UMSHA Press

AJCMI
Avicenna Journal of Clinical Microbiology and Infection

Avicenna J Clin Microbiol Infect 2026;13(2):111-118. doi:10.34172/ajcmi.3707

http://ajcmi.umsha.ac.ir

Original Article

Tracing Norovirus and *Escherichia coli O157:H7* Contaminations in Green Leafy Vegetables in Iran: A Public Health Concern

Seyed Hani Mirzadeh[1], Mojtaba Khosravi[2,3*], Hamidreza Kazemeini[1], Rahem Khoshbakht[2,3]

[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
[2]Department of Pathobiology, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran
[3]Zoonotic Diseases Research Group, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Amol, Iran

Article history:
Received: June 10, 2025
Revised: December 9, 2025
Accepted: December 28, 2025
ePublished: June 29, 2026

***Corresponding author:**
Mojtaba Khosravi,
Emails: mojtaba.kh.dvm@gmail.com; m.khosravi@ausmt.ac.ir

Abstract

Introduction: Vegetables contain many vital micronutrients, including vitamins and minerals, and are an important component of a healthy diet. However, they may be contaminated by pathogens present in contaminated soil, and water. Furthermore, the consumption of raw or insufficiently processed food pathogens, contaminated soil facilitates the transmission of enteric pathogens such as norovirus and *Escherichia coli* O157:H7 to humans.

Methods: This study aimed to assess the presence of human norovirus (HuNoV) genotypes I (GI) and II (GII), as well as *E. coli* O157:H7, in green leafy vegetables. Between December 2023 and June 2024, a total of 152 samples, including 38 lettuce, 38 watercress, 38 coriander, and 38 oregano, were collected from markets and farm gardens in the cities of Babol and Amol, located in Mazandaran Province. The reverse transcriptase polymerase chain reaction (RT-PCR) and conventional PCR were used to detect the targeted pathogens.

Results: The results revealed that 17 of 152 samples (11.18%; 95% CI: 6.65–17.30%) were positive for at least one of the target pathogens. Moreover, the contamination rates for HuNoV GI and GII were 1.97% (3/152) and 2.63% (4/152), respectively. *E. coli* O157:H7 was found in 6.58% (10/152) of the samples. Notably, the most positive samples were collected from farm-sourced vegetables. In addition, watercress exhibited the highest contamination rates for HuNoV GI (7.90%), HuNoV GII (2.63%), and *E. coli* O157:H7 (10.53%).

Conclusion: The results highlight the presence of several dangerous human enteric pathogens in leafy green vegetables. Considering the fecal-oral transmission route of these pathogens, their potential public health impact is substantial. Furthermore, the establishment of continuous, standardized surveillance systems is required to better understand environmental risk factors and to reduce contamination risks along the food supply chain.

Keywords: Human norovirus, *E. coli* O157:H7, Green leafy vegetables, Public health

Please cite this article as follows: Mirzadeh SH, Khosravi M, Kazemeini H, Khoshbakht R. Tracing norovirus and *Escherichia coli O157:H7* contaminations in green leafy vegetables in iran: a public health concern. Avicenna J Clin Microbiol Infect 2026;13(2):111-118. doi:10.34172/ajcmi.3707

Introduction

Today, vegetables are consumed in various dietary patterns, either fresh and unprocessed or incorporated into cooked dishes. Contamination of vegetables with viral and bacterial pathogens remains a significant public health concern worldwide (1), as these pathogens can contribute to foodborne illness outbreaks (2).

Food contamination arises from diverse sources. Contaminated soil and water are among the major routes of vegetable contamination, occurring during planting and harvesting activities. Livestock, wildlife, and improper food handling are the main routes of contamination (3, 4). Irrigation with water polluted by human or animal fecal matter is considered a key indicator of contamination and significantly increases the likelihood of human gastroenteritis, particularly through the consumption of raw vegetables (5)

Viruses and bacteria represent the two pathogen groups most frequently associated with foodborne outbreaks (6). Hepatitis A virus (HAV) and human noroviruses (HuNoV) are the leading viral agents responsible for gastroenteritis (7). Their role in producing clinical manifestations of gastroenteritis in humans has been well established (7). HuNoV belongs to the genus *Norovirus*

within the family *Caliciviridae*, which includes several important human and animal pathogens (8). HuNoV maintains infectivity at temperatures up to 60°C, survives freezing, shows notable resistance to widely used sanitizers and disinfectants, and can retain its infectivity for at least 61 days in groundwater (9).

