



QMRA of Waterborne Adenovirus and Rotavirus: A Source Tracking and RT-qPCR Approach

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Abstract

Surface water used for domestic purposes in under developed countries presents a critical pathway for the transmission of enteric viruses. This research combines molecular detection, faecal source tracking, and quantitative microbial risk assessment (QMRