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RESEARCH ARTICLE

Molecular Analysis of *Campylobacter* spp. Isolates from Sheep and Goats and Examination of Their Antibiotic Resistance

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Abstract

This study aimed to isolate and identify *C. jejuni, C. coli,* and *C. fetus* subsp. *fetus* from rectal swabs of diarrheic sheep and goats, characterize antimicrobial resistance and virulence genes, and determine genetic diversity using PCR-RFLP. Rectal swabs (n=374) were analyzed via selective culture and species-specific PCR. Resistance profiles, virulence genes, and *fla*A gene genetic diversity (via PCR-RFLP with *Dde*I) were evaluated. *Campylobacter* spp. was detected in 30.74% (115/374) of samples (87.82% *C. jejuni*, 12.17% *C. coli;* not determined *C. fetus* subsp. *fetus*). High susceptibility was observed to gentamicin (97.39%) and amoxicillin-clavulanic acid (93.04%). The highest resistance was against cephalothin (84.34%), reflecting intrinsic resistance. Acquired resistance to ciprofloxacin was significant (56.52%). Resistance genes 23SrRNA (100%), *gyr*A (96.92%), and *tet(O)* (89.58%) predominated. Main virulence genes were *cadF* (94.78%), *dnaJ* (92.17%), and *cdt*B (91.30%); *ggt* not determined. PCR-RFLP revealed 13 band patterns. In conclusion, *C. jejuni* was predominant in diarrheic sheep and goats. Isolates were highly susceptible to gentamicin and amoxicillin-clavulanic acid, representing efficacious antimicrobial options, though regional clinical guidelines and potential off-label use should be considered.

Keywords: Antimicrobial resistance, *Campylobacter* spp., *C. jejuni*, PCR-RFLP, Virulence gene

Introduction

Small ruminant breeding, primarily for meat, milk, and wool, is a key source of livelihood in the Siirt region and across South-eastern Anatolia, where these animals are particularly well adapted to the local climate, geography, and marginal lands [1-3]. Thermophilic *Campylobacter* spp. are the leading cause of foodborne gastroenteritis in humans, while *Campylobacter fetus* subsp. *fetus* colonises the gastrointestinal tract of small ruminants and can translocate to the urogenital system, causing abortions, underscoring the zoonotic potential of these bacteria [4].

Thermophilic *Campylobacter* spp., which are common inhabitants of the normal intestinal microbiota of domestic animals, can be transmitted to humans through the consumption of contaminated water, unpasteurized milk, or undercooked meat, particularly poultry [5]. Since *C. jejuni* and *C. coli* are frequently present in the intestinal tract of small ruminants, they can contaminate carcasses and other tissues during the slaughtering process, leading to environmental spread and public health risks [4,5]. In addition, faecal-oral transmission, aborted foetuses, and placenta are also important sources of transmission [4]. Apart from these, workers in animal shelters and animal product processing facilities, wild animals, and rodents also play a role in contamination and transmission, with flies serving as mechanical vectors [6,7].

Thermophilic *Campylobacter* spp, known to cause gastroenteritis in both human and animals, may continue to spread and infect the intestines without causing any clinical symptoms [8,9]. They may also cause death in infected animals due to abortion, weak lamb births, metritis, and diarrhoea or gastroenteritis in lambs [10]. In humans, they can cause diarrhoea, fever, and weight loss, as well as systemic complications including meningitis and myocarditis. Furthermore, they can cause significant neurological symptoms that may lead to serious conditions such as Miller Fisher syndrome and Guillain-Barré syndrome [8,11].

Treatment is rarely administered for uncomplicated *Campylobacter* infections [8]. However, antibiotics such as fluoroquinolones, tetracyclines, and macrolides may be indicated for severe or systemic cases in both

humans and small ruminants [6,12,13]. In certain regions, the historically widespread or uncontrolled use of these antibiotics has contributed to the emergence of increasing resistance mechanisms in *Campylobacter* spp [12]. This rising resistance, particularly in *C. jejuni* and *C. coli* isolates, can negatively affect the clinical efficacy of these conventional therapeutics against infection, a concern that persists globally despite varying regional antimicrobial stewardship regulations [8,12].

At the molecular level, resistance to fluoroquinolones is primarily mediated by point mutations in the *gyr*A gene, which encodes the DNA gyrase subunit A[14]. Tetracycline resistance is frequently associated with the plasmid-mediated or chromosomal *tet(O)* gene, which protects the bacterial ribosome [15]. For beta-lactams and aminoglycosides, resistance is commonly driven by the bla_{OXA-61} (encoding OXA-61 beta-lactamase) and *aphA-3-1* (encoding aminoglycoside phosphotransferase) genes, respectively [15]. Macrolide resistance, a major clinical concern, is predominantly linked to specific mutations in the 23S rRNA gene, often identified via markers such as *ERY2074* and *ERY2075* [16]. Furthermore, multidrug-resistant (MDR) phenotypes can be significantly amplified by the energy-dependent *Cme*ABC efflux pump system, encoded by the *cme*A, *cme*B, and *cme*C genes, which actively extrudes various antimicrobials out of the bacterial cell [15,16]. In addition to antimicrobial resistance (AMR) mechanisms, the pathogenicity of *Campylobacter* species is strongly governed by virulence factors responsible for motility, adherence, invasion, and toxin production [8,15]. Therefore, profiling both resistance determinants and virulence-associated genes is vital to clarifying the molecular epidemiology and pathogenic potential of small ruminants isolates [15].

Although various molecular tools are available for the characterization of *C. jejuni* and *C. coli*, their discriminatory power and epidemiological utility vary significantly [17]. Among these, *fla*A gene-based molecular techniques are widely preferred in epidemiological surveillance due to their rapidity, relative simplicity, and cost-effectiveness, while still offering acceptable discriminatory power [17,18]. Specifically, *fla*A gene-based Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) has become a well-established and widely utilized method for genotyping *Campylobacter* isolates [18]. Furthermore, while sequence analysis of short variable regions (SVRs) of *fla*A and *fla*B genes is another common alternative, particularly for short-term and localized studies, PCR-RFLP remains a highly practical and reliable approach for screening the genetic relatedness of large sample sets without requiring complex sequencing infrastructure [17].

