



Internalization and Localization of Murine Norovirus in Hydroponically Grown Green Onion (*Allium Fistulosum* L.)

Soontag Jung¹ · Hansaem Shin¹ · Daseul Yeo¹ · Hyojin Kwon¹ · Day Jung¹ · Changsun Choi¹

Received: 1 October 2025 / Accepted: 10 January 2026
© The Author(s) 2026

Abstract

Norovirus is a leading etiological agent of foodborne illnesses globally, with fresh produce serving as a significant vector for its transmission to humans. This study investigated the internalization potential of norovirus from contaminated irrigation water, focusing on the temporal distribution, persistence, and removability of murine norovirus (MNV) in hydroponically grown green onions. Our results demonstrated that MNV rapidly internalized into the root tissues of green onions within 1 h of exposure, subsequently translocating through the vascular system to the stems and leaves. Viral loads consistently peaked in the roots and persisted for at least 21 days post-inoculation. A single water exchange procedure resulted in a gradual decrease in viral load within plant tissues, although viral genomes remained detectable for up to 21 days. Repeated water exchanges effectively cleared the virus from leaves and stems but failed to eradicate it entirely from the roots. Immunofluorescence analysis localized the virus predominantly within the vascular tissues, specifically the xylem and phloem vessels, and most notably on the rough, porous surfaces of root hairs, where viral particles persisted despite rigorous washing protocols. Collectively, these findings unequivocally demonstrate that MNV can rapidly internalize and establish a sustained, long-term presence within green onion plants, particularly concentrated in the root tissues. This underscores the imperativeness of stringent pre-harvest preventive strategies to mitigate the risk of viral contamination in hydroponic cultivation systems.

Keywords Norovirus · Internalization · Hydroponic · Water exchange

Introduction

Norovirus is the predominant viral agent responsible for acute gastroenteritis globally, leading to an estimated 685 million cases of illness annually and contributing to as many as 278,000 fatalities each year (Carlson et al., 2024). Clinically, norovirus-induced gastroenteritis is characterized by the acute onset of various symptoms, including nausea, vomiting, watery diarrhea, abdominal cramping, and

low-grade fever, typically manifesting within 12–48 h following exposure to the virus, with symptom duration generally lasting 1–3 days (Robilotti et al., 2015). Despite the self-limiting and transient nature of this illness, its severity and risk of complications are heightened in vulnerable populations such as children aged < 5 years and adults aged > 80 years (Shah et al., 2018; Tsai et al., 2022). Although the overall mortality rate attributable to norovirus infection has consistently declined—now approximately 1.86 deaths per 100,000 individuals—as a result of significant advancements in medical treatment and healthcare infrastructure, there remains a concerning and disproportionate increase in mortality among older adults residing in high-income countries, reaching up to 900% (Zhang et al., 2022).

Norovirus transmission primarily occurs through the fecal–oral route via multiple pathways, including person-to-person contact, the consumption of contaminated food and water, contact with contaminated surfaces, and exposure to aerosolized viral particles (Robilotti et al., 2015). Notably, norovirus is the leading cause of foodborne illness outbreaks

Soontag Jung and Hansaem Shin have contributed equally to this work.

✉ Changsun Choi
cchoi@cau.ac.kr

¹ Department of Food and Nutrition, School of Food Science and Technology, College of Biotechnology and Natural Resources, Chung-Ang University, 4726 Seodongdaero, Daeduck-myeon, Anseong-si 17546, Gyeonggi-do, Republic of Korea

in the United States, accounting for approximately 50% of all foodborne outbreaks with a confirmed etiology (Hall et al., 2011). Foodborne transmission accounts for approximately 23% of norovirus cases, with leafy vegetables, particularly lettuce, representing the largest proportion at 33% (Hall et al., 2012; Shah et al., 2017). Norovirus contamination of fresh produce can occur both pre- and post-harvest. Post-harvest contamination often originates from infected food handlers, whether symptomatic or asymptomatic (Hardstaff et al., 2018). Pre-harvest, norovirus can be introduced into produce through contaminated irrigation water sources, such as groundwater, rivers, and lakes, or through the application of diluted pesticides (López-Gálvez et al., 2016; Potera, 2013). Notably, a meta-analysis reported that norovirus was detected in 43.5% of river water samples (Ekundayo et al., 2021).

Hydroponic cultivation systems offer significant advantages over conventional soil-based agricultural practices, including enhanced water and essential nutrient conservation, effective soil-borne phytopathogen prevention, and comprehensive control over environmental parameters throughout the growth cycle (Thapa et al., 2024). Consequently, owing to these multifaceted benefits, hydroponic systems have gained popularity and are now extensively utilized in the commercial cultivation of fresh produce, particularly leafy vegetables like lettuce and diverse culinary herbs (Brentlinger, 2005). The irrigation water utilized in hydroponic cultivation systems is subjected to stringent management protocols to ensure effective disinfection, employing various sources such as surface water, rainwater, tap water, and purified water obtained via reverse osmosis (Mielcarek et al., 2024). However, unlike bacterial contamination, viral water contamination is undetectable, and viruses can survive these management systems or be introduced by asymptomatic infected workers (Alegebeleye et al., 2023).

The contamination of fresh produce via irrigation water is a significant pre-harvest route that has garnered increasing attention in recent years, particularly owing to concerns regarding norovirus internalization within produce (Hirneisen et al., 2012). In contrast to surface contamination, which can be mitigated through cleaning and disinfection, internalized pathogens are inaccessible to conventional decontamination methods (Erickson, 2012). Therefore, this study aimed to investigate the potential for norovirus internalization in fresh produce via contaminated irrigation water, focusing on the distribution and persistence of viral particles as they migrate from irrigation water into internal plant tissues, as well as evaluating their removability through water exchange. A hydroponic system was utilized to examine norovirus internalization in green onions, selected for their widespread consumption, rapid growth characteristics, and anatomical features that facilitate

virus transmission studies. Additionally, murine norovirus (MNV) was employed as a surrogate for human norovirus to enable controlled experimental conditions.

Materials and Methods

Virus and Cell Culture

MNV-1 was generously provided by Professor Herbert W. Skip Virgin from the University of Washington. It was propagated in the murine macrophage-derived RAW 264.7 cell line (ATCC TIB-71), cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum (Gibco) and 1% antibiotic–antimycotic solution (AA, 100×; Gibco), and maintained at 37 °C in a 5% CO₂ atmosphere. To generate viral stock, confluent monolayers of RAW 264.7 cells were infected with MNV-1 at a multiplicity of infection of 0.1. Viral particles were harvested at 2 days post-inoculation (dpi) by subjecting the culture to three freeze–thaw cycles, followed by low-speed centrifugation at 1,000 × g for 30 min.

Green Onion Cultivation and Viral Inoculation

Green onions were procured from a local retail store in Anseong, South Korea, and their surfaces were thoroughly washed with sterile distilled water to eliminate potential contaminants. The hydroponic feed water was enriched with a balanced nutrient solution comprising essential macronutrients, specifically nitrogen, phosphorus, and potassium, to promote optimal plant growth. Additionally, the feed water was supplemented with 1% AA to effectively inhibit microbial contamination. Thereafter, the hydroponic feed water was inoculated with MNV-1 to introduce viral contamination. The hydroponic water reservoir, with a total volume of 200 mL, was inoculated with 5 mL of viral suspension, resulting in an initial viral titer of 2.5×10^4 plaque forming unit/mL (1.04×10^6 genome copies/mL). This contamination level was experimental to ensure reliable quantification of internalized viral RNA throughout the experiment, although it was higher than average environmental levels. Negative controls consisted of hydroponic feed water that was not inoculated with the virus. Environmental conditions were strictly maintained at a temperature of 20 °C and a relative humidity of 40% throughout the experimental period.

