

Molecular Evidences of Enteric Virus Contamination in Bangladeshi Foods

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Abstract

Enteric viruses, particularly rotavirus A (RV), norovirus GII (NV), and enteroviruses (EV), pose significant health challenges in Bangladesh, yet data on viral contamination of foods in Bangladesh remain scarce. This study investigated the presence of these fecal-oral transmitted viruses in street-vended ready-to-eat (RTE) foods and related matrices collected from high-risk locations in Dhaka using a simple, cost-effective detection approach. Four viral RNA extraction methods, TRIzol-based, PEG + NaCl precipitation, Nanotrap with Microbiome A, and direct extraction, were compared and viral RNA was detected by conventional nested RT-PCR. Bacterial indicators, including total viable counts (TVC), total coliform counts (TCC), and fecal coliform counts (FCC), were also quantified to examine their association with viral contamination. The direct washing method showed the best overall performance, combining simplicity with detection limits of approximately 3,000 and 6,800 copies/g for RV and NV, respectively. Among 205 samples, RV was detected in 6 (2.9%), with sequencing confirming two G3 genotype strains. Targeted NV and EV amplicons were detected in 7 (3.4%) and 18 (8.8%) samples, respectively. Of the 117 RTE foods, 50.4% were unsatisfactory for all three bacterial indicators, whereas only 7.7% met satisfactory criteria. No significant association was observed between bacterial indicator levels and viral detection. This study provides the first molecular evidence of enteric viral contamination in street-vended foods and related matrices in Bangladesh, providing a baseline data on foodborne viral risks and emphasizing the need for virus-specific surveillance rather than reliance on bacterial indicators alone.

Introduction

Enteric viruses are a major cause of foodborne disease and are responsible for more than half of all foodborne illnesses worldwide (Trzaskowska, Hunt, & Rodriguez-Lazaro, 2022; WHO/FAO, 2023). Although enteric viruses do not replicate in food, unlike bacteria, they remain a serious foodborne health threat because of their low infectious dose, prolonged environmental stability, and resistance to mild heat, chlorine, and pH variations, making them particularly difficult to control within food systems (Bosch et al., 2018).

Among enteric viruses, norovirus (NV), primarily genogroup II, remains the most common cause of foodborne viral outbreaks, accounting for an estimated 125 million cases and 35,000 deaths annually (WHO/FAO, 2024), while rotavirus (RV), mainly group A, remains a leading cause of severe diarrhea in young children, causing approximately 128,500 deaths annually despite widespread rotavirus vaccination efforts (Li, Sun, Fu, You, & Hartwig, 2024). In Bangladesh, 23–64% of hospitalized children with acute gastroenteritis (AGE) exhibit RV (Sharif et al., 2023), while NV is seen up to 25–28% of cases (Satter et al., 2023). In addition, Hand, foot, and mouth disease (HFMD), which has emerged in Bangladesh in recent years, is one of the many disorders linked to enteroviruses (EV), another enteric virus transmitted through fecal-oral route (S. A. Hoque et al., 2023). Thus, despite the documented environmental and clinical presence of these viruses, their direct detection in foods in Bangladesh has remained largely unexplored, representing a critical knowledge gap in foodborne viral surveillance (Hoque et al., 2019). In fact, the detection of enteric viruses in food remains limited even in developed countries because of several technical challenges, including the lack of efficient virus recovery methods capable of detecting low viral loads from complex food matrices and the absence of reliable viral enrichment technique (G. E. Greening, 2013).

Although ISO 15216:2013, revised as ISO 15216-1:2017 and ISO 15216-2:2019, provides standardized real-time RT-PCR methods for detecting NV and hepatitis A virus (HAV) in selected food matrices including (i) soft fruits, (ii) leafy, stem and bulb vegetables, (iii) bivalve molluscs, (iv) bottled water, and (v) food preparation surfaces, these protocols do not cover spicy multicomponent street foods commonly consumed in Bangladesh and require specialized reagents and real-time PCR platforms that are often unavailable in resource-limited laboratories (Lowther et al., 2019). Therefore, further investigations are ongoing to enhance the sensitivity of the detection method and expand its applicability to various food matrices (Saito, Toho, Tanaka, & Noda, 2015). For instance, Hennechart-Collette et al. (2019) has used a TRIzol-based method for recovering NV and HAV from complex foods such as dressed vegetables. The polyethylene glycol (PEG) precipitation method, recommended by ISO 15216-1:2017 for HAV and NV detection in shellfish, soft fruits, and leafy greens, has been widely applied in various food matrices including shellfish (Kingsley & Richards, 2001), oyster (Lewis & Metcalf, 1988), strawberries, raspberries, and vegetables (Butot, Putallaz, & Sanchez, 2007), as well as sewage water (S. A. Hoque, T. Kotaki, et al., 2023; S. A. Hoque, H. Saito, et al., 2023; Hoque et al., 2019). Nanotrap® Microbiome A Particles (Ceres Nanosciences) are magnetic and affinity-based hydrogel particles designed to capture a broad range of microbes including viruses, though their application for NV or RV detection from food matrices were hardly documented.