The aim present study was to identify *C. jejuni*, *C. coli*, and *C. fetus* subsp. *fetus* strains from rectal swab samples of sheep and goats raised in the Siirt region, and to determine the antimicrobial susceptibility of these isolates. The presence of genes causing AMR in isolates was investigated to determine the genetic mechanism of the resistance profile. In addition, the presence of virulence-associated genes in the obtained isolates was investigated, and the genotypic characterization of the strains was performed using the *fla*A-based RFLP method.

Material and Methods

Ethical Statement

This study was approved by Siirt University Animal Experiments Local Ethics Committee (Approval No: 2023/07/48).

Study Area and Sampling Strategy

This active surveillance study was conducted between 2024 and 2025 across traditional family farms operating in the Siirt city center and its surrounding rural districts. A convenience sampling strategy based on passive epidemiological surveillance was employed, aiming to capture all accessible clinical cases during the study timeframe. The inclusion criteria strictly required small ruminants (sheep and goats) presenting with active diarrhoeic symptoms.

A total of 374 rectal swab samples were collected from affected animals, comprising 271 sheep (Hamdani breed) and 103 goats (Hair goat breed). The higher proportion of sheep samples naturally reflects the demographic distribution of livestock in the Siirt region, where sheep farming predominantly exceeds goat production. The sampled animals ranged in age from 1 to 5 years and represented both sexes.

Sample Collection and Transport

For sample collection, rectal swabs were collected from each animal using a sterile cotton-tipped swab rotated gently against the mucosal wall. Rectal swab samples were transported in tubes containing charcoal-supplemented Cary-Blair transport medium (Cary-Blair, 104054, Türkiye) and delivered to the Laboratory of the Department of Microbiology at Siirt University Faculty of Veterinary Medicine.

Isolation and Identification of *Campylobacter* spp.

Swab samples were inoculated in modified charcoal cefoperazone deoxycholate (mCCDA) agar (Biolife, NL2403, Italy) containing 5-10% sheep blood and Campylobacter mCCDA selective supplement (Oxoid, SR0069E, UK). Petri dishes were incubated in microaerophilic environment at 41.5°C for 36-48 h. Pure cultures were obtained from the colonies formed in the medium after incubation. Following the incubation period, a single presumptive *Campylobacter*

colony was selected and subcultured onto blood agar plates in accordance with ISO 10272-1:2017 [19]. The plates were then incubated under microaerophilic conditions at 42°C for 48 h. After incubation, the colony morphology was re-examined to verify the purity of the culture. The colonies obtained were stained using the Gram's method and evaluated for their microscopic morphology, growth on MacConkey agar, motility, oxidase, catalase, and indole reactions [14].

The suspected isolates were confirmed by PCR using species-specific primers *(Table 1)*.

DNA Extraction

Genomic DNA, which will be used for the PCR confirmation of suspected isolates, was obtained by the boiling method [17].

PCR Amplification

A commercial mastermix (EcoTaq, 2X PCR Mastermix, ET5, Türkiye) was used to prepare the PCR mixture, following the manufacturer's recommendations. The PCR protocol was optimized [20] and the total volume of the reaction mixture was adjusted to 25 µL: 12.5 µL master mix, 5 µL genomic DNA, 1.5 µL (10 µM) forward primer, 1.5 µL reverse primer, and 4.5 µL PCR broth. During the amplification process, the binding temperature was optimized according to the recommendations of the company from which the primers were synthesized (Probesynthesis Biyoteknoloji, Ankara, Türkiye). Thermal cycling profiles consisted of 35 cycles, including an initial denaturation step lasting 10 min at 94°C, followed by 1 min of denaturation at 94°C, 1 min of annealing at temperatures specifically adjusted for each primer pair, and 1 min of extension at 72°C; and finally, a final extension step lasting 10 min at 72°C. Amplicons produced by PCR were electrophoresised on agarose gel and analyzed using a gel imaging system (Gen-Box ImagER, Ankara, Türkiye).

Antimicrobial Susceptibility Test

The susceptibility of the isolates towards various antimicrobial agents was determined using the disk diffusion method.

Ciprofloxacin (5 µg, Bioanalyse, Türkiye), nalidixic acid (30 µg, Bioanalyse, Türkiye), cefalotin (30 µg, Bioanalyse, Türkiye), amoxicillin-clavulanic acid (20/10 µg, Bioanalyse, Türkiye), chloramphenicol (30 µg, Bioanalyse, Türkiye), erythromycin (15 µg, Liofilmchem, Italy), tetracycline (30 µg, Bioanalyse, Türkiye), gentamicin (10 µg, Bioanalyse, Türkiye), aztreonam (30 µg, Bioanalyse, Türkiye), and streptomycin (10 µg, Bioanalyse, Türkiye) antibiotic discs were used in the test.

The results were evaluated primarily in accordance with the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2007) [21] and the *Campylobacter*-specific interpretive criteria established by Miflin et al.[22] and Luangtongkum et al.[23]. For antimicrobial agents lacking defined specific breakpoints for *Campylobacter*, the interpretive criteria for *Enterobacteriaceae* specified by the CLSI were applied. The clinical breakpoints applied are summarized in *Table 2*.

As a result of the test, the isolates were assessed as susceptible, intermediate, or resistant to the antimicrobial discs used. In addition, the isolates resistant to three or more antimicrobial groups were assessed as MDR [24].

Identification of Antimicrobial Resistance Genes

The genetic mechanism of resistance was investigated by PCR using gene-specific primers in isolates phenotypically resistant to quinolone, beta-lactam, erythromycin, tetracycline, and aminoglycoside antibiotics. *Table 3* shows the primer sequences used for the identification of AMR genes by PCR.

Identification of Virulence-Associated Genes

The presence of virulence-associated genes in the isolates was investigated using PCR with gene-specific primers. *Table 4* shows the primer sequences used for the identification of virulence genes by PCR.

