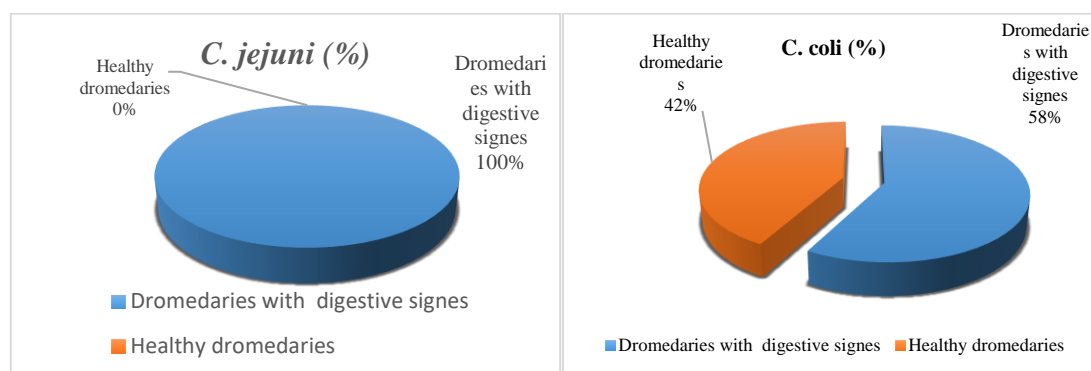
A total of 12 samples (10%) tested positive for *Campylobacter* species. Among these positive samples, *C. jejuni* was detected in 8 cases (6.7%) and *C. coli* in 4 cases (3.3%).

Most positive cases (10/12) came from dromedaries showing digestive symptoms, with a predominance of *C. jejuni* (8/10).



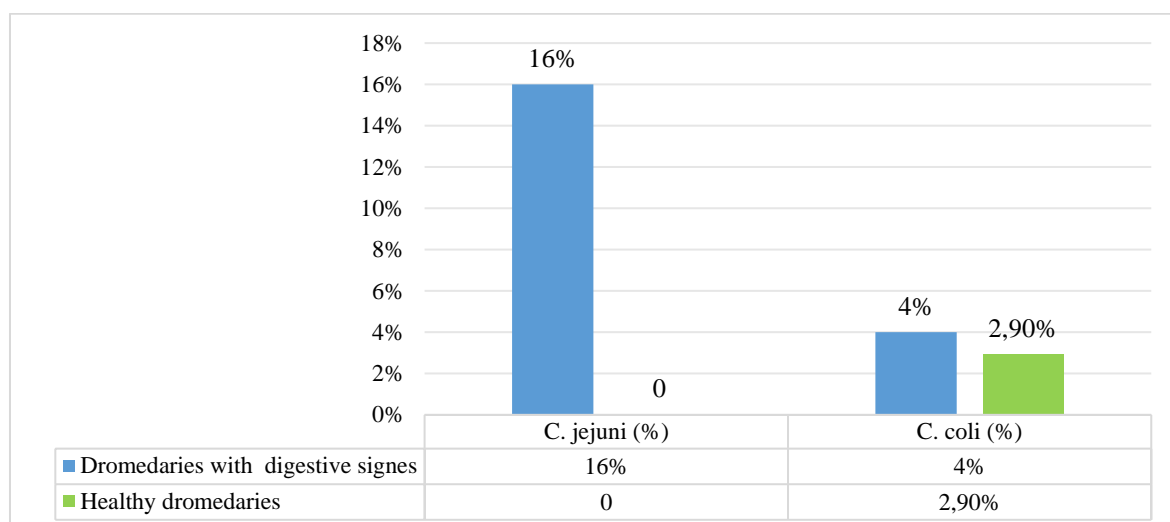
**Figure 13:** Graph demonstrating the positive cases of *Campylobacter* depending on the clinical status in camels.

This suggests a strong association between the presence of clinical signs and *Campylobacter* infection, particularly *C. jejuni*. In contrast, only two positive cases were detected in clinically healthy dromedaries.



