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Prevalence and Potential Zoonotic Risk of Campylobacter Species in Dairy Cattle from Golestan Province, Iran

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ABSTRACT

Background: Campylobacter is the primary bacterial agent responsible for gastrointestinal (GI) infections in humans. Domestic animals, including cattle, are reservoirs of this bacterium and can be one of the main sources of infection transmission to humans. This study aimed to investigate the prevalence of Campylobacter species in cattle in the Gorgan province.

Materials and Methods: A total of 200 fecal samples were collected from healthy dairy cattle and genus and species were identified using multiplex PCR.

Results: The frequency of the genus Campylobacter in 200 samples in our study was 17.5% (35 cases), C. jejuni and C. coli species were not identified in these 35 cases.

Conclusion: Isolating Campylobacter from animal fecal samples is a challenging process, but this study showed that Campylobacter contamination was relatively high in cattle in the Gorgan province, and its transmission to humans through meat consumption must be monitored.

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INTRODUCTION

In recent years, the number of foodborne illnesses caused by *Campylobacter spp*. in humans has increased dramatically. According to the World Health Organization (WHO), *Campylobacter* is one of the four major causes of diarrheal diseases worldwide (1). *Campylobacteriosis* is a foodborne disease that significantly affects human health and life and can have economic consequences for individuals, families, society, and governments (2). Because *Campylobacter ssp*. Constitute the natural microflora of the digestive tract of livestock and wild animals, these bacteria are widely distributed in the environment and are isolated from various sources, including water, soil, and food (2). Food animals such as poultry, cattle, sheep, pigs, and ostriches. Pets, including dogs and cats, are environmental sources of Campylobacteriosis in humans (3). *Campylobacter* infection has been reported through the consumption of undercooked poultry, raw meat, and even milk and its products (4). These bacteria, which live in the digestive tracts of many warm-blooded animals, are excreted in approximately 20% of cattle feces (5). Campylobacter ssp are small (0.2–0.9 µm wide and 0.2–5.0 µm long) gram-negative rod-shaped, spiral, curved, bird-wing, and gull-wing shapes and do not form spores (6). The movement of this bacterium is corkscrew-like. *Campylobacter* is a microaerophilic bacterium that requires specific conditions (10% CO2, 5% O2, and a temperature of 37-42°C) for growth (7). Pathogenic Campylobacters associated with human infections include *C. jejuni, C. concisus, C. rectus, C. hyointestinalis, C. insulaenigrae, C. sputorum, C. helveticus, C. lari, C. fetus, C. mucosalis, C. coli, C. upsaliensis, and C.* ureolyticus (8). *C. jejuni* and *C. coli* are the most common zoonotic diseases in humans and the most common cause of bacterial gastroenteritis worldwide (9). In humans, clinical symptoms of campylobacteriosis are mild to moderate with gastrointestinal symptoms, and in some cases, more severe diseases may occur such as Guillain–Barré syndrome (10). Unlike humans, *Campylobacter* infections in animals are often asymptomatic (11). Emerging *Campylobacter spp*. are currently overlooked, but the integration of molecular techniques and appropriate culture media into current diagnostic tests will help improve awareness of unusual species as relevant human and animal pathogens (12). Indirect exposure to cattle feces through environmental contamination is considered a high risk to humans (13). The longer lifespan of dairy cows compared to beef cattle could lead to permanent or prolonged shedding of *Campylobacter* by dairy cows, which act as long-term reservoirs (14). Therefore, the role of cows as reservoirs of *Campylobacter* species is important for understanding the epidemiology of this pathogen. This study aimed to investigate the prevalence of *Campylobacter* infection in dairy cows using molecular methods.

MATERIALS AND METHODS

Sample collection

The medical ethics code for this study was IR.IAU.BABOL.REC.1400.131. In this descriptive cross-sectional study, 200 feces samples were collected from November 2021 to the end of March 2022. All samples were taken from female animals that were healthy cows. The ages of the cows studied ranged from 2 to 8 years. The consistency of the collected feces also ranged from one to five, with most of them having a consistency of three (normal feces).

Molecular identification of Campylobacter spp

DNA extraction from the stool

The stool samples were transported to the microbiology laboratory using an ice pack. Due to the difficulty of culturing and biochemical methods for identifying *Campylobacter spp*., Stool DNA was extracted using a DNA extraction kit manufactured by Favorgen.

Multiplex PCR was used for the molecular identification of Campylobacter species. Primers used in this study are listed in Table 1. The total PCR reaction volume was 25 µL, comprising 12.5 µL of PCR master mix (1×), consisting of Taq DNA polymerase (0.06 U/µL), MgCl2 (1.5 mM), dNTPs (0.2 mM),1 µL each of the F and R primers, 1 µL of template DNA, and 8.5 µL of sterile deionized water. All the primers used in this study were synthesized by the German Metabion Company. The cycling conditions were as follows: initial denaturation at 95°C for 10 min; 25 cycles of 94°C for 45 s, 58°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 7 min. PCR products were electrophoresed on a 1.5% agarose gel mixed with 0.4 µl SYBR Green dye and photographed by UV irradiation.

Data Analysis

Statistical analysis was conducted using the SPSS software (version 26.0). The Chi-square test and ANOVA method were employed to calculate P values. Results were deemed statistically significant when P <0.05, across all instances.

Table 1. Primers used to detect *Campylobacter* genus and species

Species	Gene		Sequence (5′ to 3′)	Size (bp)	Ref
Genus *Campylobacter*	*16s rRNA*	C412F	5′-GGATGACACTTTTCGGAGC-3′	816	(15)
		C1228R	5′-CATTGTAGCACGTGTGTC-3′		
C. jejuni	*cj0414*	C-1	5′-CAAATAAAGTTAGAGGTAGAATGT-3′	161	(16)
		C-3	5′-CCATAAGCACTAGCTAGCTGAT-3′		
C. coli	*ask*	CC18F	5′-GGTATGATTTCTACAAAGCGAG-3′	502	(17)
		CC519R	5′-ATAAAAGACTATCGTCGCGTG-3′		

RESULTS

In this study, 200 fecal samples were collected from healthy dairy cows, 25 from traditional livestock farms, and 175 from industrial livestock farms. The age of the sampled dairy cows varied between 2 and 8 years, and the consistency of the cows' feces also ranged from one to five, with 133 samples (66.5%) having consistency of three (normal feces), 4 (2%) had loose consistency, 61 (30.5%) had medium consistency, and 2 (1%) had hard and lumpy consistency. In this study, out of a total of 200 cow feces samples, 17.5% (35 samples) contained bacteria belonging to the genus *Campylobacter*. In these 35 samples, no samples with bands related to *C. jejuni* and *C. coli species* were observed (Figure 1).

The frequency of *Campylobacter* in the feces of cows aged 2, 3, 4, 5, 6, 7, and 8 years was 20.4% (10 cases), 18.8% (13 cases), 8.3% (3 cases), 11.5% (3 cases), 18.1% (2 cases), 42.8% (3 cases), and 50% (1 case), respectively. The highest percentage of positive cases by age was in the seven and eight age groups, which were 42.8% and 50%, respectively. However, the percentage increased in the seven and eight age groups, which, given that the number of samples in this case was only seven and two samples, may be due to the limited number of samples. In the chi-square analysis, there was no significant relationship between the level of infection in the livestock and age. Additionally, based on ANOVA analysis, the mean age of positive cases was 3.6±1.7, and the mean age of negative cases was 3.5±1.3 (P=0.7), and there was no significant relationship between age and the frequency of *Campylobacter* infection (Figure 2).

In campylobacteriosis, stool appears watery (profuse); due to the limited number of watery and loose stool samples, all samples were divided into two categories: normal (normal, medium, and hard stool) and abnormal (watery and loose). The results showed that all *Campylobacter*-positive cases were in the normal stool group, and none of the abnormal samples were positive. Using the chi-square test, no statistically significant relationship was found between positive cases and stool scores (P=0.46) (Figure 3).

Out of 200 samples, only 4 were abnormal, but *Campylobacter* was negative (p=0.4). There was no significant relationship between the frequency of *Campylobacter* and the clinical signs of the cows. 25 samples were collected from local farms and 175 samples from industrial farms, of which 8 (32%) samples from local farms and 27 (15%) from industrial farms were positive for *Campylobacter* (p=0.04). There was a significant relationship between the frequency of *Campylobacter spp.* and the farm type.

DISCUSSION

Worldwide, the leading cause of foodborne illness is caused by pathogenic *Campylobacter spp*. Accounting for more than 400 to 500 million infections per year (18). *Campylobacter* species are commonly found in the intestinal tract of many domestic animals, such as poultry, cattle, sheep, goats, pigs, wild animals, and birds (19). Feces are the main source of contamination (20) and animal food products can be contaminated with this pathogen during slaughter and cutting of carcasses (21). *Campylobacter* is the leading cause of gastroenteritis in humans worldwide (22). Recently, *Campylobacter* species isolated from human and

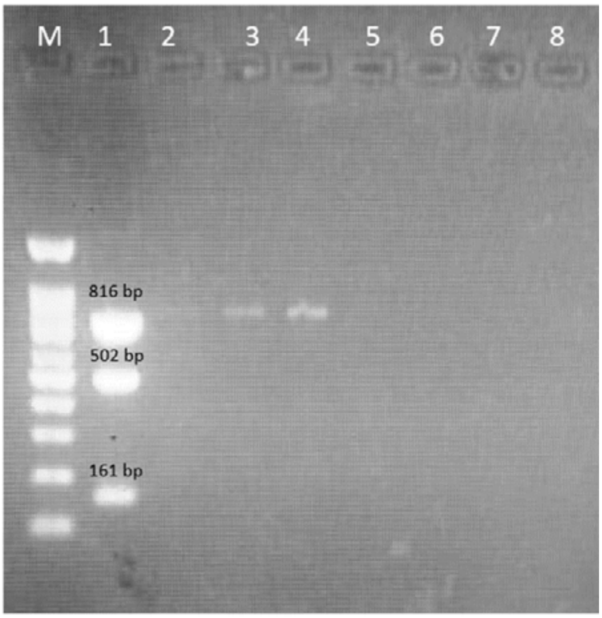

Fig 1. PCR product of feces samples from healthy dairy cows using primers specific for the genus and species of *Campylobacter* on agarose gel; M, 100bp molecular weight marker, Lanes:1, positive control containing the genera and species of *C. jejuni* and *C. coli*, Lanes 2-4: positive samples; Lanes 5- 7: negative samples; Lanes 8, negative control.

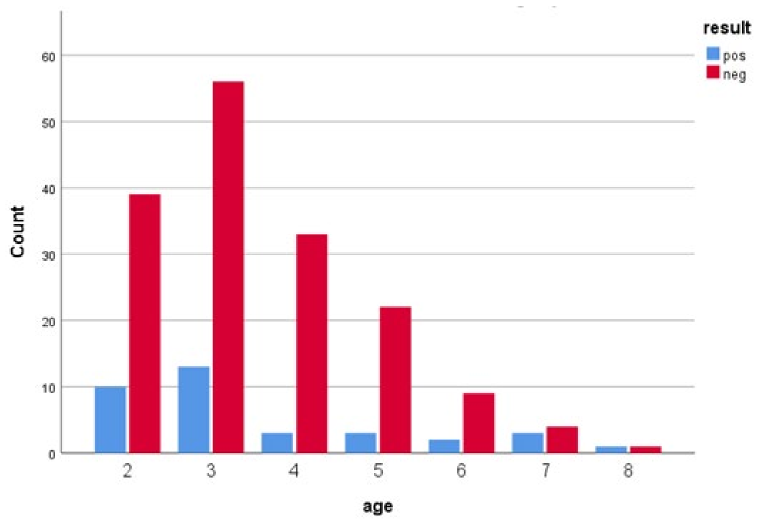

Fig 2. Distribution of Campylobacter genus abundance by age in cows in Gorgan province.