The HuNoV genome consists of a single-stranded, positive-sense RNA approximately 7.5 kb in length. It is surrounded by a non-enveloped icosahedral capsid. To date, three open reading frames have been identified in the HuNoV genome: ORF1, which encodes the nonstructural proteins (NS 1/2-7), and ORF2 and ORF3, which encode the structural proteins including viral proteins 1 (VP1) and VP2, respectively (10).

HuNoVs can cause infections at all ages and are primarily transmitted through contaminated food (11). They are responsible for numerous deaths, hospitalizations, and outpatient visits, including ambulatory visits and emergency room visits per year (12). Stool is the main route of virus shedding is stool, and viral load in infected individuals can reach up to 10^{11} particles per gram (13).

To date, 10 noroviruses genotypes (GI–GX) have been identified. According to the prior studies, only genotypes I, II, and IV, along with their subtypes, are associated with human infection and disease (8). Furthermore, molecular epidemiological studies of norovirus infections in humans indicate that genotypes I and II are the most prevalent (10).

Over the past decade, increased consumption of raw vegetables and fruits has been associated with an increase in outbreaks of enteric bacterial infection (14,15,16). *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* are recognized as major bacterial agents for foodborne diseases (17). Furthermore, several other bacterial species, such as *Bacillus cereus*, *Vibrio cholerae*, *Campylobacter* spp., *Shigella* spp., and *Clostridium* spp., have been documented by many researchers (18).

E. coli is a Gram-negative, rod-shaped bacterium belonging to the genus *Escherichia* within the family *Enterobacteriaceae*. It is a facultative anaerobic bacterium, and more than 700 serotypes have been identified based on serological differences in body (O), flagella (H), and capsular (K) antigens (19,20). Most strains (pathotypes) of *E. coli,* especially the O157:H7 strain, can develop gastroenteritis. Additionally, they constitute part of the normal intestinal flora of warm-blooded animals, contributing to vitamin K2 synthesis and impeding localization by pathogenic bacteria in the intestine (19). They show patterns similar to those of foodborne viruses and are often transmitted via the fecal route. As with viral agents, the consumption of raw or improperly washed vegetables and food play a central role in their transmission and development of gastroenteritis (21).

Currently, due to the rise in food-related illnesses, countries such as the United States and members of the European Union have implemented strict food safety regulations to reduce microbial infections (22). Comprehensive research is needed to assess the potential risks associated with the transmission of foodborne viral and bacterial agents through vegetables. Considering the high likelihood of contamination during planting and harvesting, particularly from fecal contamination of ground and surface water, as well as during processing and marketing, this study mainly aimed to trace two of the most frequently reported viral and bacterial agents in widely consumed vegetables at both farm and market levels. Therefore, this research sought to detect HuNoV and *E. coli* O157:H7 using the rapid and sensitive polymerase chain reaction (PCR) technique.

Materials and Methods

Sampling

Sampling was conducted over a seven-month period from December 2023 to June 2024. A total of 152 samples from four different types of green leafy vegetables—lettuce (*Lactuca sativa*), watercress (*Lepidium sativum*), coriander (*Coriandrum sativum*), and oregano (*Mentha pulegium*)—were collected from vegetable markets and farms located in Babol and Amol, Mazandaran Province, Iran. Sampling was performed randomly, and 38 samples were obtained from each vegetable type.

The sample size was calculated based on the prevalence of HuNoV in Tehran, as reported by Rafieepoor et al (23). A prevalence of 3%, a 95% confidence level, and a precision of 0.05 were applied, resulting in a minimum required sample size of 45 leafy vegetable samples. Ultimately, 152 samples were collected to detect two HuNoV genotypes and *E. coli* O157:H7. Then, each sample was divided into two portions and placed into two separate sterile plastic bags: one for viral analysis and the other for bacterial testing. Finally, all samples were transported to the laboratory under cold-chain conditions using ice packs. Specimens for viral and bacterial analyses were kept separately in at -40°C and 4°C, respectively.