Experimental Design

To investigate the temporal internalization and distribution patterns of the virus within the crop, the leaves, stems, and roots of green onions were collected at 1, 3, 5, and 7 h

post-inoculation (hpi) following exposure to MNV-1-contaminated hydroponic feed. Additionally, to assess the persistence of the internalized virus, leaves, stems, and roots were collected at 1, 3, 7, 14, and 21 dpi from independent samples.

Prior to the experiments, the green onions were disinfected to minimize the initial microbial load. The samples were treated with 1,000 ppm sodium hypochlorite—prepared from chlorine bleach containing 4% sodium hypochlorite—for 15 min. Thereafter, the samples were transferred to a new 200 mL beaker containing distilled water and submerged for 5 min, followed by neutralization in 0.25 M sodium thiosulfate for 5 min to remove residual chlorine. A similar disinfection procedure was also applied to the harvested samples at the conclusion of the experiment to inactivate any virus present on the surface of the green onion tissues. Each sample portion was homogenized using a mortar and pestle and resuspended in 5 mL of phosphate-buffered saline (PBS, pH 7.0). The samples were subsequently centrifuged at $1,000 \times g$ to remove cellular debris, and the supernatant was transferred to a new collection tube. Finally, 500 μ L of each sample was designated for quantitative reverse transcription polymerase chain reaction (RT-qPCR), while the remaining unhomogenized tissues were allocated for immunofluorescence (IF) analysis. Three individual green onions were used to generate data for each experimental data point.

To evaluate the impact of water exchange on internalized MNV-1, two experimental approaches were used. First, green onion surfaces were rinsed with sterile distilled water at 3 dpi and transferred to fresh feed water, with periodic observations continuing until 21 dpi. Second, to assess the potential for complete removal of the internalized virus, a separate group underwent water exchanges every 2 days for five cycles, with observations concluding after the final exchange at 10 dpi.

RT-qPCR

Total RNA was extracted from the samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany), followed by one-step RT-qPCR. One-step RT-qPCR was performed in a total volume of 25 μ L, comprising 5 μ L of 5 $\times$ UltraSense™ enzyme mix (Invitrogen, Carlsbad, CA), 1 μ L of 5 μ M MNV primers, 2 μ L of 5 μ M MNV probes, 5 μ L of RNA template, and 9.75 μ L of diethyl pyrocarbonate-treated water. Thermal cycling was conducted using a Thermal Cycler Dice Real-time System (TaKaRa, Otsu, Japan) at 50 °C for 15 min, followed by 95 °C for 2 min as well as 45 cycles of 10 s at 95 °C and 20 s at 55 °C for MNV-1 (Lee et al., 2015).

IF Analysis

Tissues from the leaves, stems, and roots of green onions were immediately fixed in 10% neutral buffered formalin. Following 48 h of fixation, the tissues were processed and embedded in paraffin. For IF analysis, tissue sections were deparaffinized and rehydrated, followed by washing in PBS containing 0.05% Tween 20 (PBS-T). Antigen retrieval was performed using Proteinase K (100 μ g/mL; Invitrogen) for 20 min at 37 °C. Non-specific binding was blocked by incubation with 1% normal goat serum (AK-5001; Vector Laboratories, Burlingame, CA) for 30 min. Subsequently, tissue sections were incubated overnight at 4 °C with rabbit anti-MNV-1 capsid protein 1 antiserum (MNV14-S; Alpha Diagnostic International, San Antonio, TX). After washing three times with PBS-T, the sections were incubated with Alexa Fluor 488-conjugated goat anti-rabbit IgG (A32731, Invitrogen) for 1 h at room temperature (20–25 °C). Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole.

Statistical Analysis

All data are expressed as the mean $\pm$ standard error, representing the results of three independent replicates and three independent experiments. One-way analysis of variance was performed to assess the decrease in MNV-1 titers over time and following water exchange, followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results

Short-term Internalization of MNV-1 in Green Onion Tissues

The temporal dynamics of MNV-1 internalization in green onion tissues (leaf, stem, and root) were assessed over a 7-h period following exposure to virus-contaminated hydroponic feed water.

Viral genome copies were quantified via RT-qPCR and expressed as $\log_{10}$ genome copies per gram of tissue (Fig. 1). At 0 hpi, no viral genomes were detected in any tissue type, confirming the absence of pre-existing contamination. Over time, a distinct tissue-dependent pattern of viral internalization emerged. The earliest and highest levels of viral internalization were observed in the roots.

Viral genome copies increased significantly from $2.34 \pm 0.43 \log_{10}$ copies/g at 1 hpi to $4.72 \pm 0.15 \log_{10}$ copies/g at 7 hpi ($p < 0.001$), indicating rapid MNV-1 uptake and accumulation in root tissues within hours of exposure.

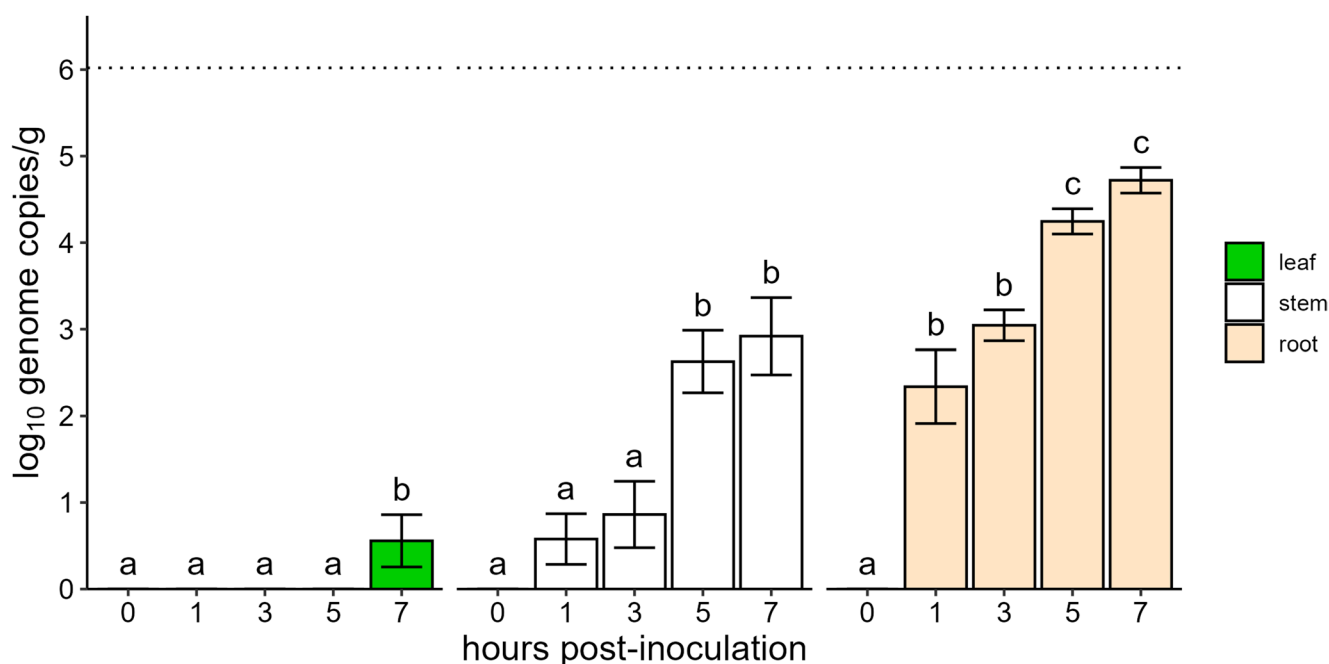


Fig. 1 Short-term distribution of MNV in hydroponically grown green onion. The horizontal dashed line indicates the initial inoculum level. Each experimental point represents the average of experiments performed in triplicate ($n=3$). Virus titer is expressed as log genome

