https://doi.org/10.17221/101/2025-CJFS

Occurrence of Norovirus genogroup II in leafy greens in the region of Marrakech using a molecular method

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Citation: Berrouch S., El Fellaki N., Biary A., Lachkar H., Rafi H., Rouane H., Goïta S., Hafid J. (2026): Occurrence of Norovirus genogroup II in leafy greens in the region of Marrakech using a molecular method. Czech J. Food Sci., 44: 123–131.

Abstract: Leafy greens are important vectors for enteric viruses, including human noroviruses (HuNoV), which are a leading cause of foodborne disease. These viruses can contaminate the agricultural environment through untreated wastewater or direct contamination. While studies on HuNoV in vegetables have been conducted, few have focused on Morocco. This study aimed to detect HuNoV in leafy greens collected in the region of Marrakech region over a fifteen-week period (March–June 2023). For this purpose, 112 samples (coriander, lettuce and parsley) were collected and analysed using the ISO 15216-2:2019 method with minor modifications, after validation. The method involved eluting viruses using an alkaline buffer, concentrating them through polyethylene glycol precipitation, and detecting viral RNA via real-time RT-PCR. The applied method yielded variable recovery rates among the tested matrices, with coriander showing the highest recovery (1.5%), followed by lettuce (1.2%) and parsley (0.6%), confirming a significant matrix-dependent variation in HuNoV recovery (ANOVA, $P < 0.001$). This method enabled the assessment of leafy green contamination, which was found to be 0.89% (1/112). This study underscores the need to enhance detection methods to better assess the risks associated to noroviruses in leafy greens, with implications for human health.

Keywords: enteric viruses, fresh vegetables; real-time RT-PCR detection; survey; market

The use of wastewater, sludge, and human or animal faeces has long been practiced in various parts of the world. This approach has been used for centuries, particularly in countries with limited water resources, to recycle nutrients and improve soil fertility (Gaskin et al. 2009). The scarcity of rainfall poses a significant challenge to global agricultural production needs. This challenge makes low-quality water, such as wastewater, a valuable resource for year-round irrigation. Consequently, wastewater allows for higher yields, year-round production, and an expanded range of crops that can be irrigated, particularly in arid and semi-arid regions (Keraita et al. 2008).

However, the use of low-quality water, such as wastewater, and excreta for irrigation and crop fertilisation has been linked to an increased risk of diarrheal diseas-

Supported by the Moroccan Ministry of Higher Education, Scientific Research and Innovation, and the OCP Foundation, Morocco, APRD – EVEFDM 2023.

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https://doi.org/10.17221/101/2025-CJFS

es, particularly in low-income regions where sanitation practices may be inadequate. Several studies report that exposure to human excreta can result in waterborne illnesses. These include diarrhoeal diseases and hepatitis caused by enteric viruses, particularly human norovirus (HuNoV) and hepatitis A virus, respectively; both remain a leading cause of morbidity and mortality (Teunis et al. 2010; Chatziprodromidou et al. 2018). HuNoVs account for the majority of foodborne illness cases, causing approximately 125 million infections annually and leading to 35 000 deaths each year (WHO and FAO 2023). They are transmitted to humans through contaminated food, water, surfaces, or hands that have come into contact with faecal matter. Numerous HuNoV outbreaks have been associated with salads and cut fruits, the former having been suspected in 15.8% of viral outbreaks (reviewed by Chatziprodromidou et al. 2018). HuNoVs are considered highly infectious at low doses (10 viral particles), making them particularly linked to environmental contamination of fresh produce (Teunis et al. 2008). Globally, numerous cases of enteric virus detection, particularly HuNoV, have been reported in fresh produce. A recent review reported that these agents were detected in fresh produce with a prevalence of 9.3% (95% confidence interval 3.7–21.7) (Ekundayo and Ijabadeniyi 2023).

In Morocco, a single study has investigated the presence of HuNoV in fresh produce. The study found a prevalence of 59% in vegetables such as lettuce, parsley, radish, and onion (Bounagua et al. 2021). The aim of this study was to investigate the presence of HuNoV in coriander, lettuce and parsley in the region of Marrakech, using the standard ISO 15216-2:2019 with minor modifications.

MATERIAL AND METHODS

Virus. The strain of human Norovirus genogroup II (HuNoV-GII) was obtained from a positive liquid stool sample of an infected patient. It was graciously provided by the laboratory of virology of the University Hospital of Saint-Etienne (France) where it was detected by immunochromatography. In our laboratory, it was stored at –20 °C, until used for spiking experiments.