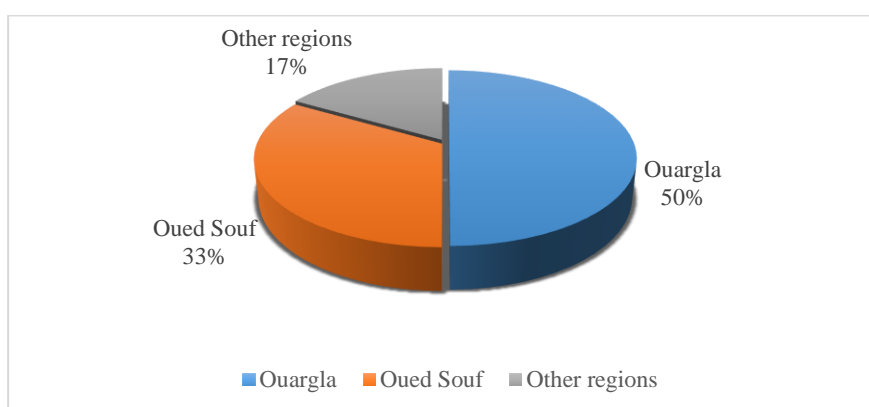
**Figure 14 :** Graph demonstrating the cases of *Campylobacter jejuni* and *Campylobacter coli* depending on the clinical status in camels.





**Figure 15:** Graph demonstrating the difference between the species of *C.jejuni* and *C.coli* depending on the clinical status in camels.

Regarding geographical distribution, the Wilaya of Ouargla showed the highest prevalence, followed by Oued Souf, which could be linked to environmental factors or specific sanitary conditions to these areas.



**Figure 16 :** Graph demonstrating the positive cases of Campylobacter depending on the geographic distribution of the samples collected from camels.

All the results obtained in our study regarding the isolation rate of Campylobacter according to the different risk factors (regions, clinical symptoms, etc.) are presented in the following table.

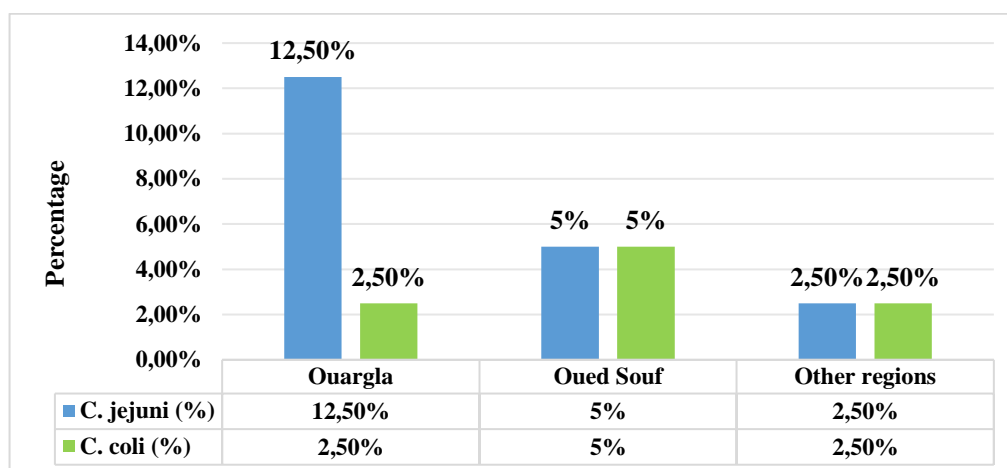
**Tableau 3:** Distribution of *Campylobacter* species according to the clinical condition of dromedaries and the Wilayas.

Variable	Number of samples	Positive cases (%)	<i>C. jejuni</i> (%)	<i>C. coli</i> (%)	P-value
<b>Clinical status</b>					
<b>Dromedaries with digestive signes</b>	50	10 (20 %)	8 (16 %)	2 (4 %)	0.001
<b>Healthy dromedaries</b>	70	2 (2.9 %)	0	2 (2.9 %)	
<b>Geographic distribution</b>					
<b>Ouargla</b>	40	6 (15 %)	5 (12.5 %)	1 (2.5 %)	0.037
<b>Oued Souf</b>	40	4 (10 %)	2 (5 %)	2 (5 %)	NS
<b>Autres régions</b>	40	2 (5 %)	1 (2.5 %)	1 (2.5 %)	NS

NS : not significant – P-values indicate significant differences between groups.