copies/g $\pm$ standard error. Different lowercase letters denote statistically significant differences in viral titer over time within the same anatomical part of the green onion ($p < 0.05$)

Viral genomes were detected in stems starting at 1 hpi (0.58 ± 0.29 log₁₀ copies/g), with a progressive increase to 2.92 ± 0.45 log₁₀ copies/g by 7 hpi. The increase from 3 hpi onward was statistically significant ($p=0.005$), suggesting viral translocation from roots to stem tissues.

Viral genomes were exclusively detected in leaves at 7 hpi, yielding a lower level (0.56 ± 0.30 log₁₀ copies/g) than that in roots and stems ($p=0.043$). At 7 hpi, significant differences in viral genome copy numbers were observed among tissue types, with roots exhibiting the highest internalization. Compared with those in roots, viral loads in stems and leaves were significantly lower ($p=0.002$ and $p < 0.001$, respectively). These results demonstrate that MNV-1 can rapidly internalize into green onion roots and subsequently translocate to stems and leaves within hours.

Long-term Internalization of MNV-1 in Green Onion Tissues

The internalization of MNV-1 in green onion tissues and hydroponic water was monitored over a 21-day period post-inoculation (Fig. 2). Viral genome copies were quantified using RT-qPCR and expressed as log₁₀ genome copies per gram of tissue or per milliliter of water.

The viral load in the contaminated hydroponic water remained relatively stable throughout the 21-day period, commencing at 6.02 ± 0.16 log₁₀ copies/mL at 0 dpi and slightly decreasing to 5.57 ± 0.14 log₁₀ copies/mL by 21

dpi. Statistical analysis indicated no significant differences in viral genome quantities over time, suggesting sustained viral presence in the aquatic environment.

MNV-1 genome remained in root tissues throughout the 21-day period, with viral genome copies ranging from 3.47 ± 0.26 log₁₀ copies/g at 1 dpi to 4.19 ± 0.26 log₁₀ copies/g at 21 dpi. Although the increase was not statistically significant, the peak viral load at 21 dpi suggests potential viral accumulation in the roots over time.

Viral genomes were detected in stem tissues starting at 1 dpi (2.24 ± 0.15 log₁₀ copies/g) and remained through 21 dpi, peaking at 7 dpi (3.18 ± 0.20 log₁₀ copies/g). Viral levels remained significantly higher than those at 0 dpi, indicating a sustained viral presence in stems throughout the study period ($p < 0.05$).

In leaves, viral genomes were intermittently detected at low levels, with the highest concentration at 3 dpi (0.96 ± 0.37 log₁₀ copies/g). While some fluctuations occurred, viral loads in leaves were generally low and did not exhibit a consistent increasing or decreasing trend over time.

Overall, these results indicate that MNV-1 can persist in hydroponic water and internalize into green onion roots and stems for at least 21 days post-exposure. The roots serve as the primary reservoir for the virus, maintaining consistently high viral loads, while stems also sustain detectable viral levels. In contrast, leaves exhibit limited long-term viral

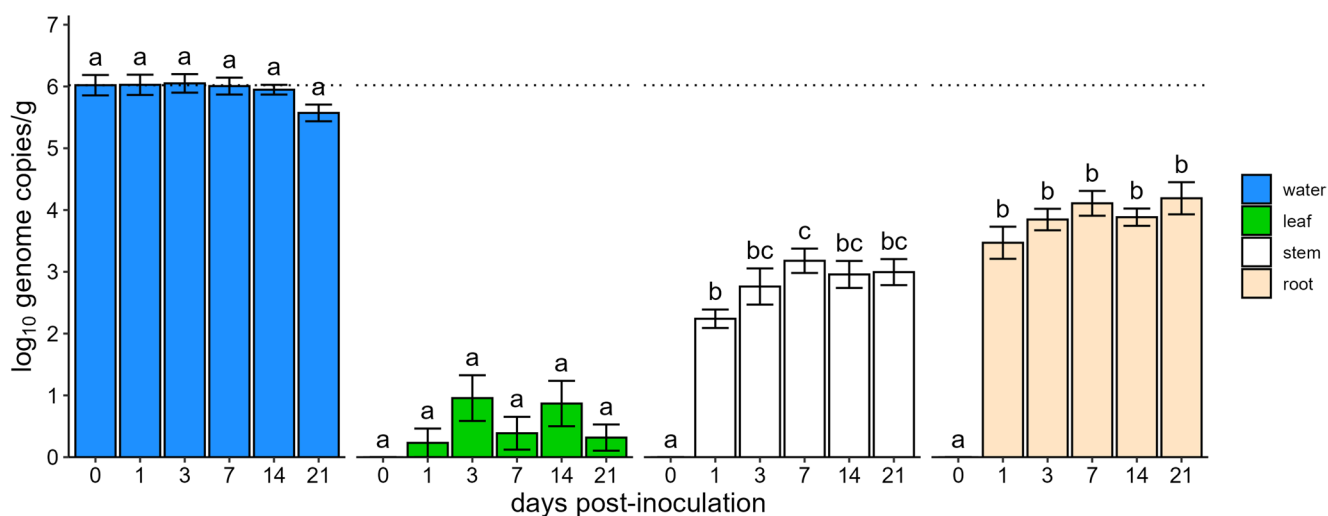


Fig. 2 MNV internalization in hydroponically grown green onion from days 1 to 21. The horizontal dashed line indicates the initial inoculum level. Each experimental point represents the average of experiments performed in triplicate ($n=3$). Virus titer is expressed as log genome

copies/g $\pm$ standard error. Different lowercase letters indicate statistically significant differences in viral titer over time within the same anatomical part of the green onion ($p < 0.05$)