Genotyping

The *fla*A gene region PCR products were analyzed by RFLP. Reactions were carried out using the *Dde*I restriction enzyme (New England BioLabs, SR0175S, Türkiye) in a thermal cycler at 37°C for 15 min. Fragments were visualized on a 2.5% agarose gel (GelRed stained, UV light) using a

Table 1. *Primer sequences used for the PCR identification of the suspected isolates*

Bacteria	Target Gene	Oligonucleotide (5'-3')	Amplicon Size (bp)	Ref.
Campylobacter spp.	23S rRNA	**F:** TATACCGGTAAGGAGTGCTGGAG **R:** ATCAATTAACCTTCGAGCACCG	816	[20]
C. jejuni	*hipO*	**F:** ACTTCTTTATTGCTTGCTGC **R:** GCCACAACAAGTAAAGAAGC	323	[20]
C. coli	*glyA*	**F:** GTAAAACCAAAGCTTATCGTG **R:** TCCAGCAATGTGTGCAATG	126	[20]
C. fetus subsp. *fetus*	*sapB2*	**F:** GCAAATATAAATGTAAGCGGAGA **R:** TGCAGCGGCCCCACCTAT	435	[20]

Table 2. *Breakpoints for Campylobacter spp.*

Antibiotic	Potency	R	I	S	Ref.
Amoxicillin-clavulanic acid	20/10 µg	≤18	19-23	≥24	[21]
Aztreonam	30 µg	≤15	16-21	≥22	[21]
Cloramphenicol	30 µg	≤11	12-22	≥23	[22]
Ciprofloxacin	5 µg	≤13	14-17	≥18	[22]
Eritromycin	15 µg	≤15	16-18	≥19	[22]
Gentamicin	10 µg	≤12	13-14	≥15	[23]
Cephalotin	30 µg	≤14	15-17	≥18	[21]
Nalidixic acid	30 µg	≤13	14-18	≥19	[23]
Streptomycin	10 µg	≤10	11-12	≥15	[21]
Tetracyclin	30 µg	≤14	15-18	≥19	[23]

Table 3. *Primer sequences used for the identification of antimicrobial resistance genes by PCR*

Target Gene		Oligonucleotide (5'-3')	Amplicon Size (bp)	Ref.
Quinolone Resistance Genes *(C. jejuni)*	CampyMAMAgryA-F	**F:** TTTTTAGCAAAGATTCTGAT	265	[25]
	CampyMAMAgyrA-R	**R:** CAAAGCATCATAAACTGCAA		
	CampyMAMAgryA1-F	**F:** TTTTTAGCAAAGATTCTGAT	368	[25]
	GZgyrA4	**R:** CAGTATAACGCATCGCAGCG		
Quinolone Resistance Genes *(C. coli)*	GZgyrACcoli3F-F	**F:** TATGAGCGTTATTATCGGTC	192	[25]
	CampyMAMAgyrA8-R	**R:** TAAGGCATCGTAAACAGCCA		
	GZgyrACcoli3F-F	**F:** TATGAGCGTTATTATCGGTC	505	[25]
	GZgyrACcoli4R-R	**R:** GTCCATCTACAAGCTCGTTA		
Beta-lactam Resistance Gene	bla_{OXA-61}	**F:** AGAGTATAATACAAGCG **R:** TAGTGAGTTGTCAAGCC	372	[26]
Erythromycin Resistance Gene	23S rRNA-F	**F:** TTAGCTAATGTTGCCCGTACCG **R:** AGCCAACCTTTGTAAGCCTCCG	697	[27]
	*ERY*2075-R	**R:** TAGTAAAGGTCCACGGGGTCGC	485	[27]
	*ERY*2074-R	**R:** AGTAAAGGTCCACGGGGTCTGG	485	
Tetracycline Resistance Gene	*tet(O)*	**F:** GCGTTTTGTTTATGTGCG **R:** ATGGACAACCCGACAGAAG	559	[26]
Aminoglycoside Resistance Gene	*aphA-3-1*	**F:** TGCGTAAAAGATACGGAAG **R:** CAATCAGGCTTGATCCCC	701	[26]
Cme ABC Efflux System	*cme*A	**F:**TAGCGGCGTAATAGTAAATAAAC **R:** ATAAAGAAATCTGCGTAAATAGGA	435	[28]
	*cme*B	**F:** AGGCGGTTTTGAAATGTATGTT **R:** TGTGCCGCTGGGAAAAG	444	[28]
	*cme*C	**F:** CAAGTTGGCGCTGTAGGTGAA **R:** CCCCAATGAAAAATAGGCAGAGTA	431	[28]

gel imaging system (Gene-Box FX, ER Biotech, Turkiye). The obtained images were analyzed with PyElph software (v.1.4). The resulting band profiles underwent phylogenetic analysis using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method.

Statistical Analysis

Minitab (v21) software was used for the statistical analysis of the data. Pearson's Chi-square (x2) method and Fischer's Exact test were used to compare the AMR of the obtained genotypes. Statistical significance level was accepted as $P<0.05$.

Results

Isolation and Identification

A total of 115 (30.74%) *Campylobacter* spp. were isolated and identified from the rectal swab samples. Through

Table 4. Primer sequences used to investigate the presence of virulence-associated genes				
Target Gene		**Oligonucleotide (5'-3')**	**Amplicon Size (bp)**	**Ref.**
Toxin Genes	*cdt*A	**F:** GGAAATTGGATTTGGGGCTATACT **R:** ATCACAAGGATAATGGACAAT	165	[29]
	*cdt*B	**F:** GTTAAAATCCCCTGCTATCAACCA **R:** GTTGGCACTTGGAATTTGCAAGGC	495	[29]
	*cdt*C	**F:** TGGATGATAGCAGGGGATTTTAAC **R:** TTGCACATAACCAAAAGGAAG	555	[29]
Adhesion-Colonization-Invasion Genes	*fla*A	**F:** GATTTCGTATTAACACAAATGGTGC **R:** CTGTAGTAATCTTAAAACATTTTG	1725	[30]
	ggt	**F:** GAGTGCTATGCTTGATCGCT **R:** TAGGTGGCGACATGGAAATG	419	[31]
	*cad*F	**F:** TTGAAGGTAATTTAGATATG **R:** CTAATACCTAAAGTTGAAAC	400	[32]
	*cia*B	**F:** TGCGAGATTTTTCGAGAATG **R:** TGCCCGCCTTAGAACTTACA	527	[33]
	*pld*A	**F:** AAGAGTGAGGCGAAATTCCA **R:** GCAAGATGGCAGGATTATCA	385	[33]
	*dna*J	**F:** ATTGATTTTGCTGCGGGTAG **R:** ATCCGCAAAAGCTTCAAAAA	177	[33]

PCR in which species-specific primers were used, 87.36% (n=83) of the 95 *Campylobacter* spp. isolates obtained from sheep rectal swab samples were identified as *C. jejuni* and 12.63% (n=12) as *C. coli*. Of the 20 *Campylobacter* spp. isolates obtained from goat rectal swab samples, 90% (n=18) were identified as *C. jejuni* and 10% (n=2) as *C. coli*. In the samples examined in the study, *C. fetus* subsp. *fetus* was not identified by PCR.

