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Molecular identification, quantification and phylogeny of non-polio enteroviruses: A wastewater-based epidemiology from northeastern Nigeria

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Abstract

Non-polio enteroviruses (NPEV) pose a major, potentially deadly global health threat. While most infections are mild or asymptomatic, certain strains cause severe complications, requiring enhanced clinical and environmental surveillance for effective management. This study utilised wastewater-based epidemiology to assess the extent of NPEV contamination across six northeastern states in Nigeria. We collected and processed 400 sewage water samples for virus isolation and identification using RD and L20B cell lines and qPCR, and genotyped them by Sanger sequencing. The overall prevalence of NPEVs was 42.3% and was significantly highest in Gombe state (55.4%, OR = 2.14, $p = 0.0372$). Remarkably, a high viral copy number of 4.28 ± 0.3 log10 was obtained at a sampling site in Adamawa State. Viral copy numbers differed significantly across sampling sites in all states ($p < 0.05$), except in Yobe and Taraba states ($p > 0.05$). Borno State had the highest mean NPEV copy numbers, followed by Taraba State, whose mean copy number was closely related to those of other states. Sequence analyses revealed genetic diversity among Echovirus E19 isolates from Borno and Yobe States. One Echovirus E19 isolate from Borno State clustered closely with another strain previously described in Ibadan, Nigeria. These findings reveal diverse and high levels of NPEV contamination in most sewage samples in northeastern Nigeria. Moreover, the relatedness of Echovirus E19 to one described over a decade ago in southwestern Nigeria highlights active local transmission and underscores the need for continuous NPEV surveillance across One Health niches.

INTRODUCTION

Human enteroviruses belong to the *Picornaviridae* and have positive-sense, single-stranded genomes of approximately 7.5 kb that contain one principal open reading frame between the 5' and 3' untranslated regions. Proteolytic processing of the encoded polyprotein generates the structural proteins VP1–VP4 and seven non-structural proteins involved in replication and host interactions (Chen, 2026; Nokhova et al., 2024). The group includes polioviruses, coxsackieviruses, echoviruses and other numbered enterovirus types (Ayyub et al., 2024a).

The enterovirus capsid consists of 60 protomers arranged around the RNA genome. VP1 contributes prominently to the external capsid surface and contains sites recognised by neutralising antibodies; consequently, VP1 sequence data are frequently used to assign enterovirus types. VP4 occupies an internal position and undergoes myristoylation (Chen, 2026; Nokhova et al., 2024).

Numerous human enterovirus types have been described, and their epidemiology varies by type, geography and season (Chen, 2026; Xie et al., 2024b). Transmission occurs through faecal–oral contact and, for some types, respiratory exposure; household and institutional contact can support spread (Li, 2026). Many infections produce no symptoms or a mild illness, although neonates and young children may be particularly vulnerable to complications (Pugliese, 2025; Li, 2026). Specific NPEV types have been associated with acute flaccid myelitis and other neurological presentations, but detecting viral RNA

in the environment does not establish that such illnesses are occurring in the contributing population (Global Polio Eradication Initiative, 2026; Gui, 2026).

Reported clinical manifestations of enterovirus infection include aseptic meningitis, encephalitis, neonatal systemic illness, etc. (Chen, 2026; Li, 2026). Associations between some enterovirus infections and pancreatic islet pathology have also been investigated. The contribution of these viruses to type 1 diabetes remains under investigation, and the evidence should not be interpreted as establishing a direct causal pathway for all cases (Pugliese, 2025; Rodriguez-Calvo et al., 2025).

Certain enterovirus and rhinovirus infections have also been associated with respiratory disease and exacerbations of pre-existing airway conditions. Their different clinical presentations and genetic diversity make both molecular characterisation and appropriately designed epidemiological surveillance relevant (Nokhova et al., 2024; Gui, 2026; Xie et al., 2024a).

Acute flaccid paralysis (AFP) surveillance remains central to poliovirus detection in Nigeria. Surveillance performance is commonly assessed using the non-polio AFP rate among children younger than 15 years and the adequacy of stool collection (Global Polio Eradication Initiative, 2026). AFP surveillance specimens may also yield non-polio enteroviruses once poliovirus has been excluded (Ben Hamida et al., 2020). Nigerian reports have identified NPEVs in AFP surveillance specimens and faecal carriage surveys, including studies by Bassey et al. (2018) and Ghamba et al. (2020). Molecular work has identified multiple enterovirus types in the country (Faleye *et al*., 2017). Because these studies used different populations and sampling designs, their percentages should not be directly compared with a wastewater sample-positivity rate.

Environmental sampling can complement clinical surveillance by identifying enteroviruses shed into wastewater, including infections that do not result in clinical testing. Sequence analysis can help describe the enterovirus types present, although wastewater results alone cannot measure person-level prevalence or establish specific chains of transmission (Bubba et al., 2023). Here, archived sewage samples from six states in northeastern Nigeria were examined for NPEV detection, quantitative molecular signals and VP1 sequence relationships.

MATERIALS AND METHODS

Study area

The study covered Adamawa, Bauchi, Borno, Gombe, Taraba and Yobe States in northeastern Nigeria. These states differ in settlement patterns, water access and sanitation arrangements. Security disruptions have also affected water and sanitation infrastructure in parts of the region (Gulumbe et al., 2023). This context provides a rationale for environmental surveillance, but the present analysis did not directly measure the effect of conflict, sanitation services or population characteristics on viral detection.

Ethical consideration

The University of Maiduguri Teaching Hospital ethics review committee evaluated and approved this study.

Study selection criteria

We performed a cross-sectional analysis of archived sewage samples collected between January 2022 and December 2023 at identifiable locations in northeastern Nigeria. Eligible specimens had accompanying site information, including location and available environmental observations. Samples with untraceable collection sites, those originating outside the study region and those collected outside the stated period were excluded.