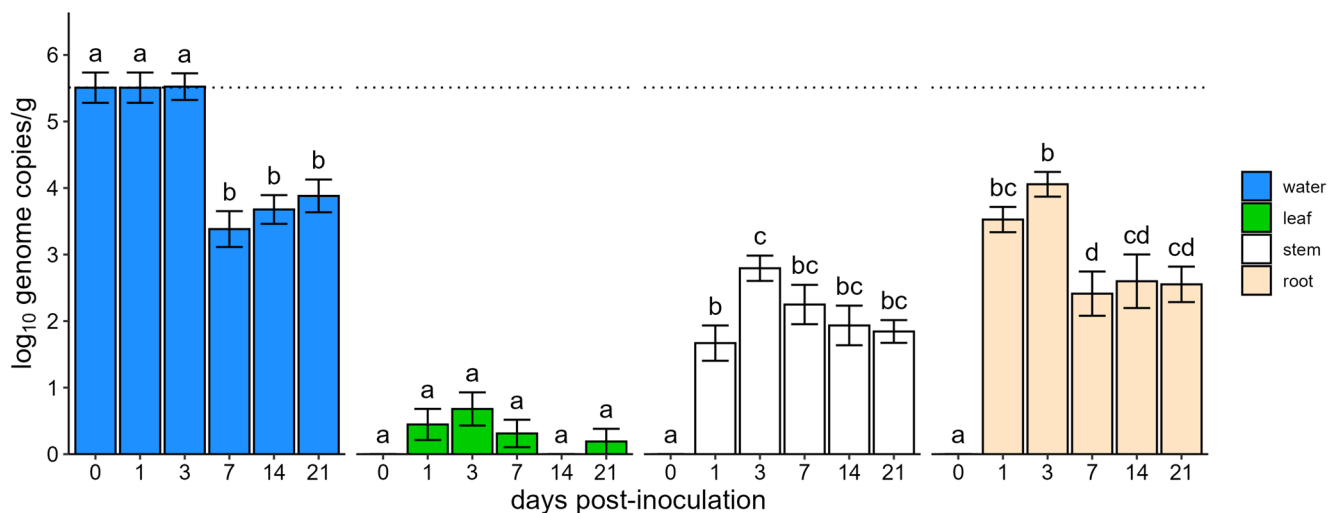


Fig. 3 Effect of water exchange on MNV internalization and persistence in hydroponically grown green onion. The arrows indicate the time points at which water exchange was performed. The horizontal dashed line indicates the initial inoculum level. Each experimental point represents the average of experiments performed in triplicate

($n=3$). Virus titer is expressed as log genome copies/g $\pm$ standard error. Different lowercase letters denote statistically significant differences in viral titer over time within the same anatomical part of the green onion ($p < 0.05$)

presence, suggesting minimal or transient viral internalization in this tissue.

Effect of Single Water Exchange on MNV Internalization

The influence of a single water exchange at 3 dpi on the internalization and persistence of MNV-1 in hydroponic water and green onion tissues was evaluated (Fig. 3). In the hydroponic water, high viral genome levels were maintained

from 0 to 3 dpi, with mean values approximating $5.5 \log_{10}$ genome copies/mL.

Following the water exchange at 3 dpi, viral genome copies sharply declined to $3.38 \pm 0.27 \log_{10}$ copies/mL by 7 dpi and subsequently remained relatively stable through 21 dpi ($3.88 \pm 0.25 \log_{10}$ copies/mL). Additionally, the slight increase in viral load up to 21 dpi likely emanated from the re-spreading of the virus from the green onions. Statistical analysis indicated significant reductions in viral loads in the water after 3 dpi ($p < 0.05$).

In leaf tissues, viral genomes were detected at low levels starting from 1 dpi ($0.45 \pm 0.23 \log_{10}$ copies/g), peaking at 3 dpi ($0.68 \pm 0.25 \log_{10}$ copies/g), and decreased slightly following the water exchange, becoming undetectable at 14 dpi. However, no significant differences in viral load occurred over time.

In stem tissue, viral genome copies increased from $1.67 \pm 0.27 \log_{10}$ copies/g at 1 dpi to a peak of $2.79 \pm 0.19 \log_{10}$ copies/g at 3 dpi ($p=0.01$). After the water exchange, viral loads gradually declined to $1.84 \pm 0.17 \log_{10}$ copies/g by 21 dpi.

Consistent with previous results, the highest viral genome copy number was observed in root tissue, with rapid accumulation reaching $4.06 \pm 0.19 \log_{10}$ copies/g at 3 dpi. This was followed by a marked decrease to $2.41 \pm 0.33 \log_{10}$ copies/g by 7 dpi, a count that subsequently remained relatively stable through 21 dpi.

Overall, the single water exchange at 3 dpi effectively reduced viral loads in the hydroponic water, accompanied by a gradual decline in viral genome copies within green onion tissues over time. However, viral genomes persisted in roots and stems up to 21 dpi, indicating MNV-1 internalization and retention within these tissues despite the water exchange.

Effect of Multiple Water Exchanges on MNV Internalization

The influence of repeated water changes on MNV-1 internalization and persistence was assessed by measuring viral genome copies in hydroponic water and green onion tissues before and after five cycles of water changes (Fig. 4). In

the hydroponic water, MNV-1 titers significantly decreased from $5.66 \pm 0.09 \log_{10}$ genome copies/mL before water changes to $2.21 \pm 0.18 \log_{10}$ copies/mL after the first cycle, further declining to below detection limits by the fourth and fifth cycles ($p<0.001$). This demonstrates effective viral clearance through repeated water changes.

Leaf tissues contained minimal viral genome copies throughout the experiment, with low levels sporadically detected (maximum: $0.23 \pm 0.23 \log_{10}$ copies/g at cycle 3) and mostly below detection limits, indicating limited viral internalization or retention.

In stem tissue, the viral load was $2.03 \pm 0.18 \log_{10}$ copies/g before the water exchange, and it slightly decreased after the first cycle to $1.82 \pm 0.24 \log_{10}$ copies/g ($p=0.436$). The viral genome was not detected from the second cycle onward, although considerably low residual levels (approximately 0.11 – $0.13 \log_{10}$ copies/g) were observed during the fourth and fifth cycles. This suggests that while the virus was effectively reduced in the stem through repeated water exchanges, it could be re-disseminated from residual virus in the roots.

Root tissues consistently harbored the highest viral genome copies, starting at $3.03 \pm 0.17 \log_{10}$ copies/g before the water change and maintaining relatively stable levels throughout the five cycles (2.40 – $2.49 \log_{10}$ copies/g). Although a gradual decline was observed over time, the virus persisted in roots despite multiple water exchanges.

Localization of MNV Internalized in Green Onions

IF analysis was conducted to compare the distribution and localization patterns of internalized MNV-1 in green onions