Antimicrobial Susceptibility

Test results showed that 97.39% (n=112) of the 115 *Campylobacter* spp. isolates were susceptible to gentamicin, and 93.04% (n=107) were fully susceptible to amoxicillin-clavulanic acid. Susceptibility rates were 91.30% for chloramphenicol, 79.13% for erythromycin, and 72.17% and 54.78% for streptomycin and aztreonam, respectively. The highest resistance was detected for cephalothin (84.34%) and ciprofloxacin (56.52%). MDR was determined in 41.73% (n=48) of the isolates. Resistance profiles at the species level (*C. jejuni* and *C. coli*) are shown in *Table 5*.

Antimicrobial Resistance Genes

The *tet(O)* gene was detected in 89.58% (43/48) of the tetracycline-resistant isolates, the bla_{OXA-61} gene in 88.34% (91/103) of the beta-lactam-resistant isolates, the *aphA*-3-1 gene in 4.34% (1/23) of the aminoglycoside-resistant isolates. Resistance-associated point mutations in the 23S

Table 5. Antimicrobial resistance distribution of C. jejuni and C. coli isolates										
Bacterial Distribution	**Antibiotic Resistance Rates (%)**									
	AMC	**ATM**	**C**	**CIP**	**ERY**	**GEN**	**KF**	**NA**	**S**	**TE**
C. jejuni (n=83) Sheep	0% (n=0)	33.73% (n=28)	6.02% (n=5)	61.44% (n=51)	22.89% (n=19)	2.40% (n=2)	90.36% (n=75)	16.86% (n=14)	14.45% (n=12)	38.55% (n=32)
C. jejuni (n=18) Goat	5.55% (n=1)	44.44% (n=8)	50% (n=9)	50% (n=9)	38.88% (n=7)	5.55% (n=1)	61.11% (n=11)	5.55% (n=1)	44.44% (n=8)	50% (n=9)
Total (%)	0.99% (n=1)	35.64% (n=36)	13.86% (n=14)	59.40% (n=60)	25.74% (n=26)	2.97% (n=3)	85.14% (n=86)	14.85% (n=15)	19.80% (n=20)	40.59% (n=41)
C. coli (n=12) Sheep	8.33% (n=1)	50% (n=6)	16.66% (n=2)	8.33% (n=1)	25% (n=3)	0% (n=0)	83.33% (n=10)	16.66% (n=2)	16.66% (n=2)	58.33% (n=7)
C. coli (n=2) Goat	0% (n=0)	0% (n=0)	0% (n=0)	50% (n=1)	50% (n=1)	0% (n=0)	100% (n=2)	0% (n=0)	0% (n=0)	0% (n=0)
Total (%)	7.14% (n=1)	42.85% (n=6)	14.28% (n=2)	14.28% (n=2)	28.57% (n=4)	0% (n=0)	85.71% (n=12)	14.28% (n=2)	14.28% (n=2)	50% (n=7)

***AMC:** Amoxicillin-clavulanic acid; **ATM:** Aztreonam; **C:** Chloramphenicol; **CIP:** Ciprofloxacin; **ERY:** Erythromycin; **GEN:** Gentamicin; **KF:** Cefalotin; **NA:** Nalidixic acid; **S:** Streptomycin; **TE:** Tetracycline*

rRNA target region were detected in 100% (24/24) of the phenotypically erythromycin-resistant isolates. Specifically, among these 24 erythromycin-resistant isolates, the *ERY2075* mutation was identified in 37.5% (9/24) of cases, and the *ERY2074* mutation in 54.16% (13/24). Among the 48 *Campylobacter* spp. isolates with MDR, *cme*A was detected in 45.83% (n=22), *cme*B in 95.83% (n=46), *cme*C in 52.08% (n=25), and all three resistance genes in 31.25% *(cmeABC) (Fig. 1)*. Furthermore, the presence of the *Thr-86-Ile* point mutation was determined in 98.33% (n=59) of 60 *C. jejuni* isolates- phenotypically determined to be quinolone-resistant and in which the *gyr*A gene was detected-, and in 80% (n=4) of 5 *C. coli* isolates.

Virulence Genes

The *cdt*A virulence gene was detected in 20.86% of *Campylobacter* spp. isolates (n=115), *cdt*B virulence gene in 91.30%, and *cdt*C virulence gene in 21.73%. In addition, the presence of the *cad*F gene was detected in 94.78% of the isolates, *dna*J gene in 92.17%, *cia*B gene in 88.69%, *fla*A gene in 79.13%, and *pld*A gene in 15.65%, while *ggt* gene was not detected *(Table 6; Fig. 2)*.

Genotyping

The restriction fragments of the 1.7 kb *fla*A gene PCR products ranged from 100 to 1000 base pairs. It was observed that 91 different *fla*A gene-positive isolates had 13 different RFLP patterns *(Fig. 3)*. Accordingly, a total of 13 (Cj1 to Cj13) different band patterns were observed in *C. jejuni* isolates. Five of these band patterns (Cc1 to Cc5) were also observed in *C. coli* isolates. The phylogenetic analysis of the band profiles is presented in *Fig. 4*.

Statistical Analysis

According to the results of the Chi-square test, the prevalence of *Campylobacter* spp. was found to be significantly higher in sheep than in goats (P<0.05).

The results revealed no relationship between *fla*A genotypes and resistance to cephalothin, streptomycin, chloramphenicol, erythromycin, nalidixic acid, and amoxicillin-clavulanic acid antimicrobials (P>0.05). However, tetracycline and ciprofloxacin AMR were higher in isolates with the Cj2 and Cj7 genotypes compared to isolates with the other genotypes (P<0.001). In isolates with Cj3 and Cj6 genotypes, aztreonam AMR was higher than in isolates with the other genotypes (P<0.05; *Table 7*). Isolates were not included in the analysis for gentamicin due to the absence of AMR.