Sample size determination

The anticipated sample size was calculated using $n = Z^2p(1 - p)/e^2$, where $Z = 1.96$, $p = 0.68$ (Hamisu *et al*., 2022), and $e = 0.05$. This yields a required sample size of roughly 334; however, this research included 400 specimens.

Sample collection

Archived wastewater specimens originated from 25 open drainage sites receiving domestic effluent in the six study states. At collection, approximately 1 L was placed in a sterile container during the early-morning sampling period (06:00–07:00). Available field records included geocoordinates and measurements of pH, temperature, turbidity and biological oxygen demand (Supplementary Table S1; Fig. 1). Specimens were transported on ice and subsequently stored at − 80°C until processing.

Sewage sample processing

Samples were concentrated by a polyethylene glycol (PEG)/dextran two-phase procedure described for environmental enterovirus surveillance (Tiwari & Dhole, 2018). Initially, 500 mL of each sample was centrifuged at 1,500 revolutions per minute (rpm) for 20 min at 4°C, and the sediment was retained at 4°C. The clarified liquid was brought to approximately pH 7.2 with 1 N NaOH and mixed with 39.5 mL of 22% dextran, 287 mL of 29% PEG 6000 and 35 mL of 5 M NaCl. After stirring for 1 h, the mixture was kept in a separation funnel overnight at 4°C. The lower phase and interface were recovered and combined with the retained sediment. Following chloroform treatment (20%), shaking for 20 min and centrifugation at 1,500 rpm for 20 min at 4°C, the supernatant was collected. A further 10% chloroform treatment was applied to the remaining turbidity. The manuscript records additions of 500 µL penicillin/streptomycin and 100 µL gentamicin to the concentrate, which was stored at − 80°C.

Virus cell culture

The human rhabdomyosarcoma (RD) and genetically modified mouse (L20B) cell lines were used to isolate enteroviruses in accordance with the Global Polio Laboratory Network (2017) recommendations. To initiate isolation, a 500 µL aliquot of extracted concentrate from sewage water samples was inoculated onto fresh, confluent monolayer (75% to 100%) cultures of RD (passage P231 + 6) and L20B (passage P19 + 6) cells within T25 cm^2 flasks. Inoculated flasks were incubated at 37°C and monitored daily by inverted microscopy for characteristic cytopathic effects (CPE).

CPE-positive cultures underwent two consecutive freeze-thaw cycles to induce cell lysis and optimise the release of intracellular viral progeny. Lysates were subsequently subjected to cross-passaging onto the alternative cell line (i.e., isolates exhibiting CPE on RD cells were passaged onto L20B monolayers, and vice versa) to differentiate polioviruses from non-polio enteroviruses (NPEVs). Inoculated cultures demonstrating no observable morphological changes over a 7-day post-inoculation window were definitively classified as negative. Conversely, samples exhibiting reproducible CPE exclusively on RD monolayers but lacking infectivity on L20B lines were designated as presumptive NPEVs (Global Polio Laboratory Network, 2017). These positive isolates were systematically cryopreserved for confirmation via quantitative polymerase chain reaction (qPCR) targeting highly conserved regions of the NPEV genome.

Viral RNA Extraction

Viral nucleic acid extraction was performed using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) following an optimised spin-column protocol tailored for low-titer clinical and culture matrices (QIAGEN, 2023). Lyophilised carrier RNA was reconstituted in Buffer AVE to a final concentration of 1 µg/µL, aliquoted, and stored at -20°C. Prior to extraction, Carrier RNA was spiked into the Buffer AVL lysis matrix at a 1:100 (v/v) ratio, and the mixture was homogenized by gentle inversion. Intracellular viral particles from tissue culture specimens were liberated via two sequential freeze-thaw cycles. The resulting lysate was clarified by centrifugation at 13,000 × g for 1 min to pellet host cell debris, and the supernatant was transferred to a sterile cryovial. Under biosafety containment, a 140 mL aliquot of the clarified supernatant was mixed with 560 mL of carrier RNA-spiked Buffer AVL, vortexed for 15 s, and incubated at room temperature for 10 min to ensure complete viral lysis and RNase inactivation (Rai et al., 2024). After incubation, adjust downstream binding conditions by adding 560 mL of absolute ethanol (96–100%) and pulse-vortexing for 15 s. The mixture was sequentially loaded onto a QIAamp Mini spin column in 630 mL increments and bound to the silica membrane via centrifugation at 7500 revolutions per minute (rpm) for 1 min, with the filtrate discarded after each pass. Impurities and potential enzymatic inhibitors were sequentially removed using two washing stages: first with 500 mL of Buffer AW1 (7500 rpm for 1 min), and subsequently with 500 mL of Buffer AW2 at high-speed centrifugation (14500 rpm for 3 min). Residual wash buffers were cleared from the column matrix with an additional dry centrifugation step (20,000 × g for 1 min). Highly purified viral RNA was eluted by applying 60 mL of

room-temperature-equilibrated Buffer AVE directly to the column membrane, followed by a 1-min incubation and centrifugation at 6,000 × g for 1 min. The eluate was divided into targeted analytical fractions, including a 25 mL aliquot reserved for non-polio enterovirus RT-PCR, and stored at -70^{0}C until molecular amplification.