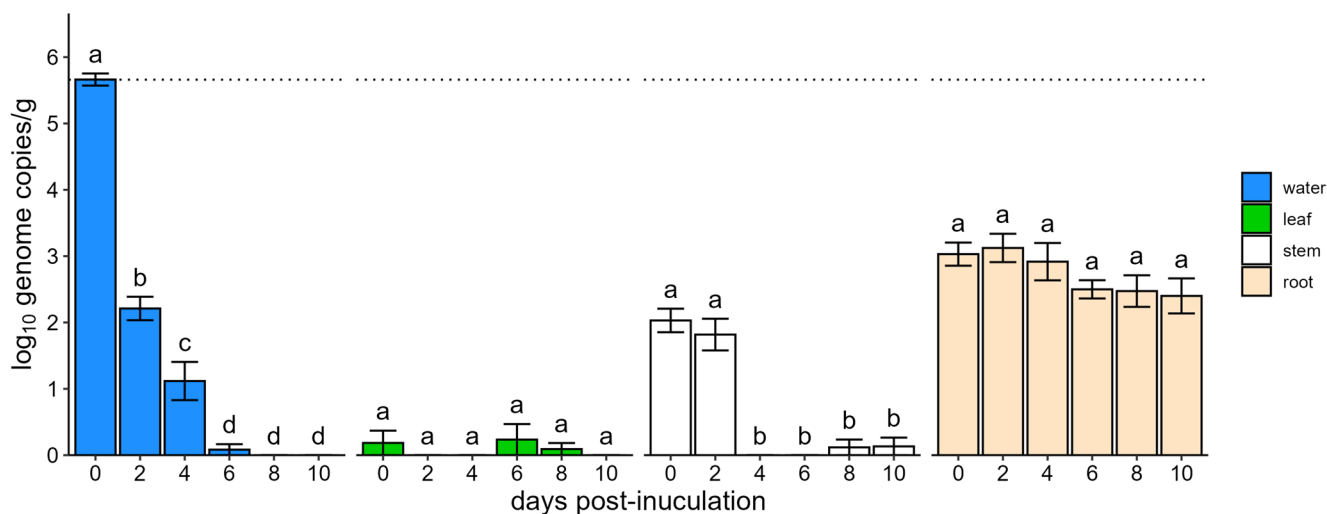
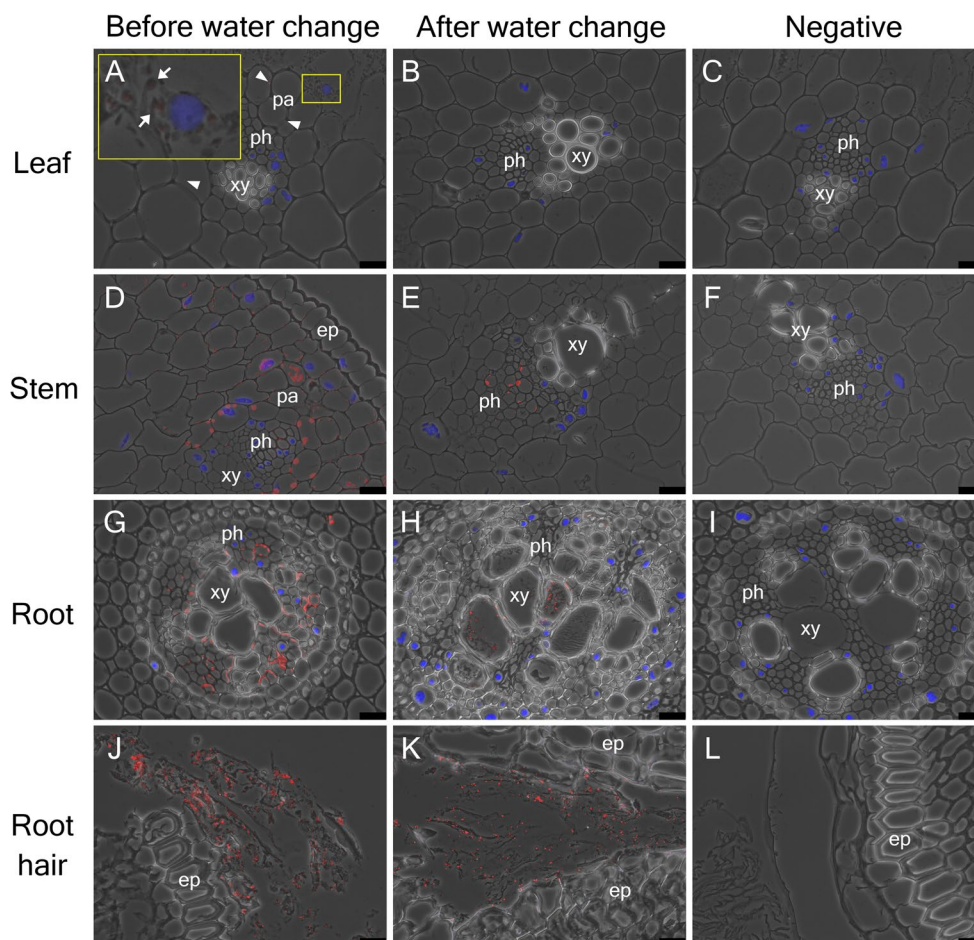


Fig. 4 Effect of multiple water exchange cycles on MNV internalization and persistence in hydroponically grown green onion. The feed water was completely exchanged with fresh, uncontaminated water at each indicated time point on the graph. The horizontal dashed line indicates the initial inoculum level. Each experimental point represents

the average of experiments performed in triplicate ($n=3$). Virus titer is expressed as $\log_{10}$ genome copies/g $\pm$ standard error. Different lowercase letters denote statistically significant differences in viral titer over time within the same anatomical part of the green onion ($p<0.05$)

Fig. 5 MNV localization in hydroponically grown green onions by plant part. Arrowheads indicate MNV localization, while the white arrow indicates MNV localized specifically to chloroplasts. Nuclei were counterstained with 4',6-diamidino-2-phenylindole. Scale bar = 25 μ m. Abbreviations: ph, phloem; xy, xylem; pa., parenchymal cell; ep, epidermis



before and after the water change (Fig. 5). In leaf tissues, which exhibited the lowest viral load, MNV-1 was localized to parenchyma cells in a few spots (arrowhead) and colocalized with chloroplasts, although the signal intensity remained weak (arrow). No MNV-1 antigen was detected after the water exchange (Fig. 5B).

In the stem, MNV-1 was localized over a wide area. Prior to washing, MNV antigens partially lined the cell walls of the phloem and were present in significant amounts within the cytoplasm of the parenchyma (Fig. 5D). After washing, MNV-1 antigens were significantly reduced and localized to some phloem and parenchyma cells (Fig. 5E).

In root tissues, where MNV-1 was most abundantly detected via RT-qPCR and persisted for an extended duration, numerous MNV-1 antigens were observed lining the cell walls of phloem and xylem vessels before the water change, as well as in the cytoplasm of parenchyma cells (Fig. 5G). After the water change, MNV-1 antigens were primarily observed within the metaxylem (Fig. 5H). A notable observation was the hairy portion of the root. Despite removing external contaminants using 1,000 ppm sodium hypochlorite during tissue harvest, extremely high levels of MNV-1 antigen were detected in the root hairs (Fig. 5J), and

they persisted even after the water change (Fig. 5K). No MNV-1 antigen was detected in any tissues of the negative control group (Figs. 5C, F, I, and L).

Discussion

The present study aimed to elucidate the potential for norovirus internalization in fresh produce via contaminated irrigation water, focusing specifically on the dynamics of MNV as a surrogate for human norovirus uptake and persistence in green onion tissues using a controlled hydroponic system. We found that MNV could reach and accumulate in the roots and stems within 1 hpi and transition from the roots to the leaves of green onions within 7 h. Although few studies have examined the degree of crop internalization by foodborne pathogens on an hourly basis, insights can be gained from the plant transport system. While time varies depending on the plant type and nutrients absorbed, it generally takes approximately 1 h per 100 cm for water absorbed through the roots to reach the leaves (Atanasov, 2021; Bodale et al., 2021). The green onions used in this study were approximately 50 cm tall, and although water

can reach the leaves in approximately 30 min, additional time is presumably necessary for MNV to accumulate to levels detectable by RT-qPCR. Internalization is known to occur when human enteric pathogens are taken up through the roots into intercellular spaces between plant cells and into the plant's vascular tissues, including the xylem and phloem (Hirneisen et al., 2012). Additionally, enteric pathogens are more easily internalized in crops when roots are damaged, and absorption is likely facilitated since some roots are cut during the distribution process of green onions (Erickson, 2012).