When AMR was evaluated based on the presence of virulence genes, only isolates that were *pld*A positive were found to have higher resistance to nalidixic acid than negative isolates (P<0.05). No difference was observed in

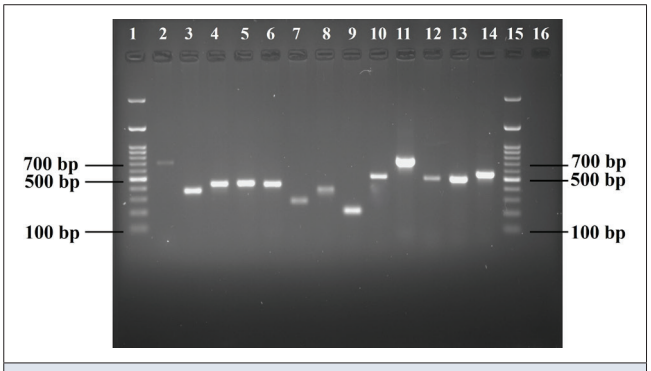

Fig 1. Agarose gel image of antimicrobial resistance-related genes obtained by PCR in the isolates [1,15: 100 bp DNA Marker; 2: *aphA*-3-1 positive isolate (701 bp); 3: *bla*OXA-61 positive isolate (372 bp); 4: *cme*A positive isolate (435 bp); 5: *cme*B positive isolate (444 bp); 6: *cme*C positive isolate (431 bp); 7: *C. jejuni* quinolone resistance gene positive isolate (265 bp); 8: *C. jejuni* quinolone resistance gene positive isolate (368 bp); 9: *C. coli* quinolone resistance gene positive isolate (192 bp); 10: *C. coli* quinolone resistance gene positive isolate (505 bp); 11: 23S rRNA gene positive isolate (697 bp); 12: *ERY*2074 gene positive isolate (485 bp); 13: *ERY*2075 gene positive isolate (485 bp); 14: *tet(O)* gene positive isolate (559 bp)]

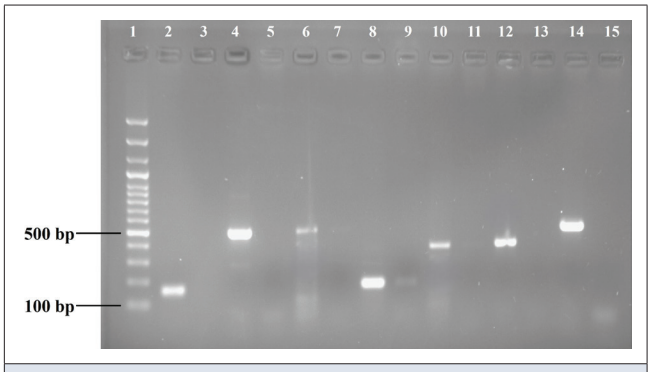

Fig 2. Agarose gel image of amplicons obtained from the screening of virulence-related genes in the isolates by PCR [1: 100 bp DNA Marker; 2: *cdt*A positive isolate (165 bp); 3: *cdt*A negative isolate; 4: *cdt*B positive isolate (495 bp); 5: *cdt*B negative isolate; 6: *cdt*C positive isolate (555 bp); 7: *cdt*C negative isolate; 8: *dna*J positive isolate (177 bp); 9: *dna*J negative isolate; 10: *pld*A positive isolate (385 bp); 11: *pld*A negative isolate; 12: *cad*F positive isolate (400 bp); 13: *cad*F negative isolate; 14: *cia*B positive isolate (527 bp); 15: *cia*B negative isolate]

Table 6. *Distribution of virulence-associated genes in C. jejuni and C. coli isolates*

Bacteria	Number of Isolates	Ratio of Virulence Genes (%)								
		*cad*F	*cdt*A	*cdt*B	*cdt*C	*cia*B	*dna*J	*fla*A	ggt	*pld*A
C. jejuni	101	98 (97.02)	22 (21.78)	97 (96.03)	25 (24.75)	96 (95.04)	96 (95.04)	84 (83.16)	0 (0)	18 (17.82)
C. coli	14	11 (78.57)	2 (14.28)	8 (57.14)	0 (0)	6 (42.85)	10 (71.42)	7 (50)	0 (0)	0 (0)
Total	115	109 (94.78)	24 (20.86)	105 (91.30)	25 (21.73)	102 (88.69)	106 (92.17)	91 (79.13)	0 (0)	18 (15.65)

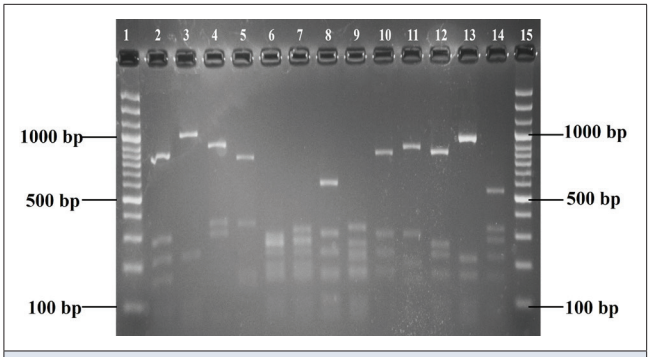

Fig 3. RFLP-PCR band patterns obtained from *C. jejuni* (Cj) and *C. coli* (Cc) isolates [1,15: 100 bp DNA Marker (M); 2: Cj1-Cc1; 3: Cj2-Cc2; 4: Cj3-Cc3; 5: Cj4-Cc4; 6: Cj5-Cj6; 7-14: Cj band patterns]