Figure 6 illustrates the relative prevalence of *Campylobacter jejuni* and *Campylobacter coli* in dromedary samples taken from different localities.

The results indicate that *C. jejuni* has the highest prevalence in the wilaya of Ouargla with more than 12% and that the prevalence of *C. coli* in the same locality is less than 2–3%. The distribution of the two bacteria is almost similar in the wilaya of Oued Souf with around 5% for each. In the other localities, the overall prevalence is low and almost equal with less than 3% for both. This result may indicate that the presence of *C. jejuni* in the wilaya of Ouargla is influenced by environmental and/or management factors.



**Figure 17:** Graph demonstrating the difference between *Campylobacter jejuni* and *Campylobacter coli* depending the geographic distribution of the samples collected from camels.

## II.2. Antimicrobial Susceptibility Profile of *Campylobacter* Isolates

Comprising *C. jejuni* and *C. coli* strains. All isolates (100%) showed resistance to at least one antimicrobial agent, indicating a high level of selective pressure in the farming environment.

However, one *C. jejuni* isolate was identified as sensitive to all five tested antimicrobials, suggesting the existence of strains not yet exposed to repeated antibiotic treatments.

One isolate (8.3%) showed resistance to a single antimicrobial agent, while four others (33.3%) were resistant to multi-agents. These results indicate variability in resistance profiles, likely reflecting differences in antibiotic exposures depending on local veterinary practices. The highest resistance rates were recorded for:

- Chloramphenicol (83.3%; 10/12 isolates),
- Followed by Amoxicillin-clavulanic acid (58.3%; 7/12 isolates).

These concerning rates could be explained by the widespread use of these molecules in dromedary farming, sometimes through self-medication or preventive treatment without accurate diagnosis. Such practices promote the selection of resistant strains. The most effective antimicrobial agents (with the lowest resistance rates) were:

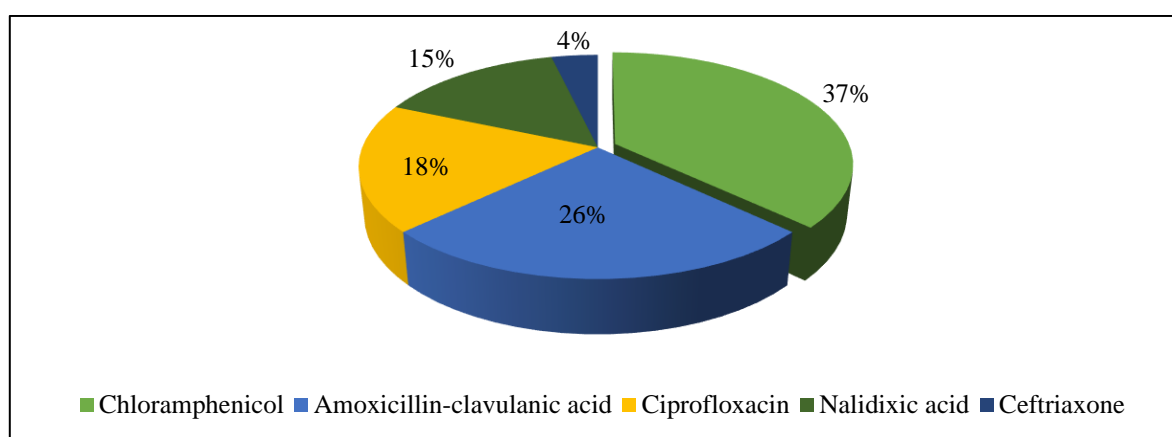
- Ceftriaxone (8.3%; 1/12),
- Nalidixic acid (33.3% each; 4/12).