Preparation of Samples

Viral isolation was carried out according to the protocol described by Baert et al (24). Briefly, vegetables were sliced into small pieces, and eight grams of each specimen were mixed with 10 mL of Trizol (Intron, South Korea) in a sterile bag. Then, the samples were rocked for 20 minutes at room temperature using a stomacher (Iul-inst, Spain) to facilitate virus elution from the vegetable surface. Then, 300 µL of the resulting elution mixture was utilized for viral genetic material extraction.

For bacterial analysis, samples were prepared following the method outlined by Shah-Ili et al (25). Ten grams of each sample were thoroughly mixed with 90 mL of sterile distilled water for two minutes. Next, one mL of the suspension was added to three mL of *E. coli* broth medium supplemented with 20 mg/L novobiocin. After incubating the mixture at 37°C for 24 hours, aliquots were transferred to the selective medium, eosin methylene blue agar (EMB), and incubated at 37°C for 24 hours. Dark-centered, flat colonies with a metallic sheen were considered presumptive *E. coli*.

Suspected *E. coli* colonies were then cultivated on sorbitol-MacConkey agar supplemented with 0.05 mg/L cefixime. Then, the culture medium was incubated at 37˚C for an additional 24 hours. Bacterial genetic material was subsequently extracted from the resulting colonies.

Isolation of Viral and Bacterial Genetic Material

The extraction of viral genetic material was performed using a viral DNA/RNA extraction kit (Intron, South Korea) following the manufacturer's instructions. After chemical and enzymatic digestion, the viral genome was eluted in 70 µL of elution buffer. To evaluate extraction efficiency, 150 mL of fecal material containing feline calicivirus (FCV) was added to each type of vegetable sample, serving as control samples. Subsequently, similar procedures were performed to prepare and extract viral genetic material for these samples.

The boiling technique, as described by Rajabzadeh Shandiz et al, was utilized to extract bacterial DNA. Briefly, several colonies were transferred from the sorbitol-MacConkey agar medium into 250 mL of sterile water, boiled for 10 minutes, then cooled at -20˚C for 10 minutes (26). The mixture was centrifuged at 14,000 rpm for three minutes, and the DNA-containing liquid portion was transferred to a sterile tube and stored at -20˚C.

Reverse Transcriptase-Polymerase Chain Reaction for Human Norovirus and Feline Calicivirus

Single-strand cDNA was synthesized using a cDNA synthesis kit (Yekta Tajhiz, Iran) in a thermocycler according to the manufacturer's protocol and temperature profile.

A semi-nested RT-PCR method was used to identify norovirus genotypes I and II. Specific primer pairs were employed for each norovirus genotype. The external primers COG1-F and SRI-1-R2 for genotype I, SRII-2-F and SRII-1-R2 for genotype II, and the internal primers COG2-F and G2SK-R for genotype I, G2SK-F and G2SK-R for genotype II were used. For FCV, the 324-bp fragment was amplified using primers ORF2-F.1386 and FCV-2R in a conventional PCR test. Detailed information about the primers used in this study is presented in Table 1.

Each PCR amplification reaction was conducted at a total volume of 25 µL. It comprised 5 µL of the cDNA template, 12.5 µL of a 2x PCR Mastermix (Yekta Tajhiz, Iran), 5.5 µL of nuclease-free water (SinaClon, Iran), and 1 µL (10 pmol) of both forward and reverse primers. PCR amplification was performed in 35 cycles, with each cycle containing three steps: denaturation at 95˚C for 1 minute, annealing at primer-specific temperatures for 1 minute, and extension at 72˚C for 1 minute. Phosphate-buffered saline (PBS) served as the negative control in the RT-PCR assays.

Conventional Polymerase Chain Reaction Detection of Escherichia coli O157:H7

Two sets of primer pairs, O157-F/O157-R and H7-F/H7-R, were employed to identify *E. coli* O157:H7. Detailed information about these primers is presented in Table 1. PCR amplification conditions were identical to those mentioned above. For positive and negative controls, the extracted genome of live *E. coli* (ATCC 43888) and PBS were used, respectively.

Polymerase Chain Reaction Product Analysis

PCR amplicons were analyzed by electrophoresis on a 1.5% agarose gel prepared in TAE buffer. The gel was stained with a safe stain, and all samples were loaded alongside a 100-bp DNA marker (SinaClon, Iran) as a size marker. Samples exhibiting bands corresponding to the expected size were interpreted as positive.