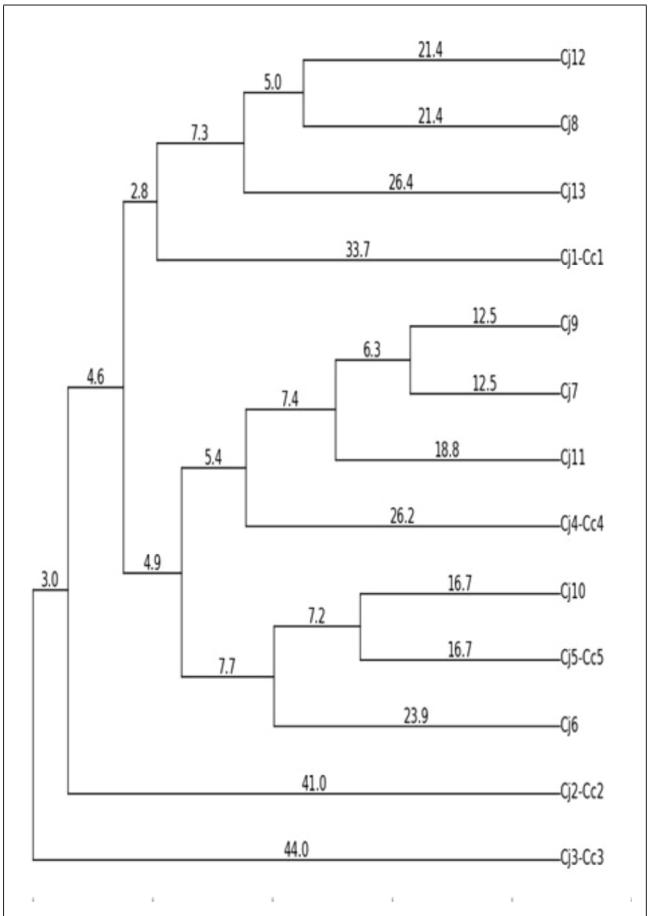

Fig 4. Phylogenetic analysis of band profiles determined by the *Dde*I in *C. jejuni* and *C. coli* isolates. Decimal numbers indicate differences between RFLP-PCR genotyping profiles (as a percentage). Cj: *Campylobacter jejuni*; Cc: *Campylobacter coli*

AMR for the *cdt*A and *cdt*C virulence genes. Because *cdt*B, *cia*B, *cad*F, and *dna*J virulence genes were positive in all isolates, they were not included in the analysis *(Table 8)*.

Discussion

The study revealed that *Campylobacter* spp. was isolated from 30.74% (115/374) rectal swab samples taken from sheep and goats in the Siirt region. Currently, there is limited data in the literature regarding the investigation of *Campylobacter* spp. in sheep and goat fecal samples in Türkiye, as studies have mostly focused on foods offered for consumption [16,17]. However, there are various studies conducted internationally for the isolation and identification of *Campylobacter* spp. When these studies were examined, it was determined that the prevalence of *Campylobacter* spp. varied between 11.3% and 98.8% [8,34-37]. When studies conducted at the species level were evaluated, it was reported that the prevalence of *C. jejuni* and *C. coli* ranged from 5.7% to 60.34% and 15.15% to 69.6%, respectively [34,35,38]. In the current study, 87.82% (n=101) of 115 (30.74%) *Campylobacter* spp. isolates obtained were identified as *C. jejuni,* 12.17% (n=14) as *C. coli*, and no positive results were obtained for *C. fetus* subsp. *fetus*. The differences observed in the study results were thought to be related to geographical conditions, animal husbandry conditions, sample size, and sampling methods.

Historically, fluoroquinolones, macrolides, tetracyclines, and aminoglycosides have been among the primary therapeutic options for treating campylobacteriosis in the veterinary field [16]. While their current use is subject to varying regional regulations and antimicrobial stewardship programs, the development of resistant strains and their potential transmission to humans through contaminated food remains a significant public health concern [15,39].

In the present study, ciprofloxacin resistance exhibited significant geographic variation across studies. The resistance rate observed in our study was lower than those reported in African and Middle Eastern settings [5,38,40,41], yet markedly higher than data reported from European livestock [42].

The observed variability in results is attributed to methodological differences and regional practices. On one hand, the exceptionally high resistance rates reported in certain studies [40,41] must be interpreted with caution due to limited sample sizes that could lead to sampling bias. In this context, our larger pool of isolates provides a more robust and representative baseline dataset. On the other hand, the discrepancy between findings in Europe [42] and our results highlights differences in antimicrobial stewardship practices within the veterinary field. While strict European restrictions on fluoroquinolone use in livestock effectively limit the development of resistance, higher resistance levels in other regions point to ongoing selective pressure resulting from unregulated antibiotic use in animal production.

Conversely, a high level of cephalothin resistance was observed in our study, aligning with previous reports [5,40]. This finding is expected, as *Campylobacter* species exhibit well-documented intrinsic resistance to first-generation

Table 7. *Relationship between flaA genotypes and antibiotic resistance status*

Genotype	AMC	ATM	C	CIP	ERY	KF	NA	S	TE
Cj1 (n=10)	0 (0%)	0[b] (0%)	0 (0%)	7[a] (70%)	0 (0%)	10 (100%)	2 (20%)	0 (0%)	0[b] (0%)
Cj2 (n=9)	1 (11.11%)	0[b] (0%)	0 (0%)	8[a] (88.88%)	2 (22.22%)	8 (88%)	0 (0%)	0 (0%)	8[a] (0%)
Cj3 (n=7)	0 (0%)	7[a] (100%)	0 (0%)	5[a] (71.42%)	1 (14.28%)	7 (100%)	1 (14.28%)	2 (28.57%)	2[b] (28.57%)
Cj4 (n=2)	0 (0%)	2[b] (100%)	0 (0%)	0[b] (0%)	0 (0%)	2 (100%)	0 (0%)	0 (0%)	2[b] (100%)
Cj5 (n=15)	0 (0%)	1[b] (6.66%)	1 (6.66%)	9[b] (60%)	3 (20%)	13 (86.66%)	1 (6.66%)	3 (20%)	6[b] (40%)
Cj6 (n=11)	0 (0%)	8[a] (72.72%)	1 (9.09%)	3 (27.27%)	2 (18.18%)	10 (90.90%)	3 (27.27%)	6 (54.54%)	4 (36.36%)
Cj7 (n=13)	0 (0%)	3[b] (2307%)	1 (7.69%)	13[a] (100%)	1 (7.69%)	13 (100%)	1 (7.69%)	3 (23.07%)	12[a] (92.30%)
Cj8 (n=2)	0 (0%)	1 (50%)	0 (0%)	1[b] (50%)	1 (50%)	2 (100%)	0 (0%)	1 (50%)	0[b] (0%)
Cj9 (n=1)	0 (0%)	0[b] (0%)	0 (0%)	1[b] (100%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	0[b] (0%)
Cj10 (n=9)	0 (0%)	5[ab] (55.55%)	0 (0%)	4[b] (44.44%)	6 (66.66%)	8 (88.88%)	3 (33.33%)	0 (0%)	0[b] (0%)
Cj11 (n=2)	0 (0%)	0[b] (0%)	0 (0%)	2[b] (100%)	1 (50%)	2 (100%)	0 (0%)	1 (50%)	1[b] (50%)
Cj12 (n=2)	0 (0%)	1[b] (50%)	0 (0%)	2[b] (100%)	0 (0%)	2 (100%)	1 (50%)	0 (%0)	0[b] (0%)
Cj13 (n=1)	0 (100%)	1[b] (100%)	0 (0%)	1[b] (100%)	0 (0%)	1 (100%)	1 (100%)	0 (0%)	1[b] (100%)
P-value	>0.05	0.000***	>0.05	0.011*	>0.05	>0.05	>0.05	>0.05	0.000***