Synthesis of Complementary DNA (cDNA)

Reverse transcription of the isolated viral RNA was conducted utilising the ProtoScript® II First Strand cDNA Synthesis Kit (New England Biolabs, Ipswich, MA, USA) in accordance with the manufacturer's established protocols (New England Biolabs, 2024). To optimise cDNA yield across complex primary transcripts and target domains with potentially high GC content or secondary structures, a template-primer mixture was prepared in sterile, RNase-free microcentrifuge tubes (Fujimoto, 2026). This mix contained 2 mL of viral RNA template, 2 mL of d(T) 23VN primer, and 4 mL of nuclease-free water. The viral target genomes were denatured in a thermal cycler at 700°C for 5 min, then collected by brief centrifugation and rapidly quenched on ice to suppress secondary-structure reannealing. For the enzymatic synthesis stage, 10 mL of M-MuLV. The Reaction Mix and 2 mL of M-MuLV Enzyme Mix were included into each sample, resulting in a cumulative volume of 20 mL. A parallel negative control (RT–) was systematically processed by substituting the enzyme mix with an equivalent volume of nuclease-free water to monitor for genomic DNA background or contamination. The assembled 20 mL cDNA synthesis reactions were incubated in a thermal cycler at 420°C for 60 min to achieve primer extension, followed by terminal enzymatic heat inactivation at 800°C for 5 min. To ensure sufficient volume for multiple downstream PCR screening applications, the raw cDNA product was diluted to a final volume of 50 mL using 30 mL of nuclease-free water (Rai et al., 2024). Spectrophotometric measurement and evaluation of nucleic acid purity (A260/A280 and A260/A230 ratios) were confirmed utilising a NanoDrop™ 2000 Spectrophotometer. Eluted cDNA products were preserved at -20°C for immediate downstream viral amplification assays.

Quantitative Reverse Transcription Polymerase Chain Reaction (RT-PCR)

One-step qRT-PCR was performed on NPEV isolates using the qScript XLT 1-Step RT-qPCR ToughMix kit (QuantaBio) according to the manufacturer’s instructions (Ng *et al*., 2022). Primers forward (5’-S GGC CCC TGA ATG CGG CTA ATC C-3’) and Pan-enterovirus reverse (5’- A GCG ATT GTC ACC ATW AGC AGY CA-3’), which are specific to the VP1 gene of the virus responsible for capsid mapping and serotype identification, along with their corresponding TaqMan hydrolysis probe (Pan-enterovirus Probe (VIC) 5’-CCG ACT ACT TTG GGW GTC CGT GT-3’), were utilized to achieve highly accurate pan-enteroviral screening (Fujimoto, 2026). Three replicates from each sampling site and three technical replicates were integrated into the RT-qPCR protocol to ensure statistical validation and assay reproducibility (Moreni et al., 2023). Briefly, the viral RNA templates and the PCR reagents were thawed at room temperature under a clean, certified biosafety cabinet. Then, 1 µL of the primer/probe mix containing the enterovirus-specific primers and probe, 10 µL of qScript XLT 1-Step RT-qPCR Tough Mix, and 8 µL of nuclease-free

water were combined to serve as a master mix in a labeled 1.5 mL Eppendorf tube. Thereafter, 19 µL of this master mix reaction solution was dispensed into individual tubes, and 1 µL of the target template was added to establish a total reaction volume of 20 µL. In-house positive and negative controls were run concurrently with the test samples to monitor amplification efficiency and detect potential cross-contamination. The reaction tubes were briefly vortexed to ensure a homogeneous mixture, and then loaded into a Bio-Rad CFX96™ thermocycler. Thermal cycling was executed under the following conditions: a holding at 50°C for 30 minutes, followed by initial denaturation at 95°C for 1 minute, and a cycling stage spanning 40 cycles of denaturation at 95°C for 15 seconds, annealing at 50°C for 45 seconds with a 25% ramp rate, and a final extension at 72°C for 5 seconds (WHO, 2004).

Enterovirus VP1 characterization

For molecular characterization and definitive serotyping of human enteroviruses (EVs), viral cDNA was amplified in a single-tube 25 mL PCR formulation comprising 3muL of template cDNA, 10 mL of HotStarTaq Master Mix to prevent non-specific mis priming, 2 mL of CL2 secondary structure resolving agent, 8 mL of sterile nuclease-free water, and 1 mL each (10 mM) of the consensus degenerate primer pairs 224-Forward (5^1-GCI ATG YTI GGI ACI CAY RT-3^1) and 011-Reverse (5^1-GCI CCI GAT TGI TGI CCG AA-3^1) targeted to the highly polymorphic structural capsid VP1 gene, which remains the gold standard target for genotype classification (Wang et al., 2024; Xie et al., 2024a).

Thermocycling kinetics were initiated with a 2-minute activation step at 94°C to release the hot-start enzyme blocking mechanism, followed by 32 cycles of denaturation (94°C for 30 seconds), low-stringency annealing (42^0C for 30 seconds) optimised to accommodate nucleotide polymorphisms across the degenerate primer binding locations, and a prolonged extension phase (60^0C for 2 minutes) designed to ensure full-length synthesis of complex structural genes.

Downstream processing involved separating the resulting distinct 660bp and 438bp amplicons via agarose gel electrophoresis, excising the targets for solid-phase silica extraction using the QIA quick Gel Extraction Kit (Qiagen), and subjecting the purified templates to automated, fluorescence-based capillary Sanger sequencing using the ABI PRISM BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems) to provide high-resolution genetic data for lineage-specific epidemiological tracking and phylogenetic classification (Wang et al., 2024).

Data analysis

Chromatograms generated from Sanger sequencing were manually inspected and edited using BioEdit software, and initial viral identities were established via the Basic Local Alignment Search Tool (BLAST) against the NCBI GenBank database. Multiple sequence alignments were performed using Clustal X version 2.0.12. Evolutionary distances and phylogenetic architectures were reconstructed using the Neighbor-Joining method in MEGA 11, with branch support and cluster confidence rigorously evaluated via bootstrap resampling with 1,000 pseudo-replicates (Tamura et al., 2021).

Continuous variables were represented as Mean ± Standard Deviation (SD) and examined with the nonparametric Kruskal-Wallis test. Additionally, bivariate regression was conducted to evaluate the correlation of NPEV prevalence in sewage samples based on geographic location (state). All evaluations were conducted using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). Statistical significance for all datasets was established as a p-value less than 0.05 at a 95% confidence interval (CI).

