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Bacteriological and Molecular Studies on Campylobacter Microorganisms Isolated from Diarrheic Cattle Calves

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Abstract

Campylobacteriosis is considered one of the most important zoonotic bacterial infections worldwide, affecting both animals and humans. *Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are recognized as leading causes of gastroenteritis in humans, and are frequently associated with enteric infections in livestock. Diarrhea in neonatal and young cattle calves represents a major economic burden due to increased morbidity, mortality, treatment costs, and reduced growth performance. The role of Campylobacter organisms as causative agents of diarrhea in calves has been reported, yet limited studies have addressed the bacteriological and molecular characterization of Campylobacter isolates recovered from diarrheic calves, especially in relation to their public health importance and antimicrobial resistance concerns.

Therefore, the current study aimed to investigate the prevalence of thermophilic Campylobacter species among diarrheic cattle calves using conventional bacteriological and biochemical methods, followed by molecular confirmation using polymerase chain reaction (PCR). Additionally, the study was designed to evaluate the antimicrobial susceptibility patterns of recovered Campylobacter isolates and determine their multiple antimicrobial resistance (MAR) indices.

A total of 120 fecal samples were collected from diarrheic cattle calves from different localities in Sharkia Governorate, Egypt, during the period from October 2024 to March 2025. All collected samples were transported under cooling conditions to the laboratory for bacteriological analysis. Samples were initially enriched in Campylo-Thioglycollate broth supple-

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mented with selective antibiotics, followed by selective cultivation on modified charcoal cefoperazone deoxycholate agar (mCCDA). Suspected Campylobacter colonies were subjected to Gram staining, motility examination, and biochemical identification including oxidase, catalase, indoxyl acetate hydrolysis, hippurate hydrolysis, and susceptibility to nalidixic acid and cephalothin.
Molecular confirmation of the recovered isolates was conducted using genus-specific PCR targeting the 23S rRNA gene, followed by species-specific PCR targeting the mapA gene for *C .jejuni* and the ceuE gene for *C. coli*. Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method against 13 antimicrobial agents representing different antimicrobial classes. The MAR indices were calculated for each isolate.
The overall prevalence of Campylobacter species among examined diarrheic calves was 23.3% (28/120). The distribution of Campylobacter species revealed that *C. jejuni* represented 16.7% (20/120), while *C. coli* represented 6.6% (8/120). PCR amplification produced characteristic amplicon sizes of 650 bp for genus Campylobacter (23S rRNA), 589 bp for *C. jejuni* (mapA), and 462 bp for C. coli (ceuE). The antimicrobial susceptibility results demonstrated high resistance rates against cefotaxime (82.1%), tetracycline (78.6%), and penicillin (75.0%), whereas the lowest resistance rates were recorded against gentamicin (21.4%) and chloramphenicol (28.6%). The MAR indices ranged between 0.31 and 0.85, indicating the presence of multidrug-resistant Campylobacter isolates.
In conclusion, the present study highlights the occurrence of Campylobacter species in diarrheic cattle calves, confirming their possible role as enteric pathogens and their importance as reservoirs for zoonotic transmission. The presence of multidrug-resistant Campylobacter isolates emphasizes the need for strict antimicrobial stewardship programs in veterinary practice and continuous surveillance for Campylobacter infections in livestock.

Introduction

Diarrhea in neonatal and young cattle calves remains one of the most significant health problems in bovine production systems worldwide. Calf diarrhea is considered a multifactorial disease involving bacterial, viral, parasitic, nutritional, and management-related causes. Among bacterial agents, *Escherichia coli*, Salmonella spp., *Clostridium perfringens*, and Campylobacter spp. have been frequently reported as causative agents of enteric disturbances in calves. Calf diarrhea causes severe economic losses due to dehydration, mortality, impaired growth, increased veterinary costs, and reduced productivity in the long term.

Campylobacteriosis is a zoonotic disease caused by Campylobacter species **(WHO, 2020).** Campylobacters are Gram-negative, curved or spiral-shaped, motile, non-spore forming bacteria that are microaerophilic in nature **(Fitzgerald, 2015).** The genus Campylobacter includes numerous species, among which *Campylobacter jejuni* and *Campylobacter coli* are the most commonly isolated thermophilic species and are strongly associated with gastroenteritis in humans **(Allos, 2001; Debruyne *et al.*, 2008).** Campylobacter infections in humans are typically transmitted through consumption of contaminated food products, particularly undercooked poultry, unpasteurized milk, and contaminated water **(Bryan and Doyle, 1995).** Additionally, direct contact with infected animals or their feces is considered an important route of transmission **(Silva *et al.*, 2011).**

The emergence of antimicrobial resistance among Campylobacter species has become a growing public health concern **(CDC, 2019).** Campylobacter isolates have demonstrated resistance to clinically important antimicrobial agents including fluoroquinolones, macrolides, tetracyclines, and beta-lactams **(Dasti *et al.*, 2007).** This resistance is often linked to excessive and uncontrolled use of antimicrobials in veterinary medicine, livestock farming, and food animal production. Multidrug-resistant (MDR) Campylobacter strains have been increasingly reported worldwide, complicating therapeutic options in both humans and animals **(Rivera-Mendoza *et al.*, 2020).**

Isolation and identification of Campylobacter species is challenging due to their fastidious

growth requirements, slow growth rates, and strict microaerophilic conditions **(Fitzgerald, 2015).** Conventional culture methods are widely used, including selective enrichment and selective media such as modified charcoal cefoperazone deoxycholate agar (mCCDA) **(ISO, 2006).** Biochemical differentiation between C. jejuni and C. coli is commonly based on hippurate hydrolysis test; however, atypical strains may lead to false results. Therefore, molecular diagnostic methods such as polymerase chain reaction (PCR) are considered reliable tools for accurate identification at both genus and species levels **(Shin and Lee, 2009).**

Accordingly, the present study was conducted to determine the prevalence of Campylobacter species in diarrheic cattle calves and evaluate antimicrobial resistance patterns of recovered Campylobacter isolates and estimate their MAR indices. As well as understanding of Campylobacter infections in cattle calves and provide valuable information regarding their zoonotic importance and antimicrobial resistance status.

Materials and Methods

Materials

Study Area and Duration

This study was conducted in Sharkia Governorate, Egypt during the period from October 2024 to Jan. 2026. Sampling was performed from different cattle farms and veterinary clinics located in Zagazig, Belbeis, and Abu Hammad districts.

Samples

A total of 120 fecal samples were collected from diarrheic cattle calves aged 2 days to 5 months showing clinical signs of diarrhea including watery feces, dehydration, weakness, and reduced suckling. Samples were collected from calves of different breeds (Baladi and Friesian cross) and both sexes.

All fecal samples were collected aseptically using sterile rectal swabs and placed immediately into sterile tubes containing Campylo-Thioglycollate (Campy-Thio) broth. Samples were transported in an ice box to the bacteriological laboratory within 3 hours for further bacteriological examination.

Table (1). Distribution of collected samples from diarrheic cattle calves during the course of the study

District	No. of Samples
Zagazig	45
Belbeis	40
Abu Hammad	35
Total	**120**

Media and Reagents

Campylo-Thioglycollate Medium Base (Campy-Thio) was used for enrichment. Campylobacter Selective Supplement-I (Blaser-Wang) was used for selective isolation. Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) was used for cultivation. Sheep blood agar was used for purification. CampyGen sachets were used for producing microaerophilic conditions.

Reference Strains

Campylobacter jejuni (NCTC 11322) and *Campylobacter coli* (NCTC 11366) were used as reference strains.

Antimicrobial Discs

Thirteen antimicrobial discs were used including penicillin, ampicillin–sulbactam, amoxicillin–clavulanic acid, erythromycin, tylosin, colistin, chloramphenicol, cefotaxime, tetracycline, gentamicin, ciprofloxacin, norfloxacin, and sulfamethoxazole–trimethoprim.

Methods

Clinical Examination: According to Constable *et al* (1998)

All calves were clinically examined. Diarrhea was defined as passage of watery to semi-liquid feces more than 3 times daily.