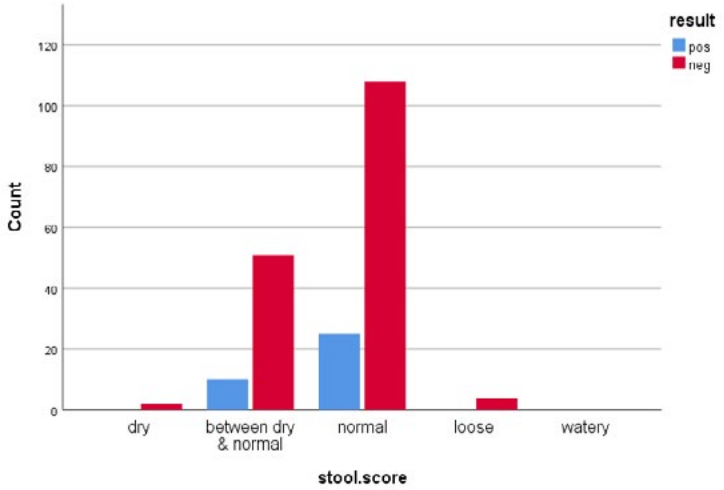

Fig 3. Frequency of *Campylobacter* genus according to clinical symptoms in healthy cows in Gorgan province

animal samples have shown increased resistance to various antibiotics (23). However, the epidemiology of *Campylobacter* remains unclear in many countries (24). In the present study, which was conducted in connection with the investigation of the frequency of *Campylobacter* species in cows in Gorgan province using the Multiplex PCR method, 17.5% Campylobacter species were isolated from a total of 200 samples examined, and none of the *C. jejuni* or *C. coli* species were observed in the samples. In 2021, Hagos et al. reported the prevalence of *C. jejuni* and *C. coli* in 384 meat samples, including 210 beef, 108 goats, and 66 chicken meat samples. Molecular methods reported 64 cases (16.67%), with a prevalence of 11.9% in beef (25). The level of contamination in beef in this study was lower than that in our study. Kagambega et al. in 2021 reported the abundance of *Campylobacter* species in 52 chicken feces samples, 18 (34.61%) of which 17 (94.45%) were *C. coli* and 1 (5.55%) were C. jejuni (24). No *C. coli* or *C. jejuni* species were isolated in this study. In 2021, Gahmanyie et al. conducted a study aimed at molecular detection of *Campylobacter* spp. in 70 human and 30 bovine stool samples. Of the 70 human stool samples, 65.7% were *Campylobacter,* and of the 30 bovine stool samples, only 20.6% were *Campylobacter* positive (26). The difference between the results of the present study and previous studies may be attributed to various factors, including adherence to hygiene principles from the time of raising livestock on the farm to the various stages of slaughter in the slaughterhouse, the prevalence in livestock herds, and climatic conditions. Igwaran et al. conducted a study in 2020 on 1238 samples with the aim of molecular identification of *C. jejuni* and *C. coli* isolated from milk and beef. 71 samples were positive by PCR, of which 35 were positive for *C. coli* and 36 for *C. jejuni* (27). Nevertheless, there are few studies documenting the occurrence of *Campylobacter* in both human and animal populations, likely because, since most healthcare facilities and veterinary clinics in developing nations do not routinely test for this pathogen (26). The reason for the difference in the prevalence of

Campylobacter in cattle can be influenced by several factors, such as season, stress, livestock management factors, diet, and immune system. The reason for this difference in the identification of *Campylobacter spp.* in our study is unknown, but one of the reasons could be the sampling season of cattle, which was conducted in winter.

CONCLUSION

Campylobacter species are one of the main causes of gastroenteritis in humans, transmitted to humans by their vectors, including livestock, such as cattle, and by consuming its meat. In this study, we reported the level of contamination by these bacteria in healthy cows in Gorgan province. However, no common species, such as *C. jejuni* and *C. coli,* were observed. Given the emergence of antibiotic resistance in *Campylobacter,* which can be a warning sign for therapeutic purposes, it is necessary to investigate the epidemiology of the spread of this bacterium and closely monitor livestock farms as a source of transmission.

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Declarations

Authors' contributions

A. B. performed the experiments, analyzed the experimental data, and wrote the manuscript. E. GH and A. Contributed to the concept of the article. A. J guided the experiments and critically revised the manuscript.

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Consent for publication

Not applicable

Availability of data and materials

All of the data generated and analyzed during this study are included in our manuscript.

Conflicts of Interest

There are no conflicts of interest associated with this manuscript.

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