In Figure 7, Chloramphenicol and amoxicillin-clavulanic acid are the least effective antibiotics against *Campylobacter* strains in this study. However, ceftriaxone remains the most effective one. These results show the alarming increase in the multi-drug resistance among

*Campylobacter* strains and it needs to be considered carefully when selecting and regulating the antibiotics used in camels.

**Tableau 4:** Antimicrobial resistance profile of the 12 *Campylobacter* isolates.

Antimicrobial	Number of resistant isolates (n)	Antibiotic resistance rate (%)
<b>Chloramphenicol</b>	10	83,3 %
<b>Amoxicillin-clavulanic acid</b>	7	58,3 %
<b>Ciprofloxacin</b>	5	41,7 %
<b>Nalidixic acid</b>	4	33,3 %
<b>Ceftriaxone</b>	1	8,3 %
<b>Multidrug-resistant (<math>\geq 3</math> classes)</b>	<b>7</b>	<b>58,3 %</b>



**Figure 18:** Graph demonstrating the antimicrobial resistance of the 12 *Campylobacter* isolates collected from camels.

These results confirm relatively good susceptibility to fluoroquinolones and third-generation cephalosporins, although regular monitoring is essential due to their critical importance in human medicine.

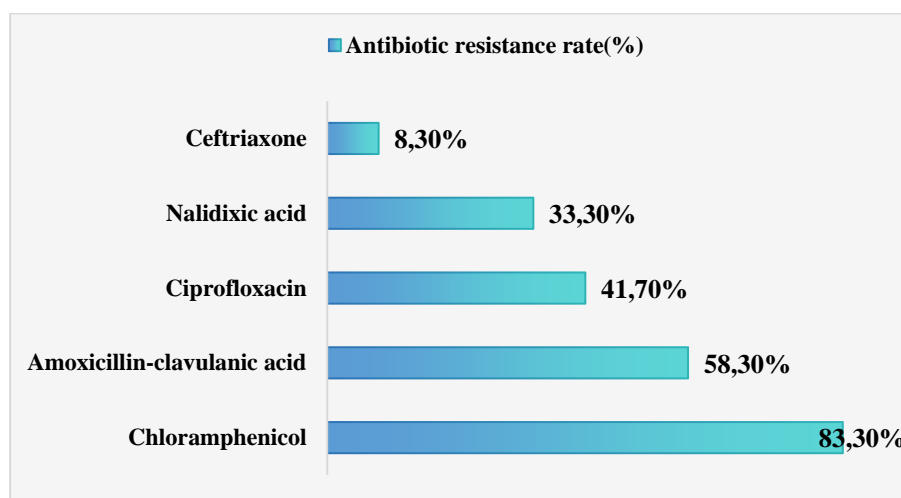
Furthermore, multidrug resistance (MDR), defined as resistance to three or more classes of antibiotics, was observed in 8 out of 12 isolates (66.7%). Among these MDR isolates:

- The majority (5 isolates; 62.5%) were *C. jejuni*,
- And the remainder (3 isolates; 37.5%) were *C. coli*.

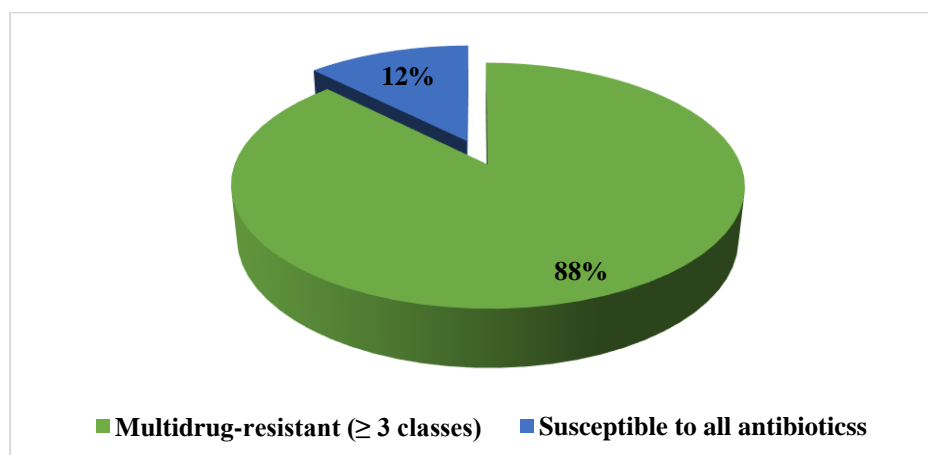
This multidrug resistance represents a major risk to public health, particularly if these strains are transmitted to humans through the food chain or the environment.

These results from figure 8 highlight the levels of antibiotic resistance in *Campylobacter* isolates from camels, reflecting their efficacy. Chloramphenicol and amoxicillin-clavulanic acid showed the lowest efficacy against ***Campylobacter*** strains in this study, while ceftriaxone

remained the most effective. This resistance rate underscores the worrying prevalence of multidrug resistance, emphasizing the importance of careful selection and regulated use of antibiotics in camel husbandry.



**Figure 19 :** Graph demonstrating the antimicrobial resistance rate in 12 *Campylobacter* isolates collected from camels.



**Figure 20 :** Graph demonstrating the antimicrobial resistance rate the multidrug and the susceptible in the 12 *Campylobacter* isolates collected from camels.

The details of the multi-resistance profiles of the different isolated strains are shown in Table 4.

**Tableau 5:** Antimicrobial resistance profile of the 12 *Campylobacter* isolates (n = 12).

Isolat	species	AMC	C	CRO	CIP	NA	Number of resistant antibiotics	MDR
1	<i>C. jejuni</i>	R	R	S	R	R	4	Oui
2	<i>C. jejuni</i>	R	R	S	R	R	4	Oui
3	<i>C. jejuni</i>	R	R	S	S	S	2	Oui
4	<i>C. jejuni</i>	S	S	S	S	S	0	Non
5	<i>C. jejuni</i>	R	R	R	R	R	5	Oui
6	<i>C. jejuni</i>	S	R	S	S	S	1	Oui
7	<i>C. jejuni</i>	S	R	S	S	S	1	Non
8	<i>C. coli</i>	R	R	S	S	R	3	Oui
9	<i>C. coli</i>	S	R	S	S	S	1	Non
10	<i>C. coli</i>	R	R	S	R	S	3	Oui
11	<i>C. coli</i>	S	S	S	S	S	0	Non
12	<i>C. jejuni</i>	R	R	S	R	S	3	Oui

### III. Discussion

The results of this study reveal an overall prevalence of 10% of *Campylobacter* infections in the dromedaries examined, with a clear predominance of *C. jejuni* (8%) over *C. coli* (4%). Although this prevalence is lower than that reported in cattle and small ruminants in certain regions (Altekruse *et al.*, 1999; Hlashwayo *et al.*, 2020), it remains concerning due to the high zoonotic potential of these bacteria, particularly *C. jejuni*, a major cause of gastroenteritis in humans (Kaakoush *et al.*, 2015).

Analysis based on the clinical status of the animals shows that 83% of positive cases were found in dromedaries showing digestive symptoms, suggesting a significant correlation between infection and clinical signs. These results corroborate those of previous studies highlighting the role of *C. jejuni* in enteritis in ruminants and its ability to cause significant intestinal lesions (Stanley and Jones., 2003).

Conversely, the low detection of **Campylobacter** (2.9%) in clinically healthy dromedaries indicates low asymptomatic carriage, or intermittent shedding of the pathogen, a

phenomenon already described in ruminants (**Sahin *et al.*, 2002**). This suggests that the symptomatic phase represents the optimal moment for the detection and diagnosis.

The geographical distribution shows a higher prevalence in the Wilayas of Ouargla and Oued Souf, probably due to environmental factors favorable to bacterial survival, including high temperatures and extensive herd management. Indeed, *Campylobacter* can persist in stagnant water and contaminated soil (**Carter *et al.*, 2009**), and its transmission is facilitated by poor hygiene and close proximity between animals (**de Boer *et al.*, 2015**).

Although this study only included fecal samples, it is important to note that the environment (such as watering points, pastures, and equipment) can play an important role in the indirect transmission of the pathogen, as demonstrated in several studies in other animal species (**Koolman *et al.*, 2015**).

The results obtained highlight concerning levels of resistance to antibiotics commonly used in livestock farming, which could limit therapeutic options in cases of clinical infection. The high prevalence of multidrug resistance aligns with other studies conducted in Maghreb countries and East Africa, which have reported MDR rates in *Campylobacter* ranging from 40% to 80% (**Hlashwayo *et al.*, 2020; Kaakoush *et al.*, 2015**).

Typically, antibiotic resistance should not be this high, especially since the use of antibiotics is not recommended. The findings indicate that camel farmers are using unprescribed antibiotics without proper precautions or diagnoses from veterinarians. This practice has led to a significant increase in antibiotic resistance and multidrug resistance in camels, which should ideally be low.

The presence of resistant strains in camel farms also suggests possible cross-transmission between animals, humans, and the environment, underscoring the need to adopt a "One Health" approach to combating antimicrobial resistance.

#### **IV. Conclusion and Recommendations**

This investigation in camel feces revealed significant and troubling findings regarding thermotolerant *Campylobacter* species. Out of 120 samples examined, 10% harbored these bacteria, indicating a noteworthy prevalence within the local camel population. Among these, *Campylobacter jejuni* was identified in 6.7% of the samples, making it more prevalent than *Campylobacter coli*, which was found in 3.3%.

This disparity raises important questions about the ecological and environmental factors in specific areas, particularly in Ouargla, where *C. jejuni* was detected in 12.5% of all samples. This localization suggests that particular environmental conditions, such as sanitation practices or the presence of contaminated sources, may favor the survival and transmission of these pathogens.

A more alarming aspect of this study is the observation of gastrointestinal symptoms in a significant proportion of infected camels. Symptoms such as diarrhea not only affirm the hypothesis that these bacteria are the causative agents of the diseases being observed but also highlight the potential for broader health impacts within animal populations. Moreover, the presence of visible symptoms in infected animals raises concerns regarding animal welfare and the economic implications for local farmers who rely on camels for their livelihoods.

The investigation also uncovered serious issues concerning antibiotic resistance. Each bacterial sample demonstrated resistance to at least one antibiotic, underscoring a growing public health concern. Alarming, 83.3% of the bacterial strains exhibited resistance to chloramphenicol, and 58.3% showed resistance to amoxicillin-clavulanic acid. Over half of the bacteria (58.3%) displayed multi-drug resistance, which severely complicates treatment strategies and raises the stakes for public health. The emergence of multi-drug-resistant bacteria not only limits therapeutic options but also poses a threat of transmission to humans, emphasizing the need for urgent attention to this issue.

This study underscores an urgent concern that the excessive and often times inappropriate use of antibiotics in the livestock industry is contributing to the alarming rise of antibiotic-resistant bacteria. Without effective management and control measures, there is a real risk that these resistant strains could spread through the food supply chain, potentially impacting not just animal health but also the well-being of humans.

This issue is particularly pressing in regions where camels are integral to agriculture and food production, highlighting the necessity for developing sustainable practices and stringent regulations to safeguard public health and animal welfare.



Long-term solutions may include promoting responsible antibiotic use, improving farm biosecurity, and implementing better hygiene and sanitation practices on farms. These measures are crucial in addressing the dual challenges of bacterial infections and antibiotic resistance in livestock populations.

## List of annex

### **Annex 1: Gram staining Technique**

#### Smear preparation

Prepare a thin bacterial smear on a clean glass slide (place a drop of physiological saline on the clean slide and collect a small amount of the culture and mix it to obtain a homogeneous suspension) and allow it to air dry

#### Heat fixation

Pass the slide briefly through a flame of burner 2-3 times to fix the bacteria onto the slide

#### Primary stain

Flood the smear with crystal violet and let sit for 1 minute. Rinse gently with water

#### Mordant:

Apply iodine solution(lugol) and let sit for 1 minute this forms a crystal violet-iodine complex. Rinse with water

#### Decolorization

Briefly apply alcohol(10-20seconds) until runoff is clear, rinse immediately with water

#### Counterstain

Apply fuchsin and let sit for 30-60 seconds, rinse with water

#### Dry and observe

Gently blot the slide dry, add a drop of immersion oil, and examine under the optical microscope (100x).