Since viral contamination in food has not yet been reported in Bangladesh, this study aimed to investigate above all three virus extraction methods comparing with a simple and cost-effective direct washing method to detect three major AGE viruses, RV, NV, and EV in street-vended ready-to-eat (RTE) foods collected from high-risk locations, with a focus on developing a convenient approach suitable for resource-limited laboratories. In addition, bacterial indicators were also examined in these samples to explore their potential association with enteric viral contamination.

Materials and Methods

Sample Collection

A total of 205 samples including 117 RTE foods, 34 vegetable wash water, 25 towel wash water, 19 whole fruits, and 10 leafy vegetables and herbs were collected between November 2024 and October 2025 from various locations across Dhaka city under a purposive sampling method that employed to select high-risk samples from crowded, less hygienic locations (e.g., busy street markets, bus terminals, hospital gates, etc.). Samples (approximately 100 g each) were purchased, placed in individual clean polyethylene bags, and transported immediately to the laboratory for further processing. Vegetable wash water, which vendors frequently use to wash or spray vegetables to maintain freshness, was collected in clean water bottles. Vendors' towels, commonly used to wipe hands or food-contact surfaces during food preparation and sale, were collected in clean polyethylene bags as a proxy for handler-mediated viral contamination. Bacterial analyses were initiated within a few hours of collection, whereas samples intended for viral detection were stored at -80°C until processing.

Spiking viruses and process control

The RV and NV strains used for spiking experiments were obtained from stool samples provided by Professor Dr. Shuvra Kanti Dey, Department of Microbiology, Jahangirnagar University, Dhaka. The sample collection and use were approved by the Biosafety, Biosecurity & Ethical Committee of Jahangirnagar University (Ref: BBEC, JU/M 2020/(10)3). The EV strain was isolated from vesicle fluid of patients with hand, foot, and mouth disease (HFMD) under approval from the Ethical Review Committee of Pabna Medical College and General Hospital, Bangladesh (Memo No. 1577, August 26, 2017) (S. A. Hoque et al., 2023). A measles-rubella (MR) vaccine obtained from the National Immunization Program Center, Dhaka, was included as a process control to generate a detectable PCR amplicon.

Sample Processing for viral investigation

Frozen RTE foods were thawed, briefly microwave-defrosted, and approximately 20 g was spiked with 30 μ L of MR vaccine as a process control before virus detection. Leafy vegetables/herbs (25 g) and whole fruits (2 apples or guavas, 100 g grapes, or 4–5 water chestnuts) were placed in clean polyethylene bags, spiked with 30 μ L of MR vaccine, and washed with 5 mL or 2 mL of autoclaved distilled water (DW), respectively. The resulting wash water was collected and processed for virus detection.

Vendor towels were soaked, agitated, and squeezed in 200 mL of sterile distilled water. Fifty mL of the rinse water or Vegetable wash water samples were processed by PEG-NaCl precipitation method as described previously for sewage water (S. A. Hoque, T. Kotaki, et al., 2023; S. A. Hoque, H. Saito, et al., 2023; Hoque et al., 2019). In brief, 50 mL of vegetable wash water was centrifuged at 3,000 $\times$ g for 3 min, and the supernatant was spiked with 30 μ L of MR vaccine, supplemented with PEG 6000 (4 g) and NaCl (1.15 g), and stirred for 4 h at room temperature. After centrifugation at 10,000 $\times$ g for 30 min, the pellet was re-suspended in 500 μ L of sterile distilled water, and 200 μ L aliquots were stored at -80°C until RNA extraction.