This study demonstrated that MNV persists stably within the leaves, stems, and roots of green onion plants for over 21 days. Consistent with these findings, previous research indicates that human norovirus and its surrogates, including MNV and Tulane virus, can be internalized within diverse leafy greens, such as romaine lettuce, kale, and mustard microgreens, remaining detectable for up to 30 days throughout the experiments (DiCaprio et al., 2012; Hirneisen et al., 2013; Wang et al., 2016; Wei et al., 2010; Yang et al., 2018). In contrast, while our findings revealed minimal MNV detection in the leaves, norovirus and its surrogates were detected at similar levels from roots to leaves in romaine lettuce, a short-growing crop (DiCaprio et al., 2012; Wei et al., 2010). Yang et al. (2018) studied norovirus internalization in three crops and found no internalization in radish (*Raphanus sativus*) at all, suggesting that crop type influences internalization. This difference in norovirus distribution between romaine lettuce and green onions is attributable to factors beyond plant length. In our study, internalized MNVs were detected solely by quantifying genome copies. However, when internalized viruses in crops were detected using PCR combined with RNase treatment to identify intact viruses, or when infectious viruses were assessed using plaque assays, the observed trends aligned with those based on genome copy numbers (DiCaprio et al., 2012; Hirneisen et al., 2013). This suggests that the internalized virus maintains high stability. Although plants possess defense mechanisms against pathogens, their immune response is unlikely activated against norovirus, which is an animal virus (Faulkner, 2016). This immunological blind spot may enable the virus to persist stably within plants for extended periods.

Hydroponic cultivation systems utilize recirculating water management strategies to maintain and optimize water quality throughout the growth cycle. In the present study, water exchange procedures were conducted either once or five times to simulate recirculation within the hydroponic system. Despite continuous water exchange, this study demonstrated that viruses internalized within plant tissues persisted and were not effectively eliminated through these interventions, thereby underscoring the critical importance

of implementing stringent preventive measures to inhibit viral contamination of irrigation water sources. Considering these findings, a series of targeted preventive measures can be recommended to growers to mitigate the risk of contamination. These measures include the stringent reinforcement of routine disinfection protocols specifically tailored for water recirculation and distribution systems, the enhancement of worker hygiene practices through comprehensive training and monitoring, and the prioritization of a holistic approach centered on proactive contamination prevention strategies (Saldinger et al., 2023).

IF analysis further demonstrated that MNV, following its absorption through the root system, subsequently migrated and accumulated predominantly within the parenchymal cells of aerial tissues, specifically concentrating in both the leaves and, notably, the stems of the plant. During the developmental stages of *Allium* species, essential nutrients, predominantly carbohydrates, accumulate in the stem region immediately beneath the leaves, which is the specific site for subsequent bulb formation (Gao et al., 2022; Kiani et al., 2022). This accumulation may be linked to the interaction between carbohydrates and norovirus. Norovirus recognizes histo-blood group antigens (HBGAs) as receptors, and its capsid protein interacts with oligosaccharide residues of the HBGA receptor (Tan et al., 2010). Furthermore, norovirus is capable of interacting with a diverse array of carbohydrate molecules, encompassing porcine gastric mucin, sialylated carbohydrates and human milk oligosaccharides, beyond the oligosaccharide structures associated with HBGA receptors. (Creutzmacher et al., 2021; Dancho et al., 2012; Weichert et al., 2016). Similarly, within plant systems, norovirus reportedly demonstrates binding affinity for specific carbohydrate components located in the cell walls of lettuce, including hemicellulose polysaccharides, which are integral structural constituents of the plant cell wall matrix (Esseili et al., 2012; Gao et al., 2016).

In this study, green onion roots constituted the plant part most prominently occupied by MNV. The roots sustained elevated viral titers over prolonged periods and retained these titers despite repeated water exchange processes. IF analysis demonstrated viral localization within the xylem and phloem vessels of the root. Moreover, MNV was notably detected on root hairs on the outer root surface, with viral particles persisting even after thorough washing with high-concentration bleach. The roots possess a rough and porous anatomical structure that plays a critical role in facilitating the efficient absorption of essential nutrients and water necessary for plant growth and development. The pore sizes within roots are approximately 30–40 nm in diameter, which closely correspond to the physical dimensions of norovirus particles, potentially influencing viral adherence and penetration (Sembada et al., 2024). Furthermore,

the combination of this roughened surface morphology, the presence of pores on crop roots, and root hairs significantly enhances the capacity for beneficial or harmful microbial attachment and colonization, thereby promoting complex interactions within the rhizospheric microenvironment (Galindo-Castañeda et al., 2024; Matthysse et al., 2001; Prieto et al., 2011). In previous research involving rotavirus, the degree of surface roughness exhibited by crop tissues demonstrated a positive correlation with the efficiency and extent of viral attachment to plant surfaces (Lu et al., 2015). Furthermore, interactions between HBGA-positive bacteria, particularly *Enterobacter*, and norovirus are well established (Yang et al., 2024). *Enterobacter* species are commonly found in soil and the root rhizosphere (Abiala et al., 2015). These interactions may arise from bacteria that are either internalized within or colonize the root tissues.

While this study provides novel insights into the internalization and persistence of MNV in green onions, several limitations should be addressed in future research. This study employed RT-qPCR to quantify viral genome copies in plant tissues. Although RT-qPCR is highly sensitive to detecting viral genomes, it cannot differentiate between intact infectious viral particles and non-infectious viral genome fragments. Ideally, cell culture-based assays would be used to confirm infectivity; however, we encountered significant technical barriers due to the inherent cytotoxicity of green onion components, such as allyl sulfide, toward the RAW 264.7 cell line. Consequently, we relied on IF analysis as a complementary tool to confirm the presence of viral antigens alongside genomic detection. Nevertheless, the long-term persistence observed in this study is based on viral RNA and protein markers, which may not fully correlate with the presence of infectious virions. Therefore, while our findings demonstrate the potential for internalization, they should be interpreted with caution regarding actual public health risks. Future research should focus on developing specialized purification protocols to neutralize plant-derived cytotoxicity while preserving viral infectivity. Additionally, research using non-surrogate human noroviruses with models such as intestinal organoids or zebrafish, where human noroviruses have demonstrated partial cultivability, should be considered to more comprehensively elucidate infectivity and risk (Sato et al., 2019; Tan et al., 2023). Although pre-disinfection protocol was applied to the green onions to minimize microbial interference, the absolute absence of bacteria was not verified. Given that certain commensal bacteria are known to enhance viral stability and uptake, the potential influence of residual microorganisms on the observed MNV-1 internalization cannot be entirely excluded and remains a limitation of this study.