Statistical Analysis

To evaluate the positive rates of HuNoV GI, HuNoV GII, and *E. coli* O157:H7 contamination, chi-square statistical tests were performed. Analyses were based on

Table 1. The Sequence of Primers Used in This Study

Pathogen	Primer Name	Sequence (3'to 5')	Product Length (bp)	Annealing Temperature	Reference
HuNVGI	COG1-F	CGYTGGATGCGNTTYCATGA	380	50	(27)
	SRI-1-R2	CCAACCCARCCATTRTACAT			
	SRI-2-F	AAATGATGATGGCGTCTA	316	50	(28)
	SRI-1-R2	CCAACCCARCCATTRTACAT			
HuNVGII	COG2-F	CARGARBCNATGTTYAGRTGGATGAG	387	50	(27)
	G2SK-R	CCRCCNGCATRHCCRTTRTACAT			
	G2SK-F	TGGGAGGGCGATCGCAATCT	344	50	
	G2SK-R	CCRCCNGCATRHCCRTTRTACAT			
E. coli O157:H7	O157-F	CGGACATCCATGTGATATGG	259	54	(29)
	O157-R	TTGCCTATGTACAGCTAATCC			
	H7-F	GCGCTGTCGAGTTCTATCGAGC	625	56	(30)
	H7-R	CAACGGTGACTTTATCGCCATTCC			
FCV	ORF2-F.1386	CTGCCTCCTACATGGGAAT	324	58	(31,32)
	FCV-2R	TATGAGTAAGGGTCAACCC			

Note. HuNV GI: Human norovirus genotype I; HuNV GII: Human norovirus genotype II; *E. coli: Escherichia coli*; FCV: Feline calicivirus.

the source of vegetables (farms or markets) and vegetable type (lettuce, watercress, coriander, and oregano), using SPSS software version 27 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $P<0.05$.

Results

Out of 152 samples, the presence of targeted pathogens was confirmed in 17 samples (11.18%; 95% CI: 6.65-17.30). HuNoV GI was found in three samples (1.97%; 95% CI: 0.41-5.66), while HuNoV GII was identified in four samples (2.63%; 95% CI: 0.72-6.60), as depicted in Figure 1. Furthermore, *E. coli* O157:H7 was identified in 10 samples (6.58%, 95% CI: 3.02-11.77), as illustrated in Figure 2. No statistically significant association was observed between the source of green leafy vegetables and viral and bacterial contamination rates. Vegetables collected from farms showed a contamination rate of 12%, whereas those purchased from markets had a contamination rate of 9.6% (Table 2). Among the seven vegetable samples contaminated with noroviruses, three (42.86%) were obtained from farms, while four (57.14%) were sourced from markets. In contrast, most *E. coli* O157:H7- positive samples were collected from farms (9 out of 10; 90%).

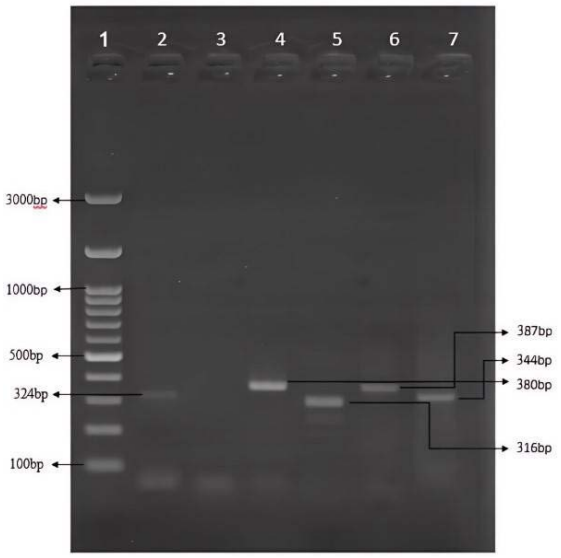

Figure 1. Representative Bands Obtained Using Semi-nested RT-PCR Assay: Lane 1: 100 bp ladder; Lane 2: extraction control (FCV); Lane 3: negative control; Lane 4: HuNV GI-positive samples, synthesized with external primers; Lane 5: HuNV GI-positive samples, synthesized with internal primers; Lane 6: HuNV GII-positive samples, synthesized with external primers; Lane 7: HuNV GII-positive samples synthesized with internal primers. *Note.* RT-PCR: Reverse transcriptase polymerase chain reactions; HuNV GI: Human norovirus genotype I; HuNV GII: Human norovirus genotype II; FCV: Feline calicivirus; pb: Base pair