Thirty microliters of stool sample (without prior preparation) were mixed with 120 µL of ultrapure water to obtain a final volume of 150 µL, which was used for nucleic acid extraction. The extract was serially diluted (10^{-3} to 10^{-8}) and each dilution was tested in duplicate as described below to evaluate the linearity of the standard curve. A single dilution corresponding to the lower genomic copy number was additionally tested in ten deposits to estimate the inoculum concentration using the Poisson distribution.

Spiking experiments for the validation of the method. To be able to interpret negative samples in our survey, the detection method used was validated in our internal conditions. To that aim, leafy greens (coriander, lettuce, and parsley) were bought from a retailer in Marrakech and damaged leaves, roots and stems were removed. The leaves were then cut into squares of about 2.5 cm side, mixed, and weighed to obtain samples of 25 g. The leaves were washed using a 0.001% sodium hypochlorite solution, placed on clean paper towels, and sterilised by exposure to ultraviolet light for 20 min on each side (Prez et al. 2018). Each sample was spiked with 30 µL of a 1/4 diluted HuNoV-GII–positive stool sample (in several spots of 5 µL maximum on leaves) and then allowed to dry in a microbiological safety cabinet overnight before being processed.

Viruses were concentrated following the ISO 15216-2:2019 method with minor modifications. Briefly, viruses were eluted from artificially contaminated samples using 40 mL of elution buffer (100 mM Tris and 50 mM glycine containing 1% beef extract; pH 9.5) in a 400 mL filter bag (BagFilter® R, Interscience, France) with gentle shaking (60 oscillations·min^{-1}, 20 min, 20 °C). The filtrates were collected in sterile 50 mL polypropylene tubes and the pH was adjusted to 7 ± 0.5, using 1 M HCl solution. A solution of 5X polyethylene glycol (PEG) 6 000/1.5 M NaCl was added to the filtrates at a 1: 4 (v/v) ratio to obtain a final concentration of 100 g·L^{-1} PEG 6 000 and 0.3 M NaCl, respectively. The mixtures were then incubated at 4 °C for 60 min under gentle agitation (60 oscillations per minute) to promote viral particle precipitation. Virus concentration was achieved by centrifugation at 10 000 × g for 30 min at 4 °C. The pellets obtained were resuspended and concentrated again by centrifugation at 10 000 × g for 5 min. The final pellets (150 µL) were then subjected to nucleic acid extraction and real-time RT-PCR. Each test was performed in four replicates. A sample of non-spiked leafy greens (coriander, lettuce, and parsley) was used as a negative process control in each experiment.

The recovery rate of the validated method was evaluated for each leafy green matrix, using the following formula:

$$Recovery\left(\%\right) = F \times 10^{\left(\frac{Cq\ revocered - Cq\ inoculum}{Slope}\right)} \times 100 \quad (1)$$

Where: F – a correction factor representing the ratio of the total pellet volume to the volume used for

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extraction, this factor was only calculated for parsley where 150 µL of the pellet out of a total of 1 000 µL was used for RNA extraction, as the solid pellet could not be completely resuspended in 150 µL.

Assessment of the level of inhibition. To assess the level of inhibition, RNA extracts from coriander, lettuce, and parsley were analysed both undiluted and at a 1 : 10 dilution. The viral RNA was detected by RT-qPCR as described below. The inhibition rate was calculated using the following equation:

$$Inhibition\ rate\ (\%) = \left(1 - 10^{\left(\frac{\Delta\ Cq\ observed - \Delta\ Cq\ expected}{Slope}\right)}\right) \times 100 \quad (2)$$

Where: ΔCq *observed* = Cq of the undiluted sample – Cq of the diluted sample; ΔCq *expected* = $\log_{10}$ (dilution factor) × *slope*.

Assessment of the limit of detection (*LOD*). The LOD_{95}, defined as the lowest dilution yielding more than 95% positive samples, was determined for the validated protocol using sterilised 25 g samples of each leafy green. While the validation step was performed using the high inoculum level, two additional dilutions were prepared from this same suspension to obtain medium and low inoculum levels. At each inoculum level, four replicates for each sterilised vegetable matrix were spiked and allowed to dry. The virus was extracted as described above. To ensure the reliability of the results, a virus-free negative control was included for each matrix. RNA extraction and RT-qPCR amplification were performed as described below.