Different Virus Extraction Methods

To identify a cost-effective, simple, rapid, convenient, and efficient method for enteric virus recovery prior to RNA extraction, we evaluated four methods. Each method was tested by spiking 20–30 g of food with 100 μ L of RV and 200 μ L of NV stool suspensions, followed by a 15-min incubation at RT to allow viral adsorption, mimicking natural contamination. Recovery efficiency was assessed by conventional RT-PCR. The methods were as follows:

A. TRIzol Method

The TRIzol extraction method was adapted with minor modifications as previously described (G. Greening, Hewitt, Hasell, & Cook, 2016; Rutjes, Lodder-Verschoor, van der Poel, van Duijnhoven, & de Roda Husman, 2006). In brief, 30 g of spiked food were mixed with 10 mL TRIzol reagent (SRL, India), homogenized for 15 min, and centrifuged at 12,000 $\times$ g for 1 min. The supernatant was mixed with chloroform (0.2 mL per 1 mL TRIzol), incubated for 3 min at room temperature (RT), and centrifuged at 12,000 $\times$ g for 15 min. The aqueous phase was mixed with an equal volume of 70% ethanol, and RNA was purified using the SV Total RNA Isolation System (Promega, WI).

B. Polyethylene Glycol (PEG)-NaCl Precipitation Method

Thirty grams of spiked food were mixed with 40 mL alkaline elution buffer (0.1 M Tris-HCl, 0.05 M glycine, 1% beef extract, pH 9.5) (Butot et al., 2007), stirred for 25 min, and centrifuged at 10,000 ×g for 30 min. Fifty milliliters of the supernatant were treated with pectinase (42 µL), PEG 6000 (4 g), and NaCl (1.15 g), stirred for 4 h, and centrifuged again (Dubois et al., 2002; S. A. Hoque, T. Kotaki, et al., 2023). The pellet was re-suspended in 2 mL TRIzol, followed by chloroform extraction as described above.

C. Nanotrap® Microbiome A Particles Method

Nanotrap® Microbiome A Particles were used according to the manufacturer's instructions (Ceres, VA) and a previously described protocol, originally developed for concentrating viruses from wastewater (Das, Gutkoska, Tadassa, & Jia, 2024; Shaffer, North, & Bibby, 2025). In brief, 30 g of spiked food were suspended in 15 mL PBS, centrifuged at 3,500 ×g for 10 min, and 10 mL of the supernatant was incubated with 150 µL Nanotrap® Microbiome A Particles for 10. After magnetic separation and washing, particles were lysed using RNA Lysis and Dilution Buffers of SV Total RNA Isolation System (Promega, WI).

D. Direct Washing Method

Twenty grams of spiked food were shaken with 1–5 mL PBS (depending on sample dryness) for 10 min and centrifuged at 3,500 ×g for 10 min. A 500 µL aliquot of the supernatant was directly used for RNA extraction with the SV Total RNA Isolation System (Promega, WI). This minimal-handling procedure (low-volume neutral buffer wash followed by direct RNA extraction) was adapted from earlier protocols successfully applied for virus detection in foods (Perrin, Loutreul, Boudaud, Bertrand, & Gantzer, 2015; Rutjes et al., 2006; Stals, Baert, De Keuckelaere, Van Coillie, & Uyttendaele, 2011).

- Molecular Analysis of Viruses

Total RNA was extracted using the silica membrane column-based SV Total RNA Isolation System (Promega, WI) with minor modifications to the manufacturer's instructions. Briefly, 350 µL of supernatant obtained either directly from the food wash or after PEG-NaCl concentration was mixed with 200 µL of RNA Lysis Buffer and 400 µL of RNA Dilution Buffer and then processed according to the manufacturer's protocol. RNA was finally eluted in 45 µL of nuclease-free water.

cDNA was synthesized using the GoScript Reverse Transcription System (Promega, WI) with random hexamer primers (Hoque et al., 2019). Partial regions of the RV VP7 gene (primer pair: sBeg9–VP7-1'), the NV capsid gene (first-round PCR: COG2F-G2SKR; nested PCR: G2SKF-G2SKR), and the EV 5' UTR (primer pair: F1-R1) were amplified by monoplex PCR as previously described (Thongprachum et al., 2018). Measles virus was detected using Sar2F and Sar2R primers, yielding a 229-bp amplicon (Mosquera Mdel, de Ory, Moreno, & Echevarria, 2002). PCR amplicons were subjected to Sanger sequencing, genotypes were determined by sequence analysis, and phylogenetic trees were constructed using the Neighbor-Joining method with 1,000 bootstrap replicates in MEGA7. These nucleotide sequences were deposited in the GenBank database for the accession number.

Bacterial Analysis

Enumeration of indicator bacteria was performed according to standard methods (APHA, 2015; FDA-BAM, 2020). Briefly, 10 g of RTE food or 1 mL of wash water was homogenized in 100 mL sterile distilled water, serially diluted, and 0.1 mL aliquots were spread onto Nutrient Agar (NA), MacConkey Agar (MC), and membrane Fecal Coliform Agar (mFC) (HiMedia, India). NA and MC plates were incubated at 37°C, and mFC plates at 44-44.5°C for 24–48 h before colony enumeration.