RESULTS

Based on CPE data on cell lines (Fig. 1) and qPCR data, the overall prevalence of NPEVs from archival sewage in northeastern Nigeria was 43.2% and was significantly highest in Gombe state, at 55.4% (OR = 2.14, 95% CI: 2.14 (1.05–4.39), $p = 0.0372$). Table 2 shows the cycle threshold (Ct) values and viral copy numbers (log10) of NPEVs by sewage sampling site in the north-eastern states of Nigeria. Mean log10 viral copy numbers differed significantly across sampling sites in all states ($p < 0.05$), except in Yobe and Taraba states (p = 0.5672 and 0.7021, respectively) (Table 2). Borno State had the highest mean copy number (log10), followed by Taraba State, whose mean copy number (log10) was similar to that of other states (Fig. 2).

The sequence analyses revealed genetic diversity among the NPEV isolates from Moduganari Bayan Tudu (MBT) and Bulabulin Gidan Zana (BGZ) in Borno State and Damaturu Central Ganye Uku (DCU) in Yobe State (Fig. 3). Based on the highest percentage similarity, the three strains are Echovirus E19 and clustered closely with another strain previously described in Ibadan, Nigeria (GenBank accession number: MH745407.1).

Table 1
Frequency and association of sewage non-polio enteroviruses by states of northeastern Nigeria

States	No. of Samples tested	No. (%) NPEV positive	OR (95% CI)	*p* value
Adamawa	49	16 (36.7)	0.84 (0.38- 1.85)	0.6619
Bauchi	69	29 (42.0)	1.25 (0.62–2.55)	0.5346
Borno	102	47 (46.1)	1.48 (0.77- 2.84)	0.2430
Gombe	65	36 (55.4)	2.14 (1.05- 4.39)	0.0372
Taraba	55	19 (34.5)	0.91 (0.42–1.95)	0.8125
Yobe	60	22 (36.7)	1	Referent
Total	400	169 (42.3)		

D

B

A

C

Table 2
Ct values and viral copy number (log_{10}) of NPEVs according to Sewage Sampling Sites in northeastern Nigeria

Sites of Sampling	No. (%) Positive for NPEV	+Ct values Mean ± SD	++Viral copy Number (log 10) Mean ± SD	+KW statistic	+p value	++KW statistic	++p value
Adamawa state							
Gadan Durbiyel	2 (33.3)	24.94 ± 0.3	2.85 ± 0.1				
Gadar Rumde	3 (33.3)	24.93 ± 1.5	2.86 ± 0.5				
Unguwan Magaji Bridge	8 (33.3)	23.06 ± 2.1	1.68 ± 4.2				
Gadar Doubeli	1 (33.3)	20.33 ± 0.9	4.28 ± 0.3				
Total	14 (33.3)	23.77 ± 3.1	2.28 ± 3.2	0.1512	0.927	8.085	0.0443
Bauchi state							
Obuna Royal site	10 (33.3)	23.64 ± 1.9	3.26 ± 0.6				
FGGC Bridge site	5 (27.8)	25.93 ± 2.8	0.86 ± 4.3				
Gwallaga Jumaat Mosque	8 (33.3)	24.79 ± 3.0	2.90 ± 0.9				
Shafa bridge site	4 (44.4)	26.08 ± 4.9	2.09 ± 1.6				
Total	27 (33.3)	23.53 ± 2.1	2.49 ± 2.3	5.69412	0.127	9.962	0.0189
Borno state							
Abbaganaram Filling Station	9 (33.3)	24.1 ± 2.0	3.11 ± 0.6				
Blind Workshop Bridge	9 (33.3)	24.57 ± 2.2	2.97 ± 0.7				
Budum Roundabout	7 (33.3)	24.06 ± 1.3	3.13 ± 0.4				

Sites of Sampling	No. (%) Positive for NPEV	+Ct values Mean ± SD	++Viral copy Number (log 10) Mean ± SD	+KW statistic	+p value	++KW statistic	++p value
Bulabulin Gidan Zana	5 (33.3)	25.53 ± 3.6	2.71 ± 1.1				
Gamboru Market Bridge	6 (33.3)	25.27 ± 2.9	2.75 ± 0.9				
Maduganari Bayan Tudu	4 (33.3)	21.64 ± 2.5	3.88 ± 0.8				
Total	40 (33.3)	24.31 ± 2.6	3.07 ± 0.8	12.33	0.031	12.11	0.0334
Gombe state							
Baba Roba Valley	8 (33.3)	23.80 ± 1.8	3.21 ± 0.6				
Dangusau Bridge	8 (33.3)	24.47 ± 2.1	3.00 ± 0.7				
Gadan Bayan Moonshine	6 (33.3)	23.34 ± 2.9	3.36 ± 0.9				
Unguwa Uku Railway Bridge	11 (33.3)	24.81 ± 2.5	0.02 ± 5.0				
Total	33 (33.3)	24.22 ± 2.4	2.12 ± 3.3	5.245	0.155	10.28	0.0163
Taraba State							
Ali Bacchi Bridge	2 (33.3)	23.72 ± 1.0	3.23 ± 0.3				
Gadan Baba Iware	8 (38.1)	23.50 ± 2.1	3.20 ± 0.6				
Gadar Bobboji	6 (28.6)	24.76 ± 6.6	2.23 ± 2.5				
Total	16 (33.3)	24.00 ± 4.3	2.81 ± 1.7	2.456	0.228	0.7073	0.7021
Yobe State							
Damaturu Central Ganye Uku	5 (33.3)	24.17 ± 3.8	3.10 ± 1.2				

Sites of Sampling	No. (%) Positive for NPEV	+Ct values Mean ± SD	++Viral copy Number (log 10) Mean ± SD	+KW statistic	+p value	++KW statistic	++p value
Hausawa Asibiti	8 (33.3)	23.65 ± 3.0	2.81 ± 2.6				
Nayinawa Unguwar Boka	6 (33.3)	24.19 ± 3.5	3.09 ± 1.1				
Yantukwane	2 (33.3)	20.54 ± 5.6	4.22 ± 1.7				
Total	21 (33.3)	23.63 ± 3.7	3.09 ± 1.9	2.025	0.567	2.025	0.5672

The values were the mean ± SD of three replicates

KW= Kruskal- Wallis H (~ X^2), NPEV = Non-Polio Enterovirus.