Samples collection. A total of 112 samples (coriander, lettuce, and parsley) were collected from four locations over a fifteen-week period (March–June 2023). The selection of these leafy greens was based on their availability in the markets and their mode of consumption (raw and/or slightly cooked). In the city of Marrakech, samples were collected from the wholesale fruit and vegetable market located in the industrial district, as well as from a supermarket in the Gueliz district. These markets are supplied with both local and imported products from various regions of Morocco. Additionally, samples were obtained from a permaculture farm situated 17 km from Marrakech in the commune of Oulad Hassoun. This farm exclusively uses organic amendments for cultivation, with crops irrigated using gravity-fed water from wells. The farm's primary activity is mixed farming, with its agricultural products, including vegetables, seasonal fruits, and aromatic herbs, intended for commercialisation. Finally, plant samples were also taken from a private garden in Marrakech, where the plants were irrigated with potable water and were not treated with any fertiliser or pesticide (Table 1).

For each vegetable, 25 g samples were prepared and processed as described above (Material and methods, paragraph 2.), and then analysed by real-time RT-PCR for HuNoV- GII.

RNA extraction and real-time RT-PCR amplification. The pellet (150 µL) was subjected to RNA extraction using the Nucleospin® RNA Virus Extraction Kit (Macherey-Nagel®, Germany) following the manufacturer's instructions. The extracted RNA (100 µL) was then analysed by real-time RT-PCR using primers and probes specific to the target virus (HuNoV-GII).

A one-step real-time RT-PCR assay was conducted using primers and a Taqman probe targeting the conserved ORF1/ORF2 junction region of the norovirus genome. The sequences of primers QNIF2d (Loisy et al. 2005) and COG2R (Kageyama et al. 2003) and probe QNIFS (Loisy et al. 2005) are listed in Table 2. The RT-PCR amplification was performed using two different kits due to reagent availability; their efficiencies were tested and found to be equivalent. The 4X CAPITAL™ One-Step qRT-PCR Probe Master Mix (Biotech Rabbit) was used for the method validation experiments, while the SensiFAST™ Probe Lo-RoX One-Step Kit (Meridian Bioscience®) was employed for the prevalence analysis.

Reactions were carried out in a final volume of 20 µL, containing RNA template (4 –5 µL), primers at 400 nM, probe at 100 nM, and RNase-free water to the final volume, following each manufacturer's instructions. Thermal cycling conditions were applied according to the protocol recommended by Da Silva et al. (2007)

Table 1. Detail of collected leafy greens in Marrakech region from March to June 2023

Sampling sites / Leafy greens	Wholesale market	Supermarket	Permaculture farm	Private garden	Total
Coriander	15	14	5	2	36
Lettuce	15	15	5	3	38
Parsley	15	15	5	3	38

https://doi.org/10.17221/101/2025-CJFS

Table 2. Primers and probe sequences, and amplification conditions for real time RT-PCR assay targeting the ORF1/ORF2 junction region of HuNoV-GII genome

Primer or probe sequence (5'–3')	Amplification conditions	References
F: ATG TTC AGR TGG ATG AGR TTC TCW GA	30 min at 50 °C	Da Silva et al. (2007), Elfellaki et al. (2024)
R: TCG ACG CCA TCT TCA TTC ACA	5 min at 95 °C	
P: FAM-AGC ACG TGG GAG GGC GAT CG-TAMRA	45 cycles of 10 s at 95 °C, 20 s at 55 °C	

F – forward primer; R – reverse primer; P – probe; FAM – 6-carboxyfluorescein; TAMRA – 6-carboxytetramethylrhodamine

as modified by Elfellaki et al. (2024) (Table 2). For quality control, each PCR plate included a negative control (RNase-free water) as well as a positive control (RNA extracted from a positive stool sample). Each sample, along with the positive and negative controls, was amplified in duplicate. A sample was deemed positive if the cycle of quantification (*Cq*) value was below 40 for both RNA deposits. Moreover, in case the RNA was detected only in one deposit, a second PCR analysis was performed using the same procedure. In this case, the positivity limit was set at least at 3/4 positive deposits.

Statistical analysis. The impact of leafy green matrix on viral detection was assessed based on *Cq* values, using ANOVA after confirmation of data normality using the Shapiro–Wilk test. Significant differences were further analysed by Tukey's post hoc test.