Isolation of Campylobacter Species: According to (ISO 10272-1, 2017)

Fecal swabs were inoculated into Campy-Thio broth and incubated at 42°C for 24–48 hours under microaerophilic conditions. After enrichment, cultures were streaked onto mCCDA plates and incubated at 42°C for 48 hours in darkness under microaerophilic conditions generated by CampyGen sachets. Suspected colonies were purified on sheep blood agar **(ISO, 2006).**

Identification of Campylobacter Isolates: According to (ISO 10272-1, 2017)

Cultural characteristics, Gram staining, motility test, biochemical tests (oxidase, catalase, nitrate reduction, indoxyl acetate, hippurate

hydrolysis), and susceptibility to nalidixic acid and cephalothin were performed **(Galate and Bangde, 2015).**

Molecular Identification by PCR

DNA was extracted using QIAamp DNA Mini Kit. PCR was performed using primers targeting 23S rRNA gene (genus), mapA gene (C. jejuni), and ceuE gene (C. coli). PCR products were visualized using 1.5% agarose gel electrophoresis.

PCR Assays and Cycling Parameters

Uniplex PCR assays were performed for amplifi cation of the 23S rRNA gene of genus Campylobacter, mapA gene of C. jejuni and ceuE gene of C. coli. All PCR reactions were done utilizing the Emerald Amp GT PCR master mix (Takara, Berkeley, CA, USA) according to the manufacturer's guidelines. The specific genes and the primer sequences for all PCR assays are shown in Table 2. All amplifi cation protocols were performed as previously described. Gel electrophoresis and ethidium bromide staining (Sigma-Aldrich, St. Louis, MI, USA) were done as previously pronounced. DNA of Campylobacter jejuni (NCTC 11322) and Campylobacter coli (NCTC 11366) were utilized as a positive controls and PCR grade water (no template DNA) was utilized as a negative control.

Table (2). Oligonucleotide primer sequences and amplified PCR products of six target genes of *Campylobacter jejuni.*

Gene	Primer sequence ('3-'5)	Reference
23S rRNA	F: TATACCGGTAAGGAGTGCTGGAG R: ATCAATTAACCTTCGAGCACCG	**Wang *et al.*, (2002)**
MapA	F: ACTTCTTTATTGCTTGCTGC R: GCCACAACAAGTAAAGAAGC	**Shin and Lee, (2009)**
ceuE	F: AATTGAAAATTGCTCCAACTATG R':TAGTTTTATTATTTGTAGCAGCG	**Shin and Lee, (2009)**

Antimicrobial Susceptibility Testing

Kirby–Bauer disk diffusion method was applied on Mueller-Hinton agar supplemented with 5% sheep blood and incubated at 42°C under microaerophilic conditions for 48 hours **(Vandepitte and Verhaegen, 2003).** The degree of sensitivity of each isolate was determined by measuring the diameter of the inhibition zone around each disc and the results were interpreted according to the clinical and laboratory standards institute **(CLSI, 2026).**

MAR Index Calculation

MAR index = number of antibiotics resisted / 13.

Statistical Analysis

Chi-square test was used for statistical analysis and p-value < 0.05 was considered statistically significant.

Results

1. Isolation and Identification of Campylobacter Species

Campylobacter colonies appeared on mCCDA as grayish, moist spreading colonies with metallic sheen. On sheep blood agar, colonies were round, convex, non-hemolytic, translucent, and exhibited typical oil drop-like appearance. Gram staining showed Gram-negative curved bacilli with characteristic gull-winged shape. Wet mount examination revealed rapid darting motility.

All isolates were oxidase positive, catalase positive, and nitrate reduction positive. All isolates were sensitive to nalidixic acid and resistant to cephalothin. Hippurate hydrolysis test was positive in C. jejuni isolates and negative in C. coli isolates.

2. Prevalence of Campylobacter Species in Diarrheic Cattle Calves

Out of 120 examined fecal samples, 28 (23.3%) Campylobacter isolates were recovered. The prevalence of *C. jejuni* was 16.7% (20/120), while *C. coli* was 6.6% (8/120).