DISCUSSIONS

Wastewater-based epidemiology (WBE) has been used for viral surveillance and, for NPEVs, is a powerful, population-level public health tool. It acts as an early warning system for outbreaks, capturing viruses that could cause severe health complications well before they are reported in clinical settings. The reported overall NPEV prevalence of 43.2% in northeastern Nigeria aligns well with environmental surveillance data from sub-Saharan Africa and South Asia, where detection rates range from 35% to 60% (Tiwari & Dhole, 2018; Ayyub et al., 2024b; Chukwuma, 2025; Hamisu *et al*., 2022; Majumdar et al., 2021; Ndiaye et al., 2024; Howard et al., 2020). Such high environmental positivity rates for NPEVs are commonly documented in urban and peri-urban contexts, reflecting continuous viral circulation sustained by environmental reservoirs such as sewage systems. The notable elevation of the odds ratio in Gombe suggests localized epidemiological drivers—including factors like population density, sanitation conditions, and climate—that facilitate enterovirus persistence and transmission. Studies from West African urban centres emphasise that variations in these determinants modulate viral detection rates and recommend focused surveillance and public health interventions tailored to local conditions (Ozawa et al., 2019).

In the global context of environmental virology, these findings illustrate a stark infrastructural divide. In high-income nations with enclosed sewage systems and tertiary wastewater treatment, environmental NPEV detection is rare and typically limited to acute seasonal spikes in summer and autumn (Xie et al., 2024b). In those highly managed settings, modern sanitation infrastructure acts as an environmental

suppressor, breaking transmission chains before pathogens can establish a permanent environmental footprint.

Quantitative PCR analyses revealing differential viral copy numbers across states—with Borno recording the highest mean viral load—underscore spatial heterogeneity in environmental contamination and viral shedding. Such variation is consistent with prior environmental surveillance research demonstrating that factors like sewage infrastructure design, water usage patterns, rainfall, and community behaviour influence enterovirus concentrations detected in wastewater samples (Girón-Guzmán et al., 2024). The relatively uniform viral loads observed in Taraba and other states, together with the lack of significant differences between Yobe and Taraba, may reflect either stable patterns of viral excretion or limitations in sample collection and detection sensitivity. These findings reinforce the value of longitudinal surveillance in capturing temporal fluctuations in viral load and more accurately mapping transmission dynamics. Moreover, studies globally have shown that integrating viral quantification with epidemiological data enhances interpretation of enterovirus spread and informs public health responses (Ozawa et al., 2019; Pellegrinelli et al., 2019).

By applying quantitative qPCR to sewage water, this study validates advanced spatial-modelling frameworks that argue that even within universally endemic municipal landscapes, distinct, hyper-local nodes drive true viral transmission (Nwaze & Ashikeni, 2026). These hotspots continuously seed the surrounding aquatic environment with high viral loads, disproportionately driving the transmission of severe NPEV-associated pathologies, including acute flaccid myelitis, aseptic meningitis, and neonatal sepsis-like syndromes (Gui, 2026; Li, 2026). Consequently, public health interventions that fail to target these micro-spatial nodes are unlikely to achieve regional suppression.

Enteroviruses readily undergo intra- and inter-typic recombination (Annan et al., 2022). The dense genetic mixing bowl within these open drainage channels provides the environmental pressure required for genetic reversion to neurovirulence, a concern strongly supported by Castro *et al*. (2024), who recently mapped the complete genome sequences of nine double-recombinant vaccine-derived polioviruses (cVDPV2) from sewage isolates in Nigeria.

The identification of genetically diverse NPEV isolates from multiple sampling sites in Borno and Yobe States affirms the molecular complexity of enterovirus populations circulating in the region. Echovirus E19's predominance is consistent with molecular epidemiology data from environmental and clinical specimens across Africa and Asia, where E19 is among the most commonly detected NPEVs (Angez et al., 2015; Faneye *et al*., 2017). The close genetic relationship with the Ibadan strain suggests dynamic interregional viral exchange, likely facilitated by human movement and environmental dissemination, a trend documented in enterovirus molecular surveillance worldwide (Salem *et al*., 2026; Itani *et al*., 2025; Ozawa et al., 2019). While historically thought to be associated with milder conditions (such as diarrhoea and mild respiratory illnesses), E19 has also frequently been identified in outbreaks complicated by severe manifestations such as myocarditis and aseptic meningitis (Fernandez-Garcia *et al*., 2017; Angez et al., 2015; Zhu et al., 2007).

Enteroviruses, including Echovirus E19, exhibit high genetic plasticity due to frequent recombination and mutation, complicating control strategies and vaccine development efforts (Jartti et al., 2024). Molecular surveillance of NPEV has shown that multiple genetically distinct strains often co-circulate within relatively confined geographic areas, reflecting ongoing viral evolution and adaptation (Itani et al., 2026). Continuous sequencing and phylogenetic investigations are therefore critical to detect emerging variants, understand evolutionary trajectories, and evaluate epidemiological risks (Vlok & Majer, 2025; Mo et al., 2026).