RESULTS

Estimation of the level of inoculum. The standard curve of the tested dilutions (from 10^{-3} to 10^{-7}) was linear (Figure 1). The mean *Cq* values of the virus RNA extracts were correlated to the logarithm of the number of spiked virus particles (R^2 = 0.997 8). The lowest detected dilution was 10^{-8}, where HuNoV-GII RNA was detected in 1 deposit out of two. The subsequent amplification of this extract in ten deposits resulted in a mean *Cq* of 35.8 ± 0.9, with 4 negative deposits out of ten.

The level of the original inoculum ($\lambda = 6.1 \times 10^{10}$ genomic copy·mL^{-1} of stool sample) was estimated using the following equation:

$$\lambda = -\ln (Po) \quad (3)$$

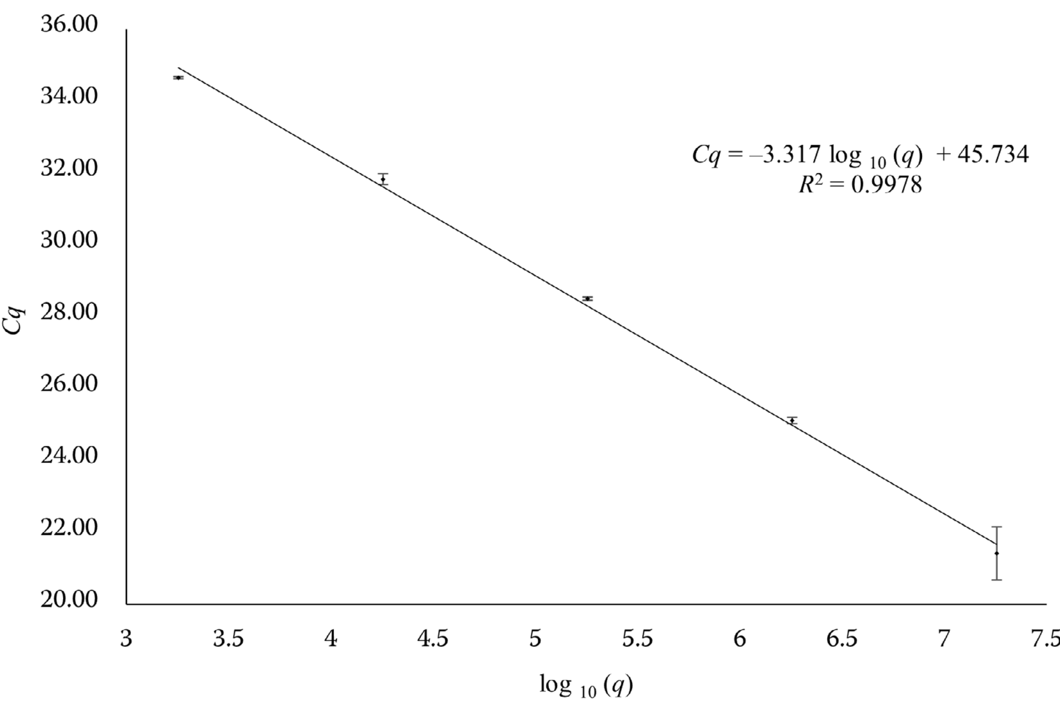

Figure 1. RT-qPCR standard curve for human norovirus genogroup II extract dilutions (from 10^{-3} to 10^{-7})
Cq – cycle of quantification

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Where: λ – the average number of target molecules per reaction; *Po* – the fraction of negative reactions.

For the spiking experiments, we used the 1/4 diluted stool sample as inoculum, corresponding to approximately 1.5×10^{10} genomic copies·mL^{-1} of stool sample.

Validation of the detection method. To evaluate the efficiency of the protocol, validation assays were conducted using three leafy greens: coriander, lettuce, and parsley. The stool sample used for spiking exhibited a *Cq* value of 12.8 ± 0.2. As shown in Table 3, all four replicates tested positive for each matrix. Mean *Cq* values obtained were 18.8 ± 0.1 for coriander, 19.2 ± 0.6 for lettuce, and 23.4 ± 0.7 for parsley, corresponding to recovery rates of 1.5, 1.2, and 0.6%, respectively. These results indicated variable recovery efficiencies among the tested matrices. The statistical analysis confirmed that the type of matrix significantly affected the method's performance (ANOVA, $P < 0.001$). Post-hoc Tukey's test revealed that parsley differed significantly from coriander and lettuce ($P < 0.001$; $\Delta Cq > 4$), whereas the difference between coriander and lettuce was not statistically significant. Inhibition assessment showed no inhibition for lettuce and parsley (inhibition rates < 75%), while coriander exhibited a high level of inhibition (76.2%), suggesting minor matrix-related effects.