Table (3). Prevalence of Campylobacter species among diarrheic cattle calves

District	No. of Examined Samples	Positive Campylobacter	Prevalence (%)
Zagazig	45	12	26.7%
Belbeis	40	9	22.5%
Abu Hammad	35	7	20.0%
Total	**120**	**28**	**23.3%**

Table (4). Distribution of Campylobacter species among positive isolates

District	Total Campylobacter isolates	*C. jejuni* No. (%)	*C. coli* No. (%)
Zagazig	12	9 (75.0%)	3 (25.0%)
Belbeis	9	6 (66.7%)	3 (33.3%)
Abu Hammad	7	5 (71.4%)	2 (28.6%)
Total	**28**	**20 (71.4%)**	**8 (28.6%)**

3. Molecular Confirmation of Campylobacter Species

PCR amplification confirmed all 28 isolates as Campylobacter genus by detecting the 23S rRNA gene at 650 bp (Wang et al., 2002). Species-specific PCR revealed that 20 isolates were positive for mapA gene at 589 bp confirming C. jejuni, while 8 isolates were positive for ceuE gene at 462 bp confirming C. coli **(Shin and Lee, 2009).**

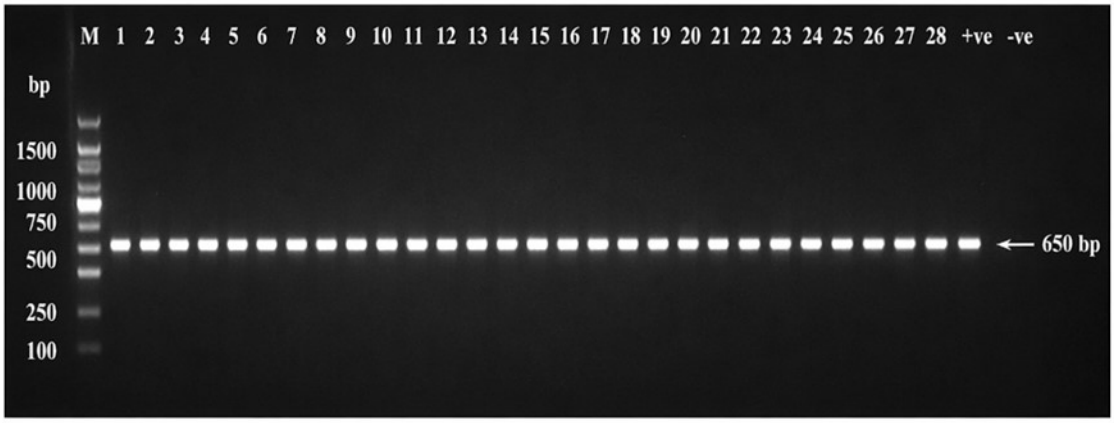

Figure (1). Agarose gel electrophoresis image showing PCR amplification of the 23S rRNA gene (650 bp) for Campylobacter genus. Lanes M: 100 bp DNA ladder, Lanes 1-28: Campylobacter isolates, +ve: Positive control (*C. jejuni* NCTC 11322). -ve: Negative control.

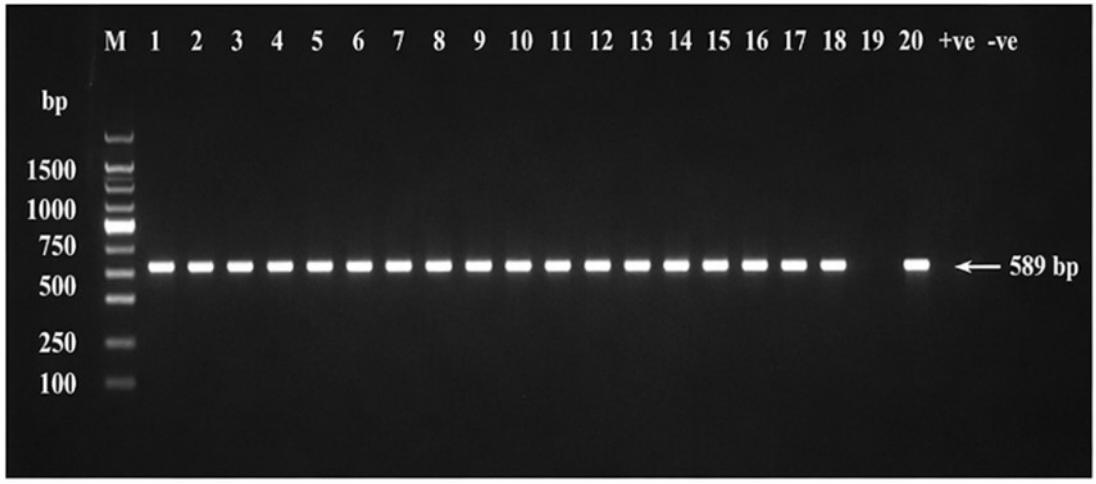

Figure (2). Agarose gel electrophoresis image showing PCR amplification of the mapA gene (589 bp) for *C. jejuni* identification. Lanes M: 100 bp DNA ladder, Lanes 1-20: C. jejuni isolates, +ve: Positive control (C. jejuni NCTC 11322) ,-ve: Negative control.