Result

Selection of Virus recovery method

Using a spike-in experiment with cooked chickpeas, four virus extraction methods were compared: (A) TRIzol-based extraction, (B) PEG-NaCl precipitation, (C) Nanotrap® Microbiome A, and (D) the direct washing method.

RV was detected by all four methods after conventional RT-PCR, whereas NV and the process control (measles virus, MV) were detected only by the direct washing method (Fig. 1A), indicating its superior sensitivity. RV was detected across all methods, likely due to its relatively higher viral load. Compared with the other methods, the direct washing method was simpler, faster, and more cost-effective. In contrast, the Nanotrap, PEG-NaCl, and TRIzol methods were more expensive, labor-intensive, technically complex, and showed lower recovery efficiency. Therefore, based on sensitivity, processing time, cost, and feasibility for resource-limited settings, the direct washing method was selected for subsequent analysis of field-collected food samples.

The detection limit for RV using the direct washing method was approximately 3,000 copies/g (Fig. 1B), with no improvement after a second round of PCR (data not shown). In contrast, the detection limit for NV was approximately 6,800 copies/g and was achieved only by nested PCR (Fig. 1B).

Testing Different Food Items

Several popular street-vended RTE foods commonly consumed without further cooking were selected for evaluation. Each food item was spiked with 100 µL of RV and tested using the direct washing method. Target amplicons were detected only in cooked chickpeas, white peas, spicy puffed rice, and coriander leaves (Fig. 1C), whereas fruit mix, pickles, cut fruits/vegetables, pizza, and salads remained negative. These negative samples were further tested using TRIzol, PEG-NaCl, and Nanotrap® Microbiome A methods, but no improvement in virus detection was observed. Cut fruits and pickles interfered with RNA extraction by clogging the silica spin columns, while pizza and puffed rice absorbed the PBS during the initial virus recovery step, necessitating the addition of extra PBS and thereby diluting the virus suspension, which likely reduced viral RNA recovery.

Detection of enteric viruses in foods

Among 205 food and environmental samples, target amplicons for RV, NV, and EV were detected in 6, 10, and 22 samples, respectively. Samples yielding clear target bands were subjected to Sanger sequencing. Two RV sequences were successfully confirmed, whereas no NV or EV sequences were identified (Table 1). Sequencing failures were likely due to low viral concentrations. Some NV and EV amplicons yielded readable sequences but either showed no significant similarity to viral sequences or matched bacterial sequences and were therefore excluded from prevalence estimation. Consequently, RV was identified in 6 (2.9%) samples, including 2 molecularly confirmed and 4 suspected positives. In contrast, 7 (3.4%) NV and 18 (8.8%) EV samples remained suspected, with no confirmed NV or EV contamination.

Table 1
Samples yielding positive bands for RV, NV, and/or EV and their bacterial indicator counts.

ID	Type	Date	Location	RV	NV	EV	Sequence	TVC*	TCC	FCC
F1	Ghughni	13.11.24	Gulistan			+		7.1	5.9	5.4
F3	Ghughni	13.11.24	Gulistan			+		6.8	5.4	3.3
F8	Ghughni	24.11.24	New Market			+		6.4	< 4	< 2
F9	Chola	24.11.24	DU Campus			+		5.5	5.3	< 2
F13	Ghughni	11.01.25	Mohakhali		+		Low Concentration	6.2	4.5	< 2
F14	Chola	11.01.25	Mohakhali	+			G3	5.5	4.3	< 2
F15	Ghughni	11.01.25	Mohakhali	+	+		Low Concentration	6.3	4.8	< 2
F16	Chola	11.01.25	Mohakhali			+	Low Concentration	5.7	< 4	< 2
F19	Chola	13.01.25	Lakshmi Bazar	+				5.7	3.9	< 2
F23	Ghughni	13.01.25	Keranigaj			+	Low Concentration	5.5	< 4	< 2
F41	Chola	20.01.25	Demra			+	Not Significant.	6.5	6.4	4.8
F43	Chola	20.01.25	Demra	+				6.5	6.3	4.8
F49	Chola	21.01.25	Karwan Bazar			+	Low Concentration	3.5	5.9	4
F53	Sweet	21.01.25	DU Campus			+	Low Concentration	3.3	3.3	< 2
F56	Papaya	22.01.25	Farmgate			+	Low Concentration	6.4	3.3	< 2
F58	Chola	22.01.25	Farmgate	+			Low Concentration	6.5	5.1	< 2
F88	Chola	29.01.25	Mirpur 10		+		Low Concentration	7	7.4	< 2
F89	Ghughni	29.01.25	Mirpur 10			+	<i>E.coli</i>	7	7.3	4.2
F96	Jhalmuri	02.02.25	Chawk Bazar			+		7.3	6.9	4.7
F97	Ghughni	02.02.25	Chawk Bazar		+		<i>Hymeno sp</i>	7.2	7.1	5.5
*Counts are shown in log ₁₀ CFU/g.										