The genetic relatedness observed between geographically distant Nigerian strains advocates for a coordinated national surveillance system that consolidates environmental and clinical data streams. Such integrated surveillance would enable rapid outbreak detection, support data-driven vaccine strategy formulation, and inform targeted public health interventions to reduce the enterovirus disease burden.

While this study provides substantive insight, its limitations include the potential bias inherent in cross-sectional sampling, which may not fully capture temporal trends in viral circulation or outbreak dynamics. The non-significant differences in viral load observed in Yobe and Taraba indicate a need for more frequent sampling and greater methodological sensitivity. Expanding molecular characterisation through whole-genome sequencing could better elucidate recombination events and evolutionary pathways. Further longitudinal and geographically expansive molecular surveillance across Nigeria would help map viral diversity comprehensively and clarify environmental and socio-demographic influences on enterovirus epidemiology. Such work is fundamental to informing public health policy and anticipating the emergence of novel or epidemiologically significant enterovirus variants.

CONCLUSION

These findings reveal diverse and high levels of NPEV contamination in most sewage samples in northwestern Nigeria. Moreover, the relatedness of Echovirus E19 to one described over a decade ago in southwestern Nigeria highlights active local transmission. The findings underscore the crucial role of environmental surveillance in capturing the diversity and circulation patterns of enteroviruses, including serotypes that are absent or under-detected in clinical surveillance. Integrating environmental sampling with advanced molecular typing offers a powerful tool for monitoring enterovirus circulation, guiding public health interventions, and mitigating disease impact. Sustained surveillance efforts and detailed molecular analyses remain critical for early detection of emerging strains and effective management of enterovirus-associated illnesses in Nigeria and comparable settings.

Declarations

Author Contribution

This research study was done in collaboration among all authors. PEG, TMH: Conceptualisation, Methodology, Validation, Software, Formal analysis, Data curation, Visualisation, Writing – original draft,

Writing – review & editing. INA, MBA, DNB, AI, WH, JF, MM, EA: Methodology, Validation, Formal analysis, Writing – original draft, Writing – review; A.O.A., I.N.A.: Supervision, Project administration. All authors read and approved the final manuscript.

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Figures

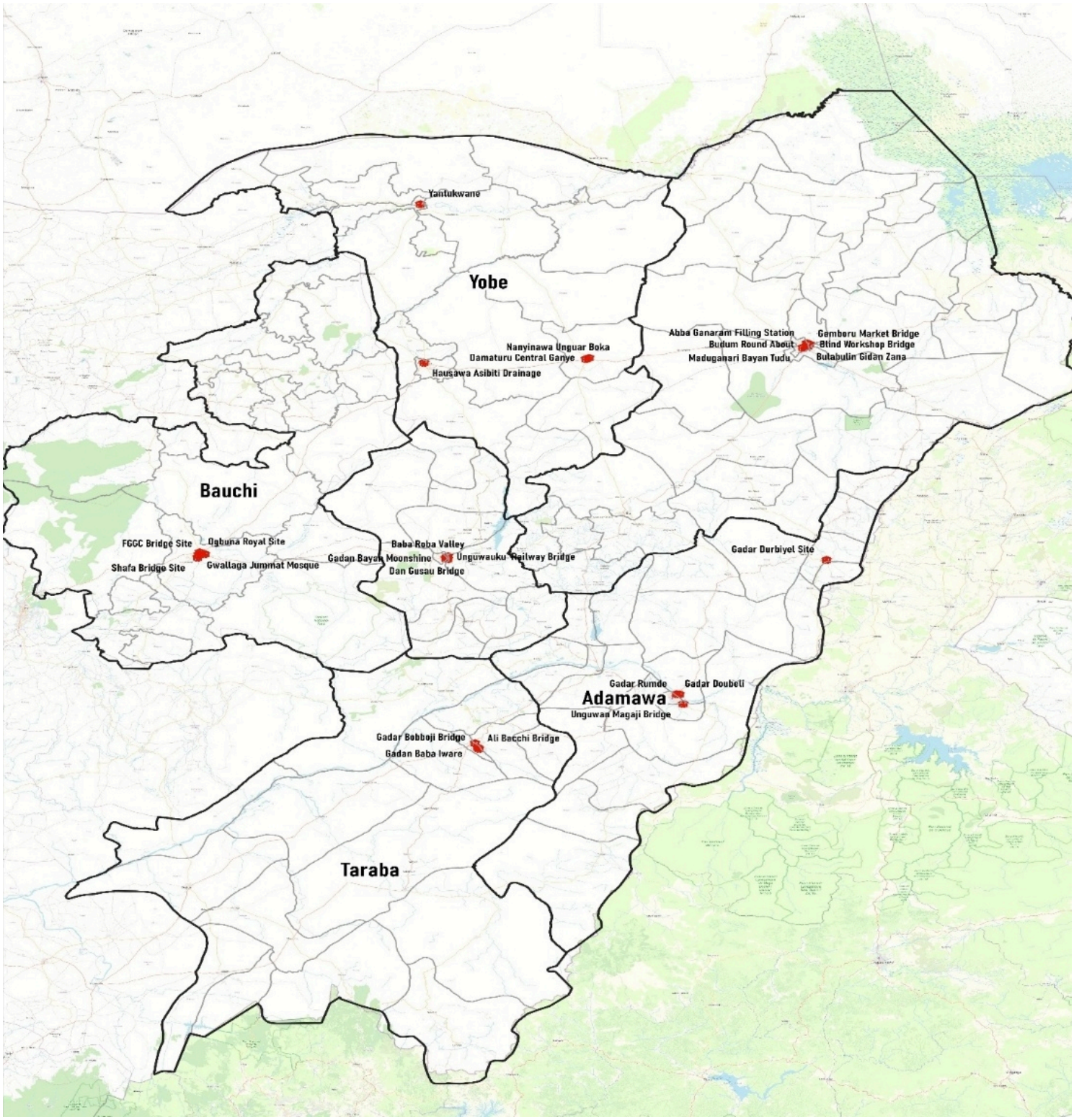


Figure 1

A map of the northeastern states of Nigeria showing the sewage sampling sites.