**P<0.05, ***P<0.001. [a,b]Different letters in the same column indicate significant differences in antibiotic resistance among the corresponding genotypes; AMC: Amoxicillin-clavulanic acid; ATM: Aztreonam; C: Chloramphenicol; CIP: Ciprofloxacin; ERY: Erythromycin; GEN: Gentamicin; KF: Cefalotin; NA: Nalidixic acid; S: Streptomycin; TE: Tetracycline*

Table 8. *Relationship between the presence of virulence genes and antibiotic resistance status*

Virulence Genes	AMC	ATM	C	CIP	ERY	KF	NA	S	TE
*cdt*A Positive (n=17)	1 (5.88%)	7 (41.17%)	0 (0%)	11 (64.70%)	4 (23.52%)	16 (94.11%)	5 (29.41%)	1 (5.88%)	4 (23.52%)
*cdt*A Negative (n=67)	0 (0%)	22 (32.83%)	3 (4.47%)	45 (67.16%)	13 (19.40%)	63 (94.02%)	9 (13.43%)	15 (22.38%)	32 (47.76%)
P-value	p>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
*cdt*C Positive (n=12)	1 (8.33%)	5 (41.66%)	0 (0%)	9 (75%)	4 (33.33%)	11 (91.66%)	4 (33.33%)	4 (33.33%)	4 (33.33%)
*cdt*C Negative (n=72)	0 (0%)	24 (33.33%)	3 (4.16%)	47 (65.27%)	13 (18.05%)	68 (94.44%)	10 (13.88%)	12 (16.66%)	32 (44.44%)
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05
*pld*A Positive (n=12)	1 (8.33%)	7 (58.33%)	1 (8.33%)	9 (75%)	3 (25%)	11 (91.66%)	5 (41.66%)	2 (16.66%)	4 (33.33%)
*pld*A Negative (n=72)	0 (0%)	22 (30.55%)	2 (2.77%)	47 (65.27%)	14 (19.44%)	68 (94.44%)	9 (12.5%)	14 (19.44%)	32 (44.44%)
P-value	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	0.012*	>0.05	>0.05

**P<0.05; AMC: Amoxicillin-clavulanic acid; ATM: Aztreonam; C: Chloramphenicol; CIP: Ciprofloxacin; ERY: Erythromycin; GEN: Gentamicin; KF: Cefalotin; NA: Nalidixic acid; S: Streptomycin; TE: Tetracycline*

cephalosporins [43]. Therefore, this particular resistance profile reflects the innate microbiological characteristics of the genus rather than acquired resistance driven by selective pressure.

It was thought that the differences in the AMR rates could be due to uncontrolled antibiotic use, differences in the number of isolates obtained, regional conditions, and differences in the methods used in the studies. Furthermore, the evaluation of the obtained data reveals the importance of the One Health perspective, which acknowledges the interconnectedness of animal health, human health, and the shared environment [43]. Small ruminants serve as important reservoirs for *Campylobacter* species, and the high rates of AMR detected in this study have significant implications for public health and food safety. Zoonotic transmission can occur through direct contact with infected animals, environmental contamination, or, most importantly, through the consumption of undercooked meat and unpasteurized dairy products. When MDR *Campylobacter* strains enter the food chain, they can lead to treatment failures, limiting the available empirical treatment options for human campylobacteriosis [15]. Therefore, the implementation of coordinated surveillance systems between the veterinary and public health sectors is vital to reduce the spread of these resistant zoonotic pathogens from farm to table.

Regarding molecular resistance determinants, this study revealed distinct gene distribution profiles. The detection of the 23S rRNA gene (which confers macrolide resistance) in every sample aligns with the observations of Gharbi et al.[15].

However, variations in the frequencies of specific point mutations *(ERY2075* and *ERY2074)* point to local genomic micro-evolution among *Campylobacter* strains, likely shaped by regional macrolide administration protocols in veterinary medicine. The detection rate of the beta-lactamase determinant *(bla_{OXA-61})* among the analyzed isolates was found to be higher compared to reports from Egypt, Indonesia, and Türkiye [15,44-46]. In contrast, the frequency of the aminoglycoside resistance gene *(aphA-3-1)* showed a lower profile compared to African isolates [44], whereas the tetracycline resistance determinant *[tet(O)]* exceeded previously reported levels [15,38,46]. These differences across studies likely reflect varying exposure profiles to specific antimicrobial classes within regional animal production systems. Methodologically, while the limited sample sizes in some studies [44,45] introduce potential selection biases, the expanded pool of isolates evaluated in this study offers greater epidemiological reliability. Furthermore, the widespread presence of multidrug resistance determinants particularly active efflux systems such as *cme*B supports current evidence highlighting the importance of efflux-mediated processes in the survival mechanisms of *Campylobacter* under persistent drug pressure [47]. When combined with antibiotic heterotolerance and phenotypic persistence [48], these dynamic survival strategies significantly complicate standard therapeutic interventions. To reconcile these regional differences and elucidate the dynamics of resistance transmission across ecosystems, future research should be supported by methods such as Whole Genome Sequencing (WGS), which enables high-resolution profiling of novel genomic variants and high-risk lineages [49].

Virulence genes play a crucial role in the colonization, adhesion, and invasion of bacterial pathogens [15]. Virulence genes, which cause an increase in the severity of infections, are also of great importance in the pathogenesis of the disease [50]. Analysis of virulence-associated determinants revealed diverse pathogenic profiles among the evaluated isolates. Regarding adhesion and colonization factors, the high detection frequency of the *cad*F gene aligns closely with observations by Lúcio et al.[51], though it presents a minor divergence from the complete detection reported by Khoshbakht et al.[52] and the lower frequency described by Hızlısoy et al.[53]. Similarly, the motility-associated *fla*A gene was present in the majority of isolates, surpassing the prevalence reported by Hızlısoy et al.[53] while remaining slightly below the figures presented by Hafez [38]. Because adherence and motility are foundational for mucosal colonization, these high prevalence rates underscore a conserved pathogenic potential across the analyzed strains.