ID	Type	Date	Location	RV	NV	EV	Sequence	TVC*	TCC	FCC
F102	Ghughni	04.02.25	Victoria Park		+		Low Concentration	> 7.5	< 4	< 2
F108	Ghughni	09.02.25	Bakshibazar		+		Low Concentration	> 7.5	7.9	3.8
F115	Ghughni	23.02.25	Dhanmondi		+		Not Significant.	> 7.5	8.9	3.9
F131	Jhalmuri	01.03.25	Diabari			+		> 7.5	7.7	4.8
F142	Ghughni	06.05.25	Shahabag		+		<i>Bacillus sp</i>	7.3	6.7	2.6
F219	Grapes	6.10.25	Jatrabari			+	Low Concentration	7.4	5.4	< 1
V1	Veg wash	10.01.25	Raja Bazar	+			G3	3.9	3.7	< 1
V9	Veg wash	11.03.25	Konapara			+		6.3	6.1	4.4
V12	Veg wash	12.03.25	Lakshmi Bazar			+		4.7	< 1	2.2
V14	Veg wash	16.03.25	Rayerbag			+		3.8	4.4	3.4
V17	Veg wash	08.04.25	Midford		+		Low Concentration	6	4.3	4.8
V18	Veg wash	09.04.25	Staff Quater			+		5.8	5.1	4.1
V24	Veg wash	29.04.25	Lalbag			+	Not Significant.	5.9	5	4.7
V29	Veg wash	05.05.25	Pilkhana			+	Not Significant.	5.3	4.1	3.9
T64	Towel	04.05.25	Farmgate			+	Low Concentration	4.5	4.4	2.7
T71	Towel	16.08.25	Mirpur		+	+	Low Concentration	4.5	4.5	1.3
*Counts are shown in log ₁₀ CFU/g.										

- Molecular characterization and origin analysis of detected RV.

The two RV strains (F14 and V1) belonged to the G3 genotype and shared 100% nucleotide identity with strain OQ507401 detected in Dhaka, Bangladesh, in 2021. Phylogenetic analysis (Fig. 2) showed 99.61% nucleotide identity with strains from Bangladesh (OP730438), India (MN414258, MH712897, OQ724497, MF563919, MF621177), and Japan (LC774631), and 99.21% identity with strains from India (MZ421325, OK334702, KY774435), Thailand (MW058283), Kenya (MZ096601), and the USA (ON737408). These findings

indicate that the detected strains were closely related to previously circulating G3 RVs in South Asia and other regions, indicating potential regional and global transmission linkages.

Bacterial analysis

In the absence of national microbiological standards, regionally relevant guidelines from ICMSF, Codex (FAO/WHO), CFS Hong Kong (2014), and FSSAI (India) were adopted. Samples were considered unsatisfactory if TVC exceeded 6 log₁₀ CFU/g, TCC exceeded 4 log₁₀ CFU/g, or FCC exceeded 2 log₁₀ CFU/g. Among the 117 RTE food samples, TVC, TCC, and FCC ranged from 3.3 to > 8.5 log₁₀ CFU/g, undetectable to 8.9 log₁₀ CFU/g, and undetectable to 6 log₁₀ CFU/g, respectively. Overall, 98 (83.8%), 90 (76.9%), and 67 (57.3%) samples remained unsatisfactory for TVC, TCC, and FCC, respectively, whereas only 9 (7.7%) met the satisfactory criteria for all three indicators and 59 (50.4%) exceeded all three thresholds (Table 2).

Table 2
Bacterial quality and viral contaminations of RTE foods.

Satisfactory	Unsatisfactory	Number	RV	NV	EV
TVC, TCC, FCC		9 (7.7%)	1	0	3
TCC, FCC	TVC	20 (17.1%)	1	1	3
FCC	TVC, TCC	29 (24.8%)	2	3	1
	TVC, TCC, FCC	59 (50.4%)	1	1	4
Total		117	5	5	11

No significant association was observed between viral detection and bacterial indicators. Suspected RV and EV were detected even in samples that met all three bacteriological criteria, whereas many samples with the highest TVC, TCC, or FCC remained negative for RV, NV, and EV. Similarly, pre-cut fruits, vegetables, and vendor towels exhibited high bacterial loads with only sporadic suspected viral detections (data not shown).