### **Annex 2: Technique for fresh preparation examination**

Collect sample with a sterile swab a small colony from agar.

Place the swab content on a microscope slide.

Add a drop of physiological saline (0,9% NaCl).

Cover with a cover slip.

Quick observe under the 40x objective with maximum light but with the diaphragm closed, or observed under the 100x objective by placing a drop of immersion oil on the coverslip.

---

**References****A**

- Altekruse, S. F., Stern, N. J., Fields, P. I., & Swerdlow, D. L. (1999). *Campylobacter jejuni*—An emerging foodborne pathogen. *Emerging Infectious Diseases*, 5(1), 28–35.

**B**

- Butzler J.P., 2004; *Campylobacter*, from obscurity to celebrity. *Clin Microbiol Infect*; 10 :868-76.

**C**

- Carter, A. M., Pacha, R. E., Clark, G. W., & Williams, E. A. (2009). Seasonal occurrence of *Campylobacter* spp. in surface waters and their correlation with standard indicator bacteria. *Journal of Water and Health*, 7(1), 129–138.
- Costas M., Owen R.J., Jackman P.J.H., 1987; Classification of *Campylobacter sputorum* and allied campylobacters based on numerical analysis of electrophoretic protein patterns. *Systematic and Applied Microbiology*; 9 (1-2): 125-31.

**D**

- De Boer, P., Wagenaar, J. A., & Achterberg, R. P. (2015). Longitudinal study on *Campylobacter jejuni* in dairy cattle and effects of hygiene measures. *Veterinary Microbiology*, 176(1–2), 188–193.
- Dekeyser P., Detrain G.M., Butzler J.P., Sternon J., 1972; Acute enteritis due to related vibrio: First positive stool cultures. *J Infect Dis*; 125: 390-92.
- Doyle L., 1944; A vibrio associated with swine dysentery. *Am J Vet Res*; 5: 3-5. 59. Doyle MP., 1981; *Campylobacter fetus* subsp. *Jejuni*: an old pathogen of new concern. *J Food Protect*; 44: 480-88.
- Dromigny E., 2007; *Campylobacter*. Ed Lavoisier ; pages: 25-29, 127-137, 168, 169, 196-201.

**E**

- Endtz H.P., Vliegthart J.S., Vandamme P., Weverink H.W., Braak N.P., Verbrugh H.A., Van B.A., 1997; Genotypic diversity of *Campylobacter lari* isolated from mussels and oysters in The Netherlands. *Int J Food Microbiol* ; 34: 79-88.
- Engberg J., Aarestrup F.M., Gerner-Smidt P., Nachamkin I., 2001; Quinolone and Macrolide Resistance in *Campylobacter jejuni* and *C. coli*: Resistance Mechanisms and Trends in Human Isolates. *Emerg Infect Dis*; 7(1): 24-34.

**F**

- Freney J., Renaud F., Leclercq R., Riegel P., 2007. Précis de bactériologie Clinique *Campylobacter*. Ed ESKA; Pages: 1349-1357.

**G**

- Ghafir Y., Daube G., 2007; Le point sur les méthodes de surveillance de la contamination microbienne des denrées alimentaires d'origine animale. *Ann Méd Vét*; 151: 79-00.

**H**

- Hald B., Madsen M., 1997; Healthy puppies and kittens as carriers of *Campylobacter* spp., with special reference to *Campylobacter upsaliensis*. *J Clin Microbiol*; 35: 3351-52.
- Hlashwayo, D. F., Sigaúque, B., & Bila, C. G. (2020). Prevalence of *Campylobacter* in animals, humans, and the environment in Africa: A systematic review and meta-analysis. *One Health*, 10, 100146.

**J**

- Jones F.S., Orcutt M., Little R.B., 1931; Vibrios (*Vibrio jejuni*) associated with intestinal disorders of cows and calves. *J Exp Med*; 53: 853-64.