Conversely, distinct patterns emerged concerning cytolethal distending toxin (cdt) cluster genes *(cdt*A, *cdt*B, and *cdt*C). The detection frequencies for *cdt*A and *cdt*C in the present investigation were comparatively lower than those reported in earlier studies [51-53]. For *cdt*B, however, the frequency exceeded data from South America [51], despite remaining below full or near-full prevalence documented in other regional reports [52,53]. These variations in toxin gene profiles likely reflect genomic instability, insertion sequences, or point mutations within the cdt operon across geographically distinct lineages, which may alter PCR binding efficiency or lead to gene loss.

Remarkably, the stress response and invasion-related determinants dnaJ and ciaB were prominently represented in the analyzed isolates, substantially surpassing the prevalence levels documented by Hafez [38] and Lúcio et al.[51]. The high conservation of *dna*J and *cia*B suggests an enhanced capacity for intracellular survival and thermal adaptation within the host environment. Methodologically, the complete absence of the *ggt* gene in our dataset indicates lineage-specific genetic divergence, as *ggt* is known to be heterogeneously distributed and primarily associated with specific strain colonization dynamics. Overall, these inter-study discrepancies highlight the genetic plasticity of *Campylobacter* virulence factors, where regional selective pressures and host-adaptation mechanisms shape the pathogenic repertoire.

The *Dde*I is known as the most widely preferred restriction enzyme worldwide for the differentiation of *Campylobacter* isolates within a specific ecological niche [54]. Within the scope of the study, RFLP analysis performed on *fla*A-positive isolates using the *Dde*I resulted in the observation of 13 different band patterns. When the related studies were reviewed, Aslantaş [17] reported that they observed 11 different band profiles in 55 *fla*A gene-positive *C. jejuni* isolates and 2 different band profiles in 24 *C. coli* isolates in their study conducted in Hatay and Adana. Yadav et al.[54] identified 15 different band patterns in 43 *C. jejuni* isolates obtained from cloacal swab samples of poultry in India.

The differences between the studies are thought to be caused by the number of isolates obtained, the diversity of variations in the bacteria, and the different resistance profiles. Thus, in this study, we observed that AMR is not randomly distributed, but rather closely associated with specific genotypes and virulence genes. In particular, tetracycline and ciprofloxacin resistance was significantly clustered in Cj2 and Cj7 genotypes ($P<0.001$), while aztreonam resistance was higher in Cj3 and Cj6 genotypes ($P<0.05$). This diverse distribution demonstrates that specific genetic profiles of *Campylobacter* spp. may be associated with resistance to critically important antibiotics. Furthermore, we identified a potential link between virulence and resistance: *pld*A-positive isolates showed significantly higher resistance to nalidixic acid ($P<0.05$). This association suggests that antibiotic

selection pressure in small ruminants may inadvertently co-select for strains carrying specific virulence markers. In conclusion, these observed resistance-virulence patterns indicate that the transmission of these resistant profiles complicate clinical treatment. Therefore, monitoring these specific molecular genotypes is vital for regional disease control and public health.

A notable limitation of this study relates to the selective culture parameters employed for isolation. Sample incubation was conducted under microaerophilic conditions at 41.5-42°C, a temperature regime that selectively favors the growth of thermophilic species such as *C. jejuni* and *C. coli*. However, non-thermophilic species-most notably *C. fetus*, which requires lower incubation temperatures (37°C) for optimal growth-may have been inhibited or underdetected during primary culture. Therefore, the absence of *C. fetus* subsp. *fetus* isolates in our findings should be interpreted with caution and should not be construed as definitive proof of its absence in the regional small ruminant population.

Additionally, another key limitation is that sampling was restricted exclusively to diarrheic animals without the inclusion of a non-diarrheic healthy control group. Because *Campylobacter* species can naturally colonize the gastrointestinal tract of small ruminants as commensal organisms, a definitive causal relationship between the isolated strains and the observed clinical diarrhoea cannot be firmly established. Furthermore, potential co-infections with other enteric pathogens (e.g., viral, parasitic, or other bacterial agents) were not systematically evaluated. Accordingly, our findings reflect the prevalence, antimicrobial resistance, and virulence profiles of *Campylobacter* strains recovered from diarrheic cases rather than proving direct etiology.

Consequently, the current study suggests that *C. jejuni* and *C. coli* are frequently recovered from diarrheic sheep and goats in the Siirt region. While high *in vitro* susceptibility to gentamicin, streptomycin, amoxicillin-clavulanic acid, and erythromycin identifies these antimicrobials as potential candidates for veterinary use, further *in vivo* clinical studies are required to confirm their therapeutic efficacy under field conditions. Notably, higher resistance tetracycline and ciprofloxacin was observed among isolates with Cj2 and Cj7 genotypes, whereas aztreonam resistance prominent in Cj3 and Cj6 genotypes. Additionally, isolates carrying the *pld*A gene combined with nalidixic acid resistance reflect specific genotype-phenotype patterns that warrant continued surveillance. Although these findings provide valuable baseline data for regional small ruminant management in Southeastern Anatolia, caution should be exercised before generalizing these results to other regions.

Furthermore, given that human isolates were not evaluated, small ruminants should be regarded as potential reservoirs rather than confirmed sources of direct zoonotic transmission. Future research utilizing comprehensive 'One Health' surveillance frameworks and comparative genomic tools will be essential to clarify transmission dynamics and safeguard public health.

Declaration

Availability of Data and Materials: The data used in this study are available from the corresponding author (MY) on reasonable request.

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Ethical Compliance Declaration: The authors declare that the study was conducted in accordance with all applicable animal welfare and research ethics principles, institutional and national/international regulations, and the requirements of the relevant ethics committees and funding/supporting institutions, where applicable. The authors accept full responsibility for the ethical integrity of the study.

Authors' Contributions: MY and ÖG contributed to the project idea, design and execution of the study. MY contributed to the acquisition of data. MY analysed the data. MY and ÖG drafted and wrote the manuscript. MY, ÖG and AOT reviewed the manuscript critically. All authors have read and approved the finalized manuscript.

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