Seasonal verification in virus detection

All six RV-positive samples were detected in January (Table 3), consistent with the well-established seasonal peak of RV-associated diarrhea during the cooler winter months (December–February) (Dey et al., 2010). Notably, a sharp increase in RV-associated diarrhea cases was observed in Dhaka from late 2024 to early 2025 (News, 2025). Although the overall detection of confirmed and suspected RV prevalence in food samples was 2.9%, it increased to 7.5% during this peak period, suggesting increased environmental and possible foodborne circulation of RV during an outbreak time.

Table 3
Seasonal verification in virus detection.

Months	Sample Number	RV	NV	EV
Nov'24	12	0	0	4 (33.3%)
Jan'25	80	6 (7.5%)	3 (3.8%)	5 (6.2%)
Feb'25	22	0	2 (9.1%)	1 (4.5%)
Mar'25	12	0	0	4 (33.3%)
Apr'25	16	0	1 (16.0%)	1 (6.2%)
May'25	20	0	0	1 (5.0%)
Jun'25	1	0	0	0
Aug'25	22	0	1 (4.5%)	1 (4.5%)
Sept'25	6	0	0	0
Oct'25	14	0	0	1 (7.1%)
Total	205	6 (2.9%)	7 (3.4%)	18 (8.8%)

In contrast, suspected NV and EV positives were detected throughout the year without a distinct seasonal pattern. Unlike RV, these viruses exhibit less pronounced seasonality (Mans, 2019), though EV often elevated during wet seasons (S. A. Hoque, T. Kotaki, et al., 2023). In this study, the highest detection rates were 16% for NV in April and 33.3% for EV in both March and November (Table 3).

Discussion

This study was conducted in Bangladesh's capital city, Dhaka, which has emerged as the world's second-most populous city in 2025 with 36.6 million inhabitants (UN, 2025). Rapid urbanization has placed considerable pressure on sanitation infrastructure, particularly in informal settlements and markets, where inadequate sanitation facilities and limited access to clean water often force street vendors to adopt poor hygiene practices. Furthermore, widespread informal food vending, the use of untreated water, and inadequate cold-chain management have increased the risk of enteric viral contamination. However, the lack of baseline data on foodborne enteric viruses continues to hinder evidence-based risk assessment and food safety policy development.

Since no previous studies have investigated enteric viruses in Bangladeshi foods, we evaluated diverse local food matrices and compared multiple virus extraction methods, including TRIzol-based extraction, PEG precipitation, Nanotrap® Microbiome A, and the direct washing method, to optimize virus recovery. To maximize the likelihood of virus detection, we also adopted several strategies, such as analyzing pooled vegetable wash water and vendor towels as representative samples to overcome the challenges of testing individual foods, and targeting high-risk street-vending locations so that the absence of viral contamination in these worst-case settings would suggest an even lower risk in more hygienic environments.

Here, the sample population primarily included RTE street foods, fresh produce, leafy vegetables, and whole fruits that are either readily eaten or eaten after little wash. Although some fruit matrices, particularly hog plum, cucumber, and pineapple, interfered with RNA extraction by clogging the silica membrane columns, others such as papaya and watermelon did not affect virus detection. The direct washing method demonstrated superior performance, likely due to fewer processing steps and reduced viral loss during extraction, and was therefore selected for subsequent analysis of field-collected samples (Fig. 1A). RV was molecularly confirmed in two samples (one RTE food and one vegetable wash water), while four additional samples yielded suspected RV bands. Several samples also produced target amplicons suggestive of NV and EV, but sequencing confirmation remained unsuccessful because of low viral loads. Notably, here, the direct method had a detection limit of around 3×10^3 copies/g for RV and 6.8×10^3 copies/g for NV, while viral contamination in foods is typically characterized by low viral load, often below 10^3 - 10^4 copies/g, yet sufficient to initiate the infection with an infectious dose as low as < 100 particles for NV or RV (Bosch et al., 2018).