Assessment of the LOD. To further evaluate the LOD of the method, two additional viral concentrations – medium and low – were tested, corresponding to 4.5×10^6 and 4.5×10^4 genomic copies (gc) per vegetable sample, respectively. As summarised in Table 4, all four replicates were positive for both concentrations across coriander, lettuce, and parsley. The mean *Cq* values (± SD) for the medium concentration ranged from 28.5 ± 0.1 to 31.1 ± 0.9, while for the low concentration they ranged from 30.6 ± 0.1 to 31.5 ± 0.7, indicating reliable detection even at the lowest tested level. Since all replicates were positive at the lowest tested concentration (4.5×10^4 gc·sample^{-1}), no fractional positivity was obtained, and therefore a statistical LOD_{95} could not be calculated. The *LOD* can be reported as $< 1.3 \times 10^3$ gc·g^{-1} of leafy greens, which represents the lowest concentration experimentally confirmed in this study.

Occurrence of HuNoV-GII in leafy greens. Leafy greens samples were analysed using the modified standard protocol. Real-time RT-PCR analysis revealed a single positive lettuce sample for HuNoV-GII (*Cq* = 36.3 ± 0.6), which was collected from a supermarket in May 2023 (Table 5).

DISCUSSION

Fresh produce is recognised as a significant vehicle for enteric viruses due to its raw consumption and minimal processing, including limited washing (Kokkinos et al. 2012). Contamination of HuNoV has been epidemiologically linked to the consumption of vegetables, salad, fruits, peas and juices (Aiyedun et al. 2021).

This study was conducted to detect HuNoV-GII using real time RT-PCR in coriander, lettuce, and parsley intended for consumption in the region of Marrakech, following the protocol described in the ISO 15216-2:2019, with minor modifications. The applied method yielded

Table 3. Results of validation of the method for detecting HuNoV-GII in leafy greens by real time RT-PCR

Leafy greens	Replicates (R)	Mean *Cq** ± SD	Overall mean *Cq*** ± SD	Recovery rate (%)	Inhibition rate (%)
Coriander	R1	18.9 ± 0.1	18.8 ± 0.1	1.5	76.2
	R2	18.9 ± 0.0			
	R3	18.8 ± 0.1			
	R4	18.7 ± 0.0			
Lettuce	R1	18.7 ± 0.1	19.2 ± 0.6	1.2	40.6
	R2	19.8 ± 0.2			
	R3	19.6 ± 0.2			
	R4	18.8 ± 0.0			
Parsley	R1	23.0 ± 0.0	23.4 ± 0.7	0.6	24.5
	R2	22.6 ± 0.1			
	R3	24.0 ± 0.1			
	R4	24.1 ± 0.1			

*average of *Cq* values for two RT-PCR deposits; **average of *Cq* values for four replicates of the method
Cq – cycle of quantification; HuNoV – human norovirus

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Table 4. Results of validation of the method for detecting medium and low level of HuNoV-GII in leafy greens by real time RT-PCR

	Replicates (R)	Medium inoculum level (4.5×10^6 gc per leafy green sample)		Low inoculum level (4.5×10^4 gc per leafy green sample)	
		mean *Cq** ± SD	overall mean *Cq*** ± SD	mean *Cq* ± SD	overall mean *Cq* ± SD
Coriander	R1	28.6 ± 0.0	28.5 ± 0.1	30.9 ± 0.0	31.2 ± 0.2
	R2	28.5 ± 0.1		31.3 ± 0.1	
	R3	28.6 ± 0.0		31.3 ± 0.2	
	R4	28.5 ± 0.2		31.3 ± 0.1	
Lettuce	R1	29.0 ± 0.0	29 ± 0.5	30.7 ± 0.1	31.1 ± 0.5
	R2	30.1 ± 0.1		31.1 ± 0.2	
	R3	28.9 ± 0.0		30.7 ± 0.2	
	R4	29.0 ± 0.1		31.8 ± 0.2	
Parsley	R1	30.0 ± 0.0	31.1 ± 0.9	31.5 ± 0.7	31.5 ± 0.7
	R2	31.8 ± 0.0		31.9 ± 0.1	
	R3	31.0 ± 0.1		32.2 ± 0.3	
	R4	31.8 ± 0.2		30.6 ± 0.1	