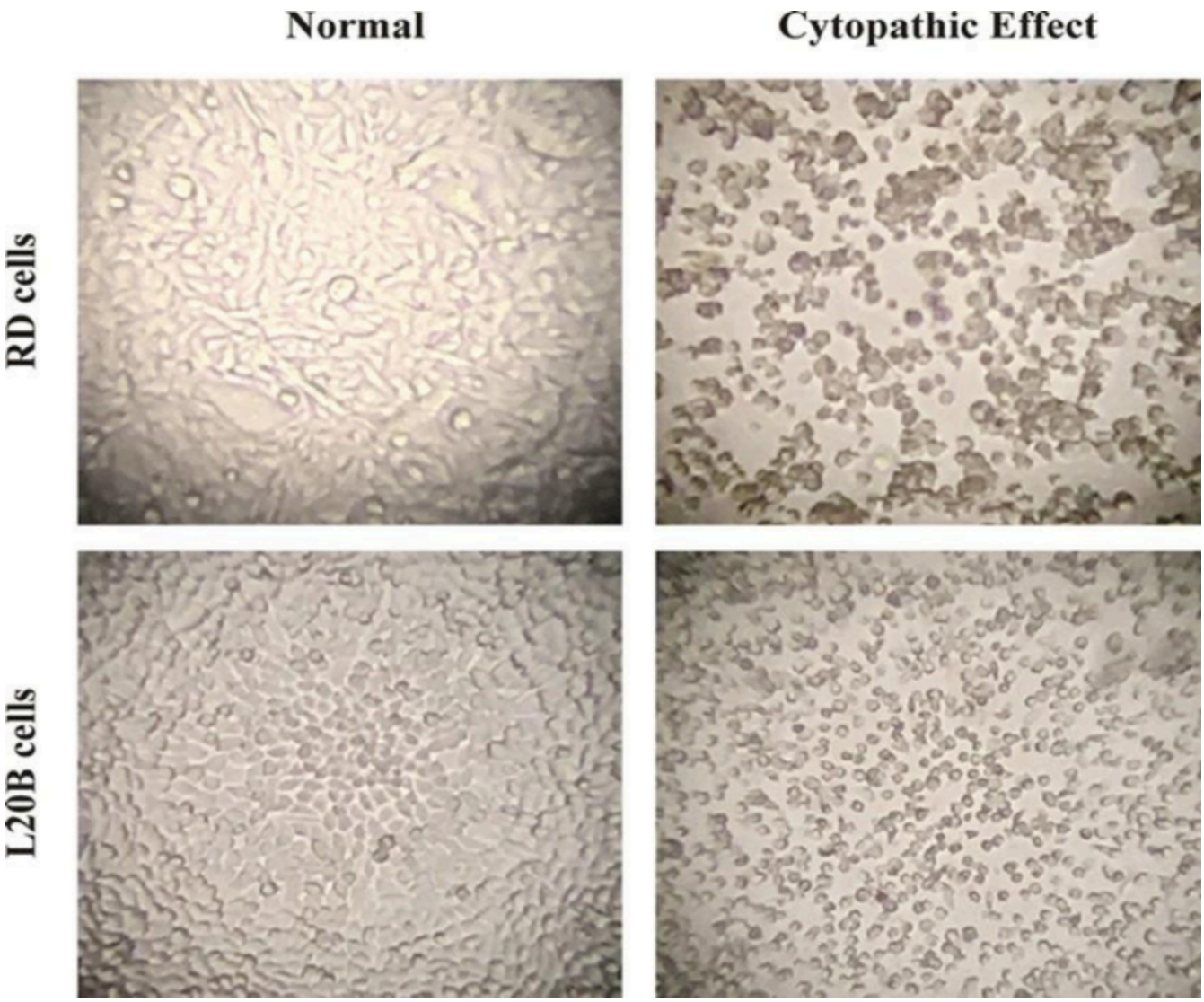


Figure 2

RD (A) and L20B (B) cell cultures before inoculation, with representative cytopathic changes shown in panels C and D. Cell morphology alone is insufficient to identify the virus; isolate classification requires interpretation of the culture and molecular results together.