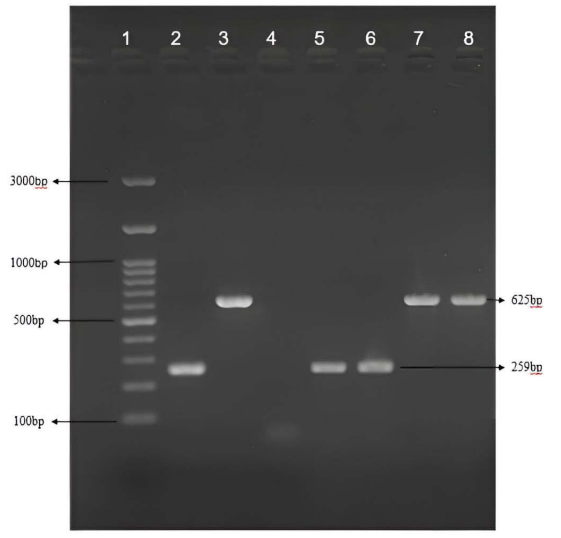

Figure 2. Representative Bands Obtained From a Conventional PCR Assay: Lane 1: 100 bp ladder; Lane 2: positive control (*E. coli* O157:H7, ATCC 43888); Lane 3: negative control; Lanes 4 and 5: *E. coli* O157:H7-positive samples, synthesized with O157-R/O157-F, Lanes 6 and 7: additional *E. coli* O157:H7-positive samples, synthesized with H7-R/H7-F. *Note.* PCR: Polymerase chain reactions; pb: Base pair; *E. coli*: *Escherichia coli*.

Table 3 presents the microbial contamination profiles of various green leafy vegetables tested in this study. Among the four vegetable types tested, all targeted pathogens were detected only in watercress. The positive rate for HuNoV GI, HuNoV GII, and *E. coli* O157:H7 in watercress were 7.90% (3/38), 2.63% (1/38), and 10.53% (4/38), respectively. HuNoV GII and *E. coli* O157:H7 were identified in 7.90% (3/38) of watercress samples. Furthermore, coriander and oregano were exclusively contaminated with *E. coli* O157:H7.

Based on the findings in Tables 1 and 3, the likelihood of contamination of vegetable samples with noroviruses and *E. coli* O157:H7 was higher in farm-grown vegetables and watercress when compared with market- purchased vegetables and lettuce (reference group). However, these associations were not statistically significant.

Similarly, although the type of green leafy vegetable was not statistically associated with the observed contamination, the highest level of contamination was recorded in watercress followed by lettuce, coriander, and pennyroyal (Table 4).

Table 2. Univariate Analysis Measuring the Association Between the Vegetable Source and the Total Prevalence of HuNoV GI, HuNoV GII, and *E. coli* O157:H7 Contamination in Green leafy Vegetables

Vegetable Source	Number Tested	Positive (%)	OR (95% CI)	*P-value*
Market[a]	52	5 (9.6)	1	-
Farms	100	12 (12)	1.28 (0.42 - 3.85)	0.65
Total	152	17 (11.18)	-	-

Note. HuNV GI: Human norovirus genotype I; HuNV GII: Human norovirus genotype II; *E. coli*: *Escherichia coli*; [a] : Reference group; OR: Odds ratio; CI: Confidence interval.

Table 3. Microbial Contamination Profiles of Various Leafy Vegetables (N = 152)

Type of Vegetables	Number Tested	Human Norovirus G1 (%)	Human Norovirus GII (%)	*E. coli* O157:H7 (%)
Lettuce[a]	38	-	3 (7.90)	3 (7.90)
Watercress	38	3 (7.90)	1 (2.63)	4 (10.53)
Cilantro	38	-	-	2 (5.26)
Oregano	38	-	-	1 (2.63)
Total	152	3 (1.97)	4 (2.63)	10 (6.58)

Note. E. coli: Escherichia coli; [a]: Reference group; OR: Odds ratio; CI: Confidence interval.