*average of *Cq* values for two RT-PCR deposits; **average of *Cq* values for four replicates of the method
Cq – cycle of quantification; gc – genomic copies

Table 5. Results of the occurrence of HuNoV-GII by real-time RT-PCR, in leafy greens in Marrakech

Leafy greens	Sampling sites	No. analysed samples	Positive deposits *Cq* < 40				No. positive samples (mean *Cq* values ± SD)
			1st amplification		2nd amplification		
			deposit 1 (*Cq*)	deposit 2 (*Cq*)	deposit 1 (*Cq*)	deposit 2 (*Cq*)	
Coriander	WM	15	1 (31.8)	0	0	0	0
	SM	14	0	0	Na	Na	0
	PF	5	0	0	Na	Na	0
	PG	2	0	0	Na	Na	0
	Total	36	1	0	0	0	0
Lettuce	WM	15	1 (31.0)	0	0	0	0
	SM	15	1 (36.6)	0	1 (36.6)	1 (35.6)	1 (36.3 ± 0.6)
	PF	5	0	0	Na	Na	0
	PG	3	0	0	Na	Na	0
	Total	38	2	0	1	1	1
Parsley	WM	15	1 (33.1)	0	0	0	0
	SM	15	0	0	Na	Na	0
	PF	5	0	0	Na	Na	0
	PG	3	0	0	Na	Na	0
	Total	38	1	0	0	0	0

Cq – cycle of quantification; PF – permaculture farm; PG – private garden; SM – supermarket; WM – wholesale market; HuNoV – human norovirus

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variable recovery rates among the tested matrices, with coriander showing the highest recovery (1.5%), followed by lettuce (1.2%) and parsley (0.6%), confirming a significant matrix-dependent variation in HuNoV recovery (ANOVA, $P < 0.001$). These differences are likely due to matrix-specific factors such as leaf structure, surface composition, and the presence of secondary metabolites, all of which can affect the efficiency of viral elution and concentration. Randazzo et al. (2018) highlighted similar issues in their study that reported variability in hepatitis E virus detection across different leafy greens using the ISO 15216-1:2017 method. Their study reported recovery rates of 1.3% for lettuce, 0.5% for spinach, and 4.0% for pepper, underscoring how differences in leaf structure can affect the effectiveness of virus detection methods.

High PCR inhibition (76%) was observed in coriander, exceeding the recommended threshold of 75%, while no inhibition was observed in lettuce or parsley. This finding suggests the presence in coriander of matrix-derived inhibitors, such as polyphenols or other secondary metabolites, consistent with previous reports (Suther and Moore 2019). According to Wilson (1997), diluting samples before detection by RT-qPCR is often useful for diluting PCR inhibitors, but it also dilutes the RNA targets, which may affect the sensitivity of the technique. In contrast, Raymond et al. (2021) reported higher recovery rates and no inhibition in coriander using a magnetic silica bead-based extraction method. Their study demonstrated that the use of silica beads effectively mitigates PCR inhibitors present in certain matrices, highlighting the importance of extraction protocols in enhancing detection sensitivity.

Furthermore, the PEG concentration method used in the present study was commonly adopted for viral recovery from various food matrices at a rate reaching 17.0% in leafy greens (Dubois et al. 2002; Stoufer et al. 2024). However, a meta-analysis reported that a highest prevalence of enteric viruses was detected by membrane filtration followed by PEG precipitation, suggesting that combining multiple methods may be more effective for concentrating and extracting the virus (Barril et al. 2022).

The current study shows a low presence of HuNoV-GII 0.89% (1/112) in leafy greens. A meta-analysis study reported an overall prevalence of HuNoV of 9.3% (95% confidence interval [CI] 3.7–21.7 in fresh produce (fruits and vegetables), with fruits being more contaminated (9.8% [3.7–23.5]) than vegetables (9.4% [3.1–25.3]). Various prevalence rates of HuNoV were reported worldwide: they varied from Africa (25.8%, [5.7–66.7]), Asia (23.2% [4.6–65.0]), Europe (5.6% [0.7–33.9]), North America (12.4% [1.8–52.7]) to South America (7.5% [0.0–100.0]) (Ekundayo and Ijabadeniyi 2023).