In conclusion, our findings demonstrate that MNV rapidly internalizes into green onion roots within hours of

exposure and subsequently translocate to stems and leaves, with roots serving as the primary reservoir sustaining viral loads for at least 21 days post-inoculation. Notably, a single water exchange partially reduced the presence of the virus in plant tissues, whereas repeated water exchanges effectively removed the virus from leaves and stems; however, the virus persisted and was not eliminated from the roots. This study enhances the current understanding of norovirus transmission via pre-harvest contamination routes by providing detailed temporal and spatial insights into viral internalization and persistence within edible plant tissues.

Acknowledgements This work was supported by the National Research Foundation of Korea (NRF2018R1A6A1A03025159) and Chung-Ang University Research Grants in 2025.

Author Contributions Data curation, Formal analysis, Visualization, Writing – original draft: Soontag Jung; Experimentation, Data curation, Formal analysis, Writing – editing: Hansaem Shin; Data curation: Daseul Yeo, Hyojin Kwon, and Day Jung; Funding acquisition, Supervision, Writing – review and editing: Changsun Choi All authors have read and approved the final manuscript.

Funding Open Access funding enabled and organized by Chung-Ang University

Data Availability All data underlying the results are available in the article, and no additional source data are required.

Declarations

Competing Interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

- Abiala, M. A., & Odebo, A. C. (2015). Rhizospheric *Enterobacter* enhanced maize seedling health and growth. *Biocontrol Science and Technology*, 25(4), 359–372. <https://doi.org/10.1080/09583157.2015.1008838>
- Alegbeleye, O., & Sant'Ana, A. S. (2023). Microbiological quality of irrigation water for cultivation of fruits and vegetables: An overview of available guidelines, water testing strategies and some

- factors that influence compliance. *Environmental Research*, 220, Article 114771. <https://doi.org/10.1016/j.envres.2022.114771>
- Atanasov, S. (2021). Methodology for irrigation water uptake time estimation based on RGB colorimetric measurements of leaves (A visual-graphical observation). In *IOP Conference Series: Materials Science and Engineering* (Vol. 1031, p. 012016). IOP Publishing. <https://doi.org/10.1088/1757-899X/1031/1/012016>
- Bodale, I., Mihalache, G., Achiței, V., Teliban, G.-C., Cazacu, A., & Stoleru, V. (2021). Evaluation of the nutrients uptake by tomato plants in different phenological stages using an electrical conductivity technique. *Agriculture*, 11(4), Article 292. <https://doi.org/10.3390/agriculture11040292>
- Brentlinger, D. (2005). New trends in hydroponic crop production in the US. In *International Conference and Exhibition on Soilless Culture: ICESC 2005* (pp. 31–33).
- Carlson, K. B., Dilley, A., O'Grady, T., Johnson, J. A., Lopman, B., & Viscidi, E. (2024). A narrative review of norovirus epidemiology, biology, and challenges to vaccine development. *NPJ Vaccines*, 9(1), Article 94. <https://doi.org/10.1038/s41541-024-00477-9>
- Creutzmacher, R., Maass, T., Ogrissek, P., Wallmann, G., Feldmann, C., Peters, H., Lingemann, M., Taube, S., Peters, T., & Mallagaray, A. (2021). NMR experiments shed new light on glycan recognition by human and murine norovirus capsid proteins. *Viruses*, 13(3), Article 416. <https://doi.org/10.3390/v13030416>
- Dancho, B. A., Chen, H., & Kingsley, D. H. (2012). Discrimination between infectious and non-infectious human norovirus using porcine gastric mucin. *International Journal of Food Microbiology*, 155(3), 222–226. <https://doi.org/10.1016/j.ijfoodmicro.2012.03.024>
- DiCaprio, E., Ma, Y., Purgianto, A., Hughes, J., & Li, J. (2012). Internalization and dissemination of human Norovirus and animal caliciviruses in hydroponically grown Romaine lettuce. *Applied and Environmental Microbiology*, 78(17), 6143–6152. <https://doi.org/10.1128/AEM.01074-12>
- Ekundayo, T. C., Igere, B. E., Oluwafemi, Y. D., Iwu, C. D., & Olaniyi, O. O. (2021). Human norovirus contamination in water sources: A systematic review and meta-analysis. *Environmental Pollution*, 291, Article 118164. <https://doi.org/10.1016/j.envpol.2021.118164>
- Erickson, M. C. (2012). Internalization of fresh produce by foodborne pathogens. *Annual Review of Food Science and Technology*, 3(1), 283–310. <https://doi.org/10.1146/annurev-food-022811-101002>
- Esseili, M. A., Wang, Q., & Saif, L. J. (2012). Binding of human GII.4 norovirus virus-like particles to carbohydrates of romaine lettuce leaf cell wall materials. *Applied and Environmental Microbiology*, 78(3), 786–794. <https://doi.org/10.1128/AEM.06967-11>
- Faulkner, C. (2016). Defense mechanisms in plants. In M. J. H. Ratcliffe (Ed.), *Encyclopedia of Immunobiology* (pp. 389–396). Academic Press. <https://doi.org/10.1016/B978-0-12-374279-9.01084-8>
- Galindo-Castañeda, T., Hartmann, M., & Lynch, J. P. (2024). Location: Root architecture structures rhizosphere microbial associations. *Journal of Experimental Botany*, 75(2), 594–604. <https://doi.org/10.1093/jxb/erac004>
- Gao, X., Esseili, M. A., Lu, Z., Saif, L. J., & Wang, Q. (2016). Recognition of histo-blood group antigen-like carbohydrates in lettuce by human GII.4 norovirus. *Applied and Environmental Microbiology*, 82(10), 2966–2974. <https://doi.org/10.1128/AEM.00239-16>
- Gao, S., Kong, Y., Lv, Y., Cao, B., Chen, Z., & Xu, K. (2022). Effect of different LED light quality combinations on the content of vitamin C, soluble sugar, organic acids, amino acids, antioxidant capacity, and mineral elements in green onion (*Allium fistulosum* L.). *Food Research International*, 156, Article 111329. <https://doi.org/10.1016/j.foodres.2022.111329>
- Hall, A. J., Vinjé, J., Lopman, B., Park, G. W., Yen, C., Gregoricus, N., & Parashar, U. (2011). Updated norovirus outbreak management and disease prevention guidelines. *MMWR Recommendations and Reports*, 60(RR-3), 1–18.
- Hall, A. J., Eisenbart, V. G., Etingüe, A. L., Gould, L. H., Lopman, B. A., & Parashar, U. D. (2012). Epidemiology of foodborne norovirus outbreaks, United States, 2001–2008. *Emerging Infectious Diseases*, 18(10), 1566–1573. <https://doi.org/10.3201/eid1810.120392>
- Hardstaff, J. L., Clough, H. E., Lutje, V., McIntyre, K. M., Harris, J. P., Garner, P., & O'Brien, S. J. (2018). Foodborne and food-handler norovirus outbreaks: A systematic review. *Foodborne Pathogens and Disease*, 15(10), 589–597. <https://doi.org/10.1089/fpd.2018.2445>
- Hirneisen, K. A., & Kniel, K. E. (2013). Comparative uptake of enteric viruses into spinach and green onions. *Food and Environmental Virology*, 5(1), 24–34. <https://doi.org/10.1007/s12560-012-9116-5>
- Hirneisen, K. A., Sharma, M., & Kniel, K. E. (2012). Human enteric pathogen internalization by root uptake into food crops. *Foodborne Pathogens and Disease*, 9(5), 396–405. <https://doi.org/10.1089/fpd.2011.0969>
- Kiani, Z., Mashayekhi, K., Mousavizadeh, S. J., & Nezhad, K. Z. (2022). Changes in carbohydrate and phytochemical content in the bulbs and leaves of ten onion accessions between two developmental stages. *Italus Hortus*, 29, 47–55.
- Lee, M., Seo, D. J., Seo, J., Oh, H., Jeon, S. B., Ha, S.-D., Myoung, J., Choi, I.-S., & Choi, C. (2015). Detection of viable murine norovirus using the plaque assay and propidium monoazide-combined real-time reverse transcription-polymerase chain reaction. *Journal of Virological Methods*, 221, 57–61. <https://doi.org/10.1016/j.jviromet.2015.04.008>
- López-Gálvez, F., Truchado, P., Sánchez, G., Aznar, R., Gil, M. I., & Allende, A. (2016). Occurrence of enteric viruses in reclaimed and surface irrigation water: Relationship with microbiological and physicochemical indicators. *Journal of Applied Microbiology*, 121(4), 1180–1188. <https://doi.org/10.1111/jam.13274>
- Lu, L., Ku, K.-M., Palma-Salgado, S. P., Storm, A. P., Feng, H., Juvik, J. A., & Nguyen, T. H. (2015). Influence of epicuticular physicochemical properties on porcine rotavirus adsorption to 24 leafy green vegetables and tomatoes. *PLoS One*, 10(7), Article e0132841. <https://doi.org/10.1371/journal.pone.0132841>
- Matthysse, A. G., & McMahan, S. (2001). The effect of the *Agrobacterium tumefaciens* attR mutation on attachment and root colonization differs between legumes and other dicots. *Applied and Environmental Microbiology*, 67(3), 1070–1075. <https://doi.org/10.1128/AEM.67.3.1070-1075.2001>
- Mielcarek, A., Kłobukowska, K., Rodziejewicz, J., Janczukowicz, W., & Bryszewski, K. L. (2024). Water nutrient management in soilless plant cultivation versus sustainability. *Sustainability*, 16(1), Article 152. <https://doi.org/10.3390/su160100152>
- Potera, C. (2013). Pesticide spraying may spread Norovirus. *Environmental Health Perspectives*, 121(5), a148–a148. <https://doi.org/10.1289/ehp.121-a148>
- Prieto, P., Schilirò, E., Maldonado-González, M. M., Valderrama, R., Barroso-Albarracín, J. B., & Mercado-Blanco, J. (2011). Root hairs play a key role in the endophytic colonization of olive roots by *Pseudomonas* spp. with biocontrol activity. *Microbial Ecology*, 62(2), 435–445. <https://doi.org/10.1007/s00248-011-9920-5>
- Robilotti, E., Deresinski, S., & Pinsky, B. A. (2015). Norovirus. *Clinical Microbiology Reviews*, 28(1), 134–164. <https://doi.org/10.1128/CMR.00075-14>
- Sato, S., Hisaie, K., Kurokawa, S., Suzuki, A., Sakon, N., Uchida, Y., Yuki, Y., & Kiyono, H. (2019). Human Norovirus propagation in human induced pluripotent stem cell-derived intestinal epithelial