It is expected that the detection rate would have been higher if a more sensitive detection method like one-step real-time RT-qPCR (often $< 10^2$ copies/g) had been used however, resource limitations precluded its application. Although conventional RT-PCR has successfully detected trace enteric viruses in sewage in our previous studies (S. A. Hoque, T. Kotaki, et al., 2023; S. A. Hoque, H. Saito, et al., 2023; Hoque et al., 2019), its lower sensitivity together with the complexity of food matrices, likely limited virus detection in food samples. Additional limitations include the relatively small sample size and limited food types examined. In fact, being the first study of this sort in Bangladesh, opportunities to optimize protocols and evaluate a broader range of food matrices were limited. Finally, detection of viral nucleic acids by RT-PCR may not necessarily indicate the viability or infectivity of the viruses. Nonetheless, such molecular evidence offers crucial evidence of contamination and possible risk of exposure.

Despite these drawbacks, to our knowledge, this study provides the first molecular evidence of confirmed and suspected enteric viral contamination in Bangladeshi street foods and related matrices, together with practical recommendations for future foodborne virus surveillance studies. In addition to viral investigation, simultaneous bacteriological analysis was another strength of this study, providing an overall assessment of the microbial quality of these foods. Notably, although stricter microbiological limits, such as TVC $> 5 \log_{10}$ CFU/g and TCC $> 3 \log_{10}$ CFU/g, are often recommended, regionally relevant guidelines from ICMSF, Codex (FAO/WHO), CFS Hong Kong (2014), and FSSAI (India) were considered more practical because sampling targeted high-risk street-vending locations. While elevated TVC, TCC, and FCC levels in most samples reflected poor hygienic conditions in low-hygiene street-vending environments (Table 2), a substantial proportion of samples still met satisfactory bacteriological criteria even under these worst-case conditions, representing an encouraging finding while highlighting the need for continued improvement.

Importantly, no significant association was observed between viral detection and bacterial indicators, consistent with previous findings (Stals et al., 2011) indicating that bacterial hygiene indicators are poor predictors of enteric viral contamination as viruses exhibit greater environmental persistence and resistance to common sanitizers compared to bacteria.. These findings highlight the need to incorporate direct viral detection into routine food safety surveillance rather than relying solely on bacterial indicators.

Finally, the confirmed and suspected detection of enteric viruses in Bangladeshi street foods and related matrices highlights the potential risk of foodborne viral transmission and underscores the need for greater public health attention to the informal food sector. As the first molecular evidence of its kind in Bangladesh, these findings provide an important baseline for future surveillance, risk assessment, and the development of evidence-based strategies to improve the viral and microbiological safety of street-vended foods.

Declarations

Conflicts of Interest

The authors declare that they have no conflict of interest.

Author Contribution

SAH, planned the study, performed the experiment, analyzed the results, and wrote the manuscript. TH, MM, AT, UNS also performed the experiment and analyzed the results. TK did the viral load quantification. SAH, SMS, TK, and HU involved in funding. SJH, ZH, SKA, and HU provided critical feedback. SAH and HU conceived the original idea and supervised the whole project. All authors critically reviewed the article and approved the final version of the manuscript.