Table 4. Univariate Analysis Measuring the Association Between Vegetable Type and the Total Prevalence of HuNoV GI, HuNoV GII, and *E. coli* O157:H7 Contamination in Green Leafy Vegetables

Type of Vegetables	Number Tested	Positive (%)	OR (95% CI)	*P-value*
Lettuce[a]	38	6 (15.78)	1	0.07
Watercress	38	8 (21.05)	1.42 (0.44 - 4.58)	0.55
Cilantro	38	2 (5.26)	0.29 (0.05 – 1.57)	0.15
Oregano	38	1 (2.63)	0.14 (0.01 – 1.26)	0.08
Total	152	17 (11.18)	-	-

Note. HuNV GI: Human norovirus genotype I; HuNV GII: Human norovirus genotype II; *E. coli*: *Escherichia coli*; [a]: Reference group; OR: Odds ratio; CI: Confidence interval.

Discussion

Vegetables are a vital food group, which can be contaminated by enteric pathogens through multiple routes from farm to fork (33). Earlier studies have confirmed their key role as crucial vehicles for transmitting foodborne illnesses (34). Several important enteric pathogens, such as HuNoVs, *S. Typhimurium*, and *E. coli* O157:H7, can internalize within leafy vegetables, including lettuce, spinach, and green onions (35). Other risk factors include the use of pathogen-contaminated irrigation water, inadequate processing, and improper washing of products before raw consumption, all of which increase the risk of pathogen transmission to human (35).

HuNoVs and *E. coli* O157:H7 are among the most significant and prevalent viral and bacterial pathogens contributing to foodborne contamination, respectively. Their high resistance to environmental conditions and their ability to develop severe disease contribute to their widespread prevalence and substantial public health impact (36).

The present study assessed the presence of HuNoV GI, HuNoV GII, as well as *E. coli* O157:H7 in the four types of green leafy vegetables, including lettuce, watercress, coriander, and oregano, collected from farms and vegetable markets. In addition, extraction controls were used to validate the efficiency of genome recovery and to confirm the absence of PCR amplification inhibitors in the samples.

A systematic review by Chatziprodromidou et al reported 152 viral outbreaks associated with the fresh produce consumption, with an overall positive rate of 48.7% for different viral outbreaks. HuNoV GI and HuNoV GII were detected in 12–37% and 25–58% of reported cases, respectively (37). These results underscore the contamination of fresh vegetables with HuNoVs.

In this study, the overall positive rate for HuNoV and *E. coli* O157:H7 in vegetable samples was determined to be 17 out of 152 (11.18%). Numerous studies have investigated the prevalence of HuNoVs in leafy greens in many countries, including Canada, Italy, France, the United Kingdom, and Belgium, with rates varying from 3.33% to 50% (38,39,40,41,42,43).

Furthermore, HuNoVs were detected in 7 out of 152 samples (4.6% of the total). In line with our results, Torok et al reported a relatively low prevalence (2% to 2.2%) of HuNoV and HAV in Australian fresh products, including leafy greens, strawberries, and blueberries (44).

The prevalence of HuNoV GII (2.63%) in our study was slightly higher than that of HuNoV GI (1.97%), aligning with findings from Elmahdy et al and Terio et al (2, 3). They also observed a high prevalence of HuNoV GII (3,6). In an Iranian study by Rafieepoor et al, a higher frequency rate of HuNoV GII was reported. HuNoV GII and GI were detected in 3.15% and 2.5% of samples, respectively. These samples contained leafy green vegetables such as parsley, basil, watercress, lettuce, and spinach, all collected from farms, food distribution centers, and irrigation water in Tehran, Iran (23). In contrast, Cook et al reported a higher positive rate for HuNoV GI with 33/1152 (2.86%) compared to HuNoV GII with 19/1152 (1.65%) in a sample size comprising 1152 samples of berry fruits and salad vegetables collected in the United Kingdom (43). Therefore, several factors, such as the type of samples and the experimental approaches employed for pathogen detection, contribute to the observed prevalence rates of HuNoV GI and GII in food products. To obtain a comprehensive understanding of HuNoV GI and HuNoV II contamination, a broader sample size containing more food product types and using similar experimental approaches is proposed. However, our findings offer valuable insights into the dissemination of norovirus genotype in fresh vegetables in Iran.