- cells. *Cellular and Molecular Gastroenterology and Hepatology*, 7(3), 686–688. <https://doi.org/10.1016/j.jcmgh.2019.01.004>
- Sela Saldinger, S., Rodov, V., Kenigsbuch, D., & Bar-Tal, A. (2023). Hydroponic agriculture and microbial safety of vegetables: Promises, challenges, and solutions. *Horticulturae*, 9(1), Article 51. <https://doi.org/10.3390/horticulturae9010051>
- Sembada, A. A., & Lenggono, I. W. (2024). Transport of nanoparticles into plants and their detection methods. *Nanomaterials*, 14(2), Article 131. <https://doi.org/10.3390/nano14020131>
- Shah, M. P., & Hall, A. J. (2017). Global disease burden of foodborne illnesses associated with norovirus. *The Norovirus* (pp. 3–19). Elsevier. <https://doi.org/10.1016/B978-0-12-804645-0.00001-5>
- Shah, M. P., & Hall, A. J. (2018). Norovirus illnesses in children and adolescents. *Infectious Disease Clinics*, 32(1), 103–118. <https://doi.org/10.1016/j.idc.2017.10.001>
- Tan, M., & Jiang, X. (2010). Norovirus gastroenteritis, carbohydrate receptors, and animal models. *PLoS Pathogens*, 6(8), Article e1000983. <https://doi.org/10.1371/journal.ppat.1000983>
- Tan, M. T. H., Gong, Z., & Li, D. (2023). Use of zebrafish embryos to reproduce human Norovirus and to evaluate human Norovirus infectivity decay after UV treatment. *Applied and Environmental Microbiology*, 89(4), e00115–00123. <https://doi.org/10.1128/aem.00115-23>
- Thapa, U., Hansda, N. N., Kundu, S., Giri, A., Tamang, D., & Rahaman, A. O. (2024). Advancements in hydroponic systems: A comprehensive review. *Archives of Current Research International*, 24(11), 317–328. <https://doi.org/10.9734/acri/2024/v24i11170>
- Tsai, H., Yune, P., & Rao, M. (2022). Norovirus disease among older adults. *Therapeutic Advances in Infectious Disease*, 9, Article 20499361221136760. <https://doi.org/10.1177/20499361221136760>
- Wang, Q., & Kniel, K. E. (2016). Survival and transfer of murine Norovirus within a hydroponic system during kale and mustard microgreen harvesting. *Applied and Environmental Microbiology*, 82(2), 705–713. <https://doi.org/10.1128/AEM.02557-15>
- Wei, J., Jin, Y., Sims, T., & Kniel, K. E. (2010). Manure- and biosolids-resident murine Norovirus 1 attachment to and internalization by Romaine lettuce. *Applied and Environmental Microbiology*, 76(2), 578–583. <https://doi.org/10.1128/AEM.01973-09>
- Weichert, S., Koromyslova, A., Singh, B. K., Hansman, S., Jennewein, S., Schroten, H., & Hansman, G. S. (2016). Structural basis for Norovirus inhibition by human milk oligosaccharides. *Journal of Virology*, 90(9), 4843–4848. <https://doi.org/10.1128/JVI.00040-16>
- Yang, Z., Chambers, H., DiCaprio, E., Gao, G., & Li, J. (2018). Internalization and dissemination of human Norovirus and Tulane virus in fresh produce is plant dependent. *Food Microbiology*, 69, 25–32. <https://doi.org/10.1016/j.fm.2017.05.003>
- Yang, Y., An, R., Lyu, C., & Wang, D. (2024). Interactions between human norovirus and intestinal microbiota/microbes: A scoping review. *Food Microbiology*, 119, Article 104456. <https://doi.org/10.1016/j.fm.2023.104456>
- Zhang, X., Chen, C., Du, Y., Yan, D., Jiang, D., Liu, X., Yang, M., Ding, C., Lan, L., Hecht, R., & Yang, S. (2022). Global burden and trends of norovirus-associated diseases from 1990 to 2019: An observational trend study. *Frontiers in Public Health*, 10, Article 905172. <https://doi.org/10.3389/fpubh.2022.905172>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.