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Figures

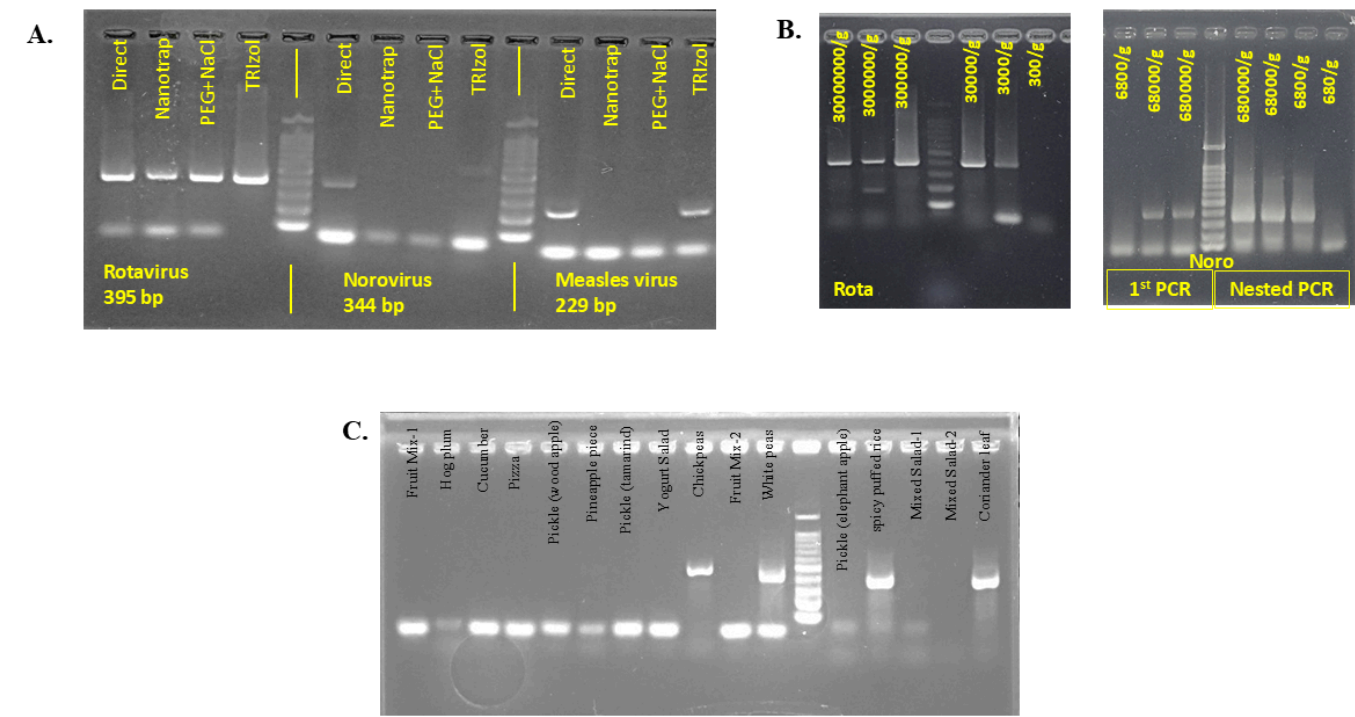


Figure 1

Figure 1

(A) Comparison of virus extraction methods. Rotavirus (395 bp), norovirus (344 bp), and measles virus (229 bp) amplicons were visualized by agarose gel electrophoresis. (B) Detection limits of RV and NV at different viral concentrations using the direct washing method. (C) Detection of spiked RV in different ready-to-eat food matrices using the direct washing method.

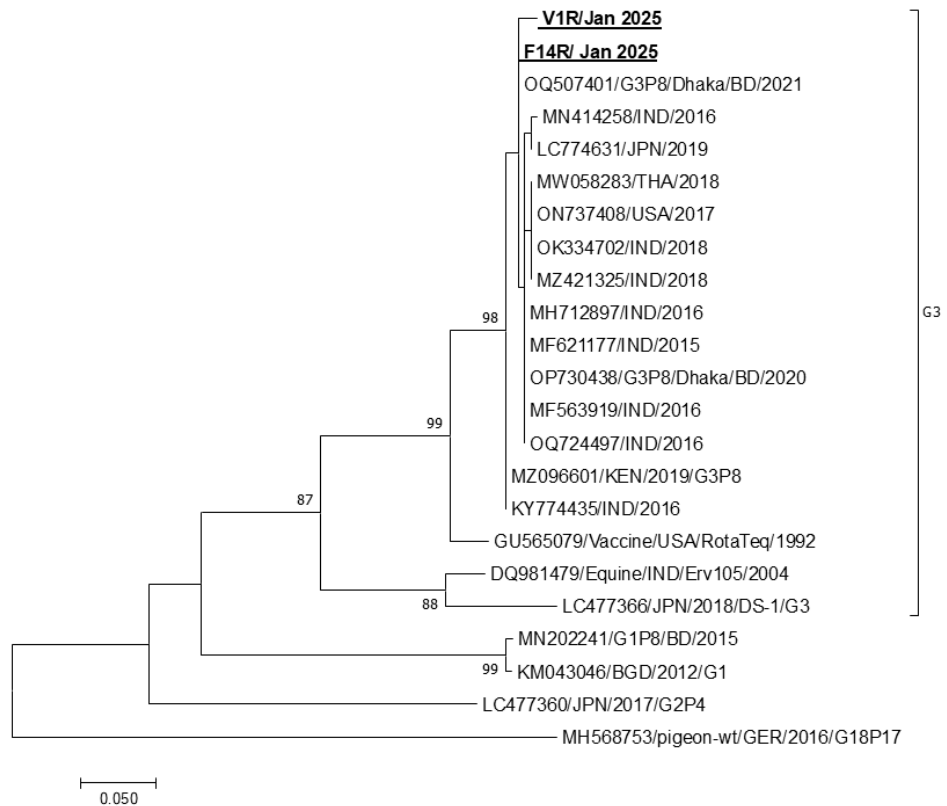


Figure 2

Figure 2

Phylogenetic analysis of RV based on partial VP7 region. The tree was constructed by the Neighbor-Joining method. The tree was rooted with the G18 strain and included the study strains (bold and underlined) and reference strains from GenBank. Bootstrap values $\geq 80\%$ (1,000 replicates) are shown.