**K**

- Kaakoush, N. O., Castaño-Rodríguez, N., Mitchell, H. M., & Man, S. M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, 28(3), 687–720.
- King E.O., 1957; Human infections with *Vibrio fetus* and a closely related *Vibrio* isolated from cases of human vibriosis. *J infect Dis*; 101: 119-28.
- King E.O., 1962; The laboratory recognition of *Vibrio fetus* and a closely related *Vibrio* isolated from cases of human vibriosis. *Ann NY Acad Sci*; 98: 700-11.
- Koolman, L., Whyte, P., & Bolton, D. (2015). An investigation of the molecular diversity of *Campylobacter* species in Irish retail poultry. *International Journal of Food Microbiology*, 210, 239–245.

**L**

- Levy A.J., 1946; A gastro-enteritis outbreak probably due to a bovine strain of *Vibrio*. *J Infect Dis*; 18:243-58.

**M**

- Marshall B.J., 1986; *Campylobacter pyloridis* and gastritis. *J Infect Dis*; 153 (4): 650-57.
- Megraud F., Bultel C., 2004; Appréciation des risques alimentaires liés aux campylobacters. Application au couple poulet/*Campylobacter jejuni*, Rapport de AFSSA ; 96 pages.

- 
- Megraud F., Bultel C., 2004; Appréciation des risques alimentaires liés aux campylobacters. Application au couple poulet/*Campylobacter jejuni*, Rapport de AFSSA; 96 pages.
  - Mentor Aliber L., 2012; Développement d'un essai PCR pour l'identification des espèces de *Campylobacter*. Mémoire présentée à la Faculté des études supérieures et postdoctorales de l'Université Laval dans le cadre du programme de maîtrise en Microbiologie-Immunologie pour l'obtention du grade de Maître ès sciences. Département de microbiologie-immunologie, faculté de médecine, université Laval (QUÉBEC), 87 pages.
  - Miller W.G., Bates A.H., Horn S.T., Brandl M.T., Wachtel M.R., Mandrell R.E., 2000 ; Detection on surfaces and in Caco-2 cells of *Campylobacter jejuni* cells transformed with new gfp, yfp, and cfp marker plasmids. *Appl Environ Microbiol*; 66: 5426-36.

**N**

- Ng L.k., Sherburne R., Taylor D.E., Stiles M.E., 1985; Morphological forms and viability of *Campylobacter* species studied by electron microscopy. *J Bacteriol* ; 164 (1): 338-43.

**P**

- Parkhill J.B.W., Wren K., Mungall J.M., Ketley C., Churcher D., Basham T., Chillingworth R.M., Davies T., Feltwell S., Holroyd K., Jagels A.V., Karlyshev S., Moule M.J., Pallen C.W., Penn M.A., 2000; The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature*; 403 : 665-68.

**S**

- Sahin, O., Morishita, T. Y., & Zhang, Q. (2002). *Campylobacter coli* in turkeys: Antimicrobial resistance and molecular typing. *Avian Diseases*, 46(4), 1036–1041.
- Sebald M., Veron M., 1963; Teneur en base de l'ADN et classification des vibrions. *Ann Inst Pasteur*; 105: 897-910.
- Sebald M., Veron M., 1963; Teneur en base de l'ADN et classification des vibrions. *Ann Inst Pasteur*; 105: 897-910.
- Stanley, K., & Jones, K. (2003). *Campylobacter* and public health. *British Medical Bulletin*, 66, 21–38.

**V**

- Vandeplas S., Dubois-Dauphin R., Palm R., Beckers Y., Thonart P., Théwis A., 2008; Contamination of poultry flocks by the human pathogen *Campylobacter* spp. and strategies to reduce its prevalence at the farm level. *Biotechnol. Agron. Soc. Environ*; 12 (3): 317-34.
- Vincent R., Dumas J., Picard N., 1947 ; Septicémie grave au cours de la grossesse due à un *Vibrio*. Avortement consécutif. *Bull Acad Nat Med Paris*; 131: 90-

---

---