More specifically, among the various types of vegetables studied, watercress exhibited the highest contamination rate for both HuNoV GI (7.9%, 3/38) and GII (2.63%, 1/38), followed by lettuce, in which only one HuNoV GII was detected. This contrasts with findings by Mattison et al, who reported a relatively high positive rate of HuNoV at 28.2% (181/641) in lettuce samples (45). Conversely, Kokkinos et al found a relatively low positive rate for HuNoV GI (1.3%, 12/149) and GII (0.8%, 1/126) in lettuce samples collected from markets in Canada (46). As previously mentioned, a study by Raeefipour et al indicated variable HuNoV positive rates in lettuce, ranging from 0.1% (3/30) to 19.23% (20/104) during two sampling phases.

Based on our results, the positive rate of *E. coli* O157:H7 in the studied samples was 6.58%, highlighting an ongoing public health concern associated with the consumption of contaminated green leafy vegetables. This finding aligns with recent reports emphasizing the association between green leafy vegetables and recurrence of *E. coli* O157:H7 outbreaks (47, 48). In an Iranian study, Ahmadi et al confirmed the shiga-like toxigenic E. coli in 14.4% of vegetable samples. In addition, a positive rate of 12% also was reported for E. coli contamination in vegetables (49).

As stated earlier, food contamination can occur at multiple stages, from cultivation to distribution, a point acknowledged by many researchers. In the present study, the frequency of HuNoV and *E. coli* O157:H7 contaminations were slightly higher in farm-sourced vegetables (12%) compared to market-purchased samples (9.6%), but this difference was not statistically significant.

This confirms the importance of hygienic practices in food supply chain. The higher positive rate in farm-sourced products suggests the critical importance of hygiene during growth and harvesting. Gao et al revealed a relatively higher prevalence of HuNoV in farm-sourced products (18%) compared to market-sourced samples (4%) (50). In addition, Huvarova et al reported a higher prevalence rate of foodborne pathogens in vegetable and herb samples originating from farm (51). Therefore, enhancing monitoring systems to assess the quality and hygienic conditions of irrigation water and soil fertilizers, especially those of animal origin, has been proposed for cultivated vegetables (52).

This study is the first report of the simultaneous detection of two important human enteric pathogens in the northern part of Iran. However, some limitations are acknowledged in this study, such as low sample diversity and the nature of PCR products obtained from the molecular approach for sequencing (52). Moreover, our analysis did not show statistically significant associations between contamination rates and vegetable type or source, which could be attributed to the type of sampling strategy and sample size employed (53). In this study, viral contamination was analyzed using a molecular assay. These methods could not discriminate between infectious and non-infectious virus particles. Consequently, the detection of the viral genome cannot confirm the presence of infectious particles or prove the transmission of infectious virus particles via the studied vegetables (54). Despite these constraints, this study provides valuable data about foodborne pathogens (HuNoV and *E. coli* O157:H7) in green leafy vegetables, which remains a major public health concern.

Conclusion

The findings of this study underscore the necessity of addressing the risks associated with microbial and viral contamination of leafy green vegetables and the potential transmission of the studied pathogens through the consumption of raw vegetables. Thus, further studies are proposed to elucidate the role of environmental factors, such as the hygienic quality and condition of soil and irrigation water, as well as handling methods. Additionally, establishing a routine surveillance system based on more sensitive and reliable detection methods for foodborne viruses and bacterial contaminants is necessary. To date, a limited number of surveys have been conducted on vegetables and fresh foods to assess viral and bacterial pathogens in Iran. Finally, due to the vast geographical size of the country, local comprehensive food safety measures should be taken.

Acknowledgments

This study is supported by Amol University of Special Modern Technologies, Amol, Iran.

Authors' Contribution

Conceptualization: Mojtaba Khosravi, Rahem Khoshbakht.
Data curation: Seyed Hani Mirzadeh.
Formal analysis: Mojtaba Khosravi.
Investigation: Seyed Hani Mirzadeh.
Methodology: Mojtaba Khosravi, Rahem Khoshbakht.
Project administration: Mojtaba Khosravi, Rahem Khoshbakht, Hamidreza Kazemeini.
Supervision: Mojtaba Khosravi, Hamidreza Kazemeini,
Writing–original draft: Mojtaba Khosravi, Seyed Hani Mirzadeh,
Writing–review & editing: Mojtaba Khosravi, Rahem Khoshbakht.

Competing Interests

The authors declare no conflict of interests.

Ethical Approval

The study received ethical approval from the Ethics Committee of Amol Special modern technologies, the code IR.ausmt.rec.1405.2.

Funding

No funding was obtained for this study.

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