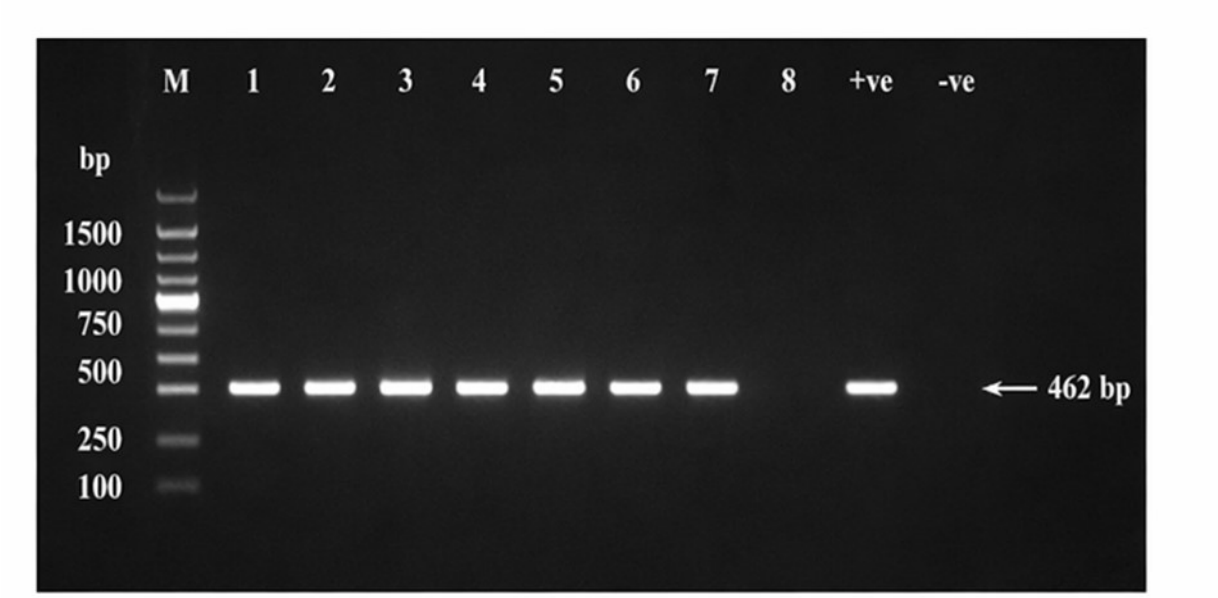

Figure (3). Agarose gel electrophoresis image showing PCR amplification of the ceuE gene (462 bp) for *C. coli* identification. Lanes M: 100 bp DNA ladder, Lanes 1-8: C. coli isolates, +ve: Positive control (*C. coli* NCTC 11366), -ve: Negative control.

4. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of Campylobacter isolates (n = 28) against 13 antimicrobial agents revealed high resistance levels to cefotaxime (82.1%), tetracycline (78.6%), penicillin (75.0%), and colistin (71.4%). Moderate resistance was detected against erythromycin (60.7%), ciprofloxacin (53.6%), and norfloxacin (50.0%). Lower resistance rates were observed against gentamicin (21.4%) and chloramphenicol (28.6%).

Table (4). Antimicrobial resistance pattern of Campylobacter isolates recovered from

Antimicrobial Agent	Resistant No. (%)	Sensitive No. (%)
Penicillin	21 (75.0)	7 (25.0)
Ampicillin-sulbactam	12 (42.9)	16 (57.1)
Amoxicillin-clavulanic acid	15 (53.6)	13 (46.4)
Cefotaxime	23 (82.1)	5 (17.9)
Tetracycline	22 (78.6)	6 (21.4)
Erythromycin	17 (60.7)	11 (39.3)
Tylosin	16 (57.1)	12 (42.9)
Colistin	20 (71.4)	8 (28.6)
Ciprofloxacin	15 (53.6)	13 (46.4)
Norfloxacin	14 (50.0)	14 (50.0)
Gentamicin	6 (21.4)	22 (78.6)
Chloramphenicol	8 (28.6)	20 (71.4)
SXT	13 (46.4)	15 (53.6)

5. Multidrug Resistance and MAR Index

Out of 28 Campylobacter isolates, 25 (89.3%) were found to be multidrug resistant (MDR). The MAR indices ranged from 0.31 to 0.85, with a mean MAR index of 0.62.