It is important to interpret the negative results in parsley and coriander in light of the method's performance obtained for these two matrices. According to ISO 15216-2:2019, extraction efficiencies below 1% and inhibition levels above 75% fall outside the validity criteria of the method. In this study, parsley showed an extraction efficiency of only 0.6%, while coriander exhibited a PCR inhibition rate of 76%. Both values exceed the limits recommended by the standard, meaning that the analytical results for these matrices could not fully reflect their true contamination status. Recovery efficiency below 1% can underestimate viral load by up to two orders of magnitude, and inhibition above 75% can markedly reduce amplification efficiency. Therefore, the non-detection of HuNoV in parsley and coriander should be interpreted with caution, as low-level contamination may have gone undetected due to limited sensitivity of the technique. These methodological constraints likely contributed to lettuce being the only matrix in which HuNoV was detected.

In Morocco, only one study has examined the presence of HuNoV in fresh produce. It has reported a HuNoV prevalence of 59.0% (47/80) in vegetables including lettuce, parsley, radish, and onion, with leafy greens showing particularly high levels of contamination 32.5% (26/80). The study has also highlighted the influence of irrigation water quality, with 68.0% (27/40) of contaminated samples being irrigated with wastewater treatment plant effluents, compared to 50.0% (20/40) irrigated with well water (Bounagua et al. 2021). Interestingly, the role of irrigation water in the viral contamination of vegetables was also highlighted by the results of the present study in which HuNoV was not detected in any samples from the private garden and permaculture farm, both of which used well water for irrigation. Unfortunately, the source of contamination in the positive sample could not be determined, as the origin of vegetables from the wholesale market and supermarket was unidentified due to a lack of traceability.

Furthermore, it is also possible that during the sampling period, HuNoV was not present in significant quantities in the environment, particularly in water sources. This could be due to various factors, such as changes in agricultural practices or seasonal fluctuations in virus prevalence. Some studies have reported that HuNoV persists longer during colder months (winter and early spring) (Sorensen et al. 2021) and have observed a correlation between the presence of the virus in lettuce and seasonality in Japan, with higher prevalence noted in colder seasons (Xie et al. 2021). However, other research works conducted in Australia (Torok et al. 2019), the United Kingdom (Cook et al. 2019), and Egypt

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(Shaheen et al. 2019) found no significant seasonality in detecting HuNoV in fresh produce.

The disparity in reported prevalence across several studies may also be attributed to the efficiency of the detection methods used. While most studies adopted the ISO 15216 standard for HuNoV detection in fresh produce due to its ease of implementation, cost-effectiveness, and reliability, some have introduced innovative and improved techniques. For instance, a recent study developed a bentonite-coated activated carbon RT-qPCR method for HuNoV detection in fresh produce, yielding viral extraction efficiencies ranging from 53.4% to 95.6%, which was 10 to 100 times more sensitive than the ISO 15216-1:2017 standard (Tang et al. 2023).

In the current study, the real-time RT-PCR method used was unable to distinguish between infective and non-infective viral particles. The presence of viral RNA in the samples may be attributed to non-infective viruses, which suggest that the products were at some point contaminated with HuNoV particles during their production process, but do not confirm an actual health risk for consumers.

CONCLUSION

The presence of norovirus in raw-consumed fresh produce represents a significant concern for food safety. Preventing the introduction of this virus into fresh produce is crucial for ensuring food safety and minimising the risk of transmission to consumers, particularly by managing the quality of irrigation water. This study enhances our understanding of the risks associated with norovirus and highlights the matrix-dependent nature of HuNoV recovery and PCR inhibition when using the ISO 15216-2:2019 method. Adjustments to extraction or concentration procedures, along with consideration of matrix-specific characteristics, are crucial for optimising viral detection in leafy greens. Although, the results indicated a low prevalence of HuNoV-GII in leafy green samples, further assessment of the infectivity of detected viruses is crucial, as this likely corresponds more closely with the actual risk encountered by consumers.

Acknowledgement. We thank Professor Thomas Bourlet and Dr. Sylvie Pillet from the Virology Laboratory at Saint- Etienne University Hospital, France, for generously providing HuNoV-GII positive stool samples.

We also thank Professor Bruno Pozzetto for his professional review and correction of the English language in this manuscript.

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Received: June 28, 2025
Accepted: January 29, 2026
Published online: April 13, 2026