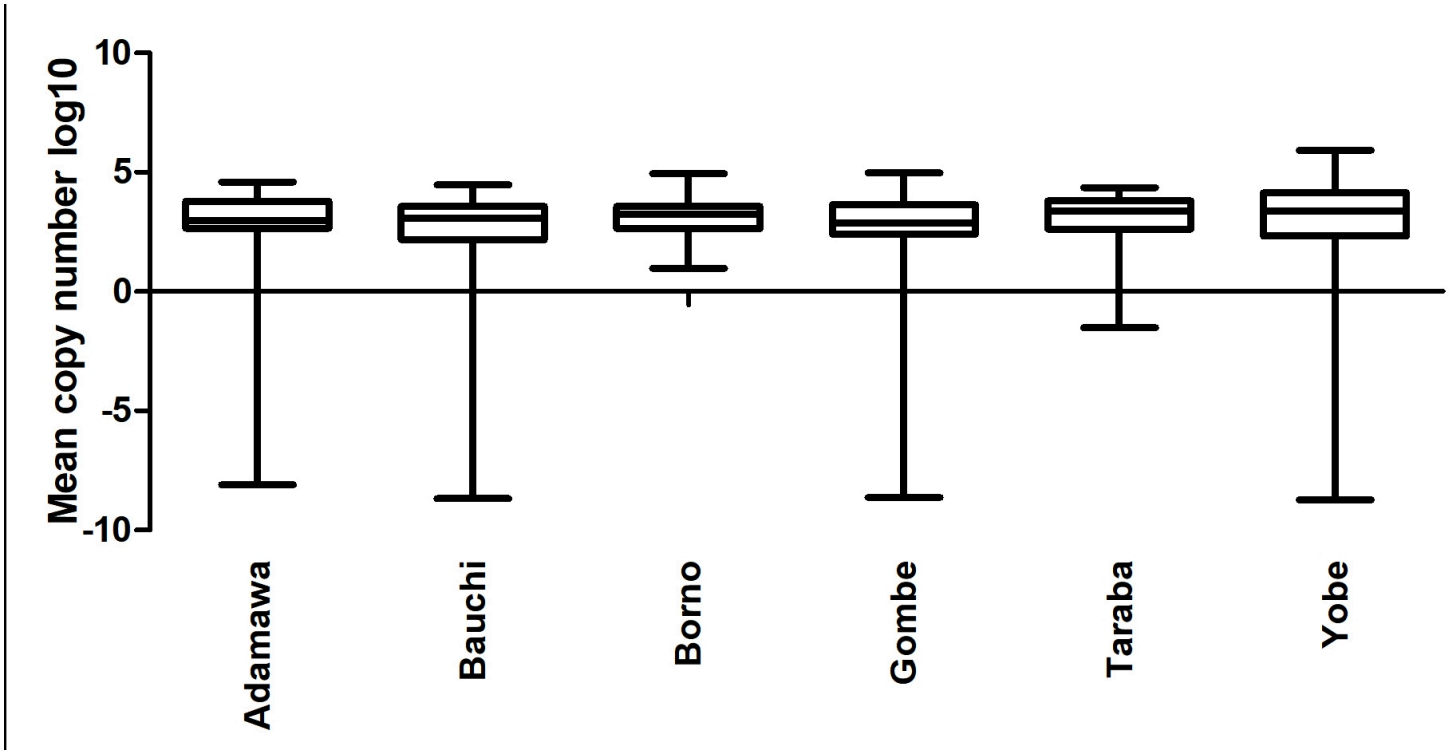


Figure 3

Distribution of reported log10 enterovirus copy-number estimates by sampling location and state.

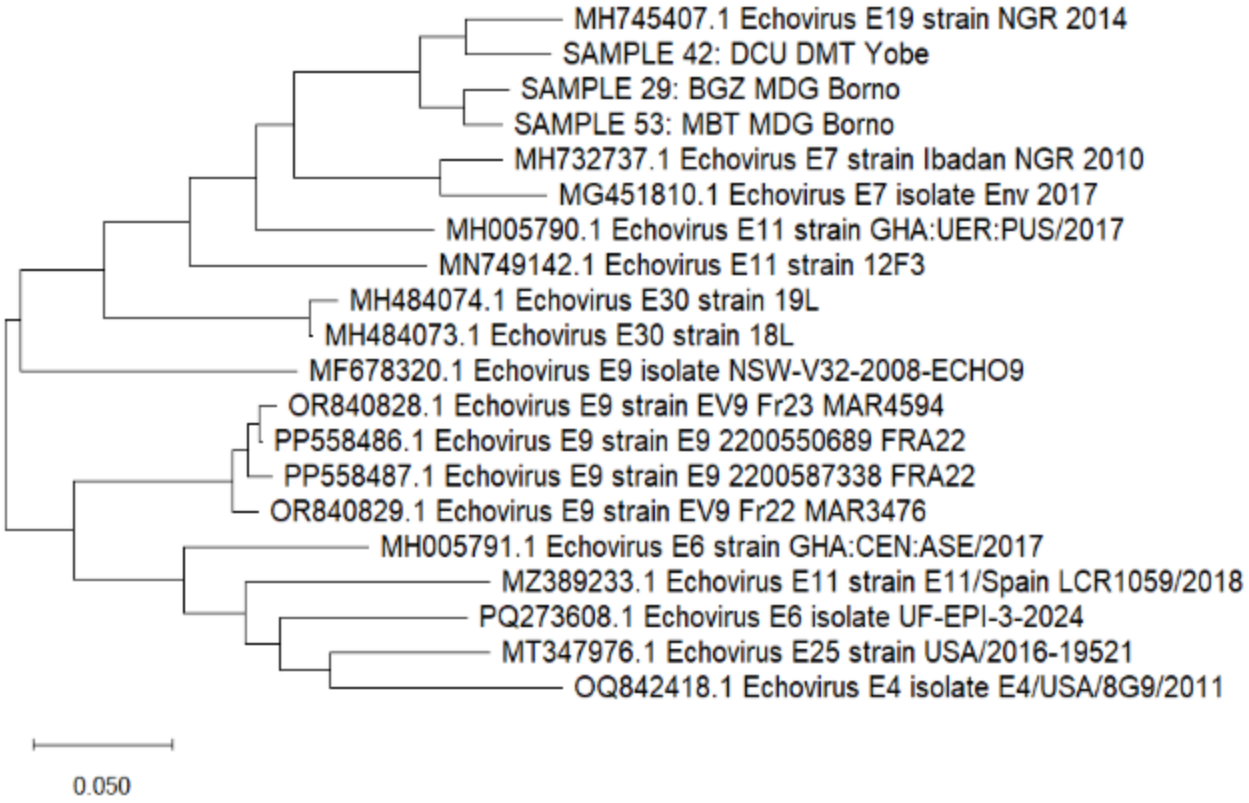


Figure 4

Neighbour-joining phylogenetic analysis of three study-derived VP1 sequences and selected reference sequences. Bootstrap values at internal nodes indicate the percentage support obtained from 1,000 replicates. Evolutionary distances were estimated using the Kimura two-parameter model.

Supplementary Files

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- SupplTableS1.docx