Discussion

The current study investigated the prevalence and molecular characterization of thermophilic Campylobacter species isolated from diarrheic cattle calves in Sharkia Governorate, Egypt. Campylobacteriosis is recognized as a major zoonotic disease, and cattle are considered important reservoirs contributing to environmental contamination and possible transmission to humans **(WHO, 2020; Goni *et al.*, 2017).**

In this study, Campylobacter species were isolated from 23.3% of examined diarrheic calf fecal samples. This finding suggests that Campylobacter organisms may play a role in calf enteritis and diarrhea, either as primary pathogens or as opportunistic organisms in association with other enteric agents. Similar prevalence rates have been documented in previous studies reporting Campylobacter recovery from cattle feces, confirming their widespread distribution in bovine populations **(Chon *et al.*, 2014).**

Regarding species distribution, *C. jejuni* represented 71.4% of recovered isolates, while C. coli represented 28.6%. These findings agree with several reports indicating that *C. jejuni* is the dominant Campylobacter species in cattle. The higher prevalence of *C. jejuni* may be attributed to its superior ability to colonize the bovine intestinal tract compared to other Campylobacter species **(Debruyne *et al.*, 2008).**

Bacteriological identification in the present study depended on enrichment and cultivation on mCCDA medium, which successfully supported the growth of thermophilic Campylobacter species. Colonies exhibited typical grayish spreading morphology on mCCDA and oil drop-like appearance on blood agar. Microscopic examination revealed characteristic curved Gram-negative rods with darting motility. Biochemical tests confirmed oxidase and catalase positivity. Species differentiation was performed using hippurate hydrolysis test, which revealed that hippurate-positive isolates were identified as C. jejuni, whereas hippurate-negative isolates were identified as C. coli. However, due to possible false results of biochemical differentiation, PCR was conducted for molecular confirmation **(ISO, 2006).**

PCR results confirmed the accuracy of conven-

tional identification. All isolates were positive for the 23S rRNA gene (650 bp), confirming genus Campylobacter **(Wang *et al.,* 2002).** Additionally, 20 isolates were positive for mapA gene (589 bp) confirming C. jejuni, while 8 isolates were positive for ceuE gene (462 bp) confirming *C. coli* **(Shin and Lee, 2009).** These results emphasize the importance of PCR as a reliable diagnostic method for Campylobacter species identification.

Antimicrobial susceptibility testing demonstrated high resistance rates to cefotaxime, tetracycline, penicillin, and colistin. Such high resistance may reflect the extensive use of these antimicrobial agents in veterinary medicine for treatment of bacterial infections and as growth promoters. Resistance to tetracycline and beta-lactams has been widely reported among Campylobacter isolates from livestock, and resistance to colistin is particularly alarming due to its importance as a last-resort antimicrobial in human medicine **(Dasti *et al.,* 2007).**

Notably, 89.3% of Campylobacter isolates in this study were MDR, showing resistance to three or more antimicrobial classes. The MAR indices ranged from 0.31 to 0.85, indicating high antimicrobial exposure and suggesting that cattle farms may represent high-risk environments for emergence and dissemination of resistant Campylobacter strains. Such findings represent a major public health concern, as resistant Campylobacter strains may be transmitted to humans through direct animal contact, environmental contamination, or food chain exposure **(CDC, 2019; Rivera-Mendoza *et al.,* 2020).**

In conclusion, the current study confirms that diarrheic cattle calves in Sharkia Governorate harbor Campylobacter species, mainly C. jejuni. The detection of MDR Campylobacter isolates with high MAR indices highlights the urgent need for antimicrobial stewardship programs, strict regulation of antibiotic use in livestock, and continuous surveillance for Campylobacter infections in cattle **(WHO, 2020).**

Ethics approval

The study received approval from the Zagazig University Institutional Animal Care and Use Committee (ZU-IACUC) under approval number ZU-IACUC/7/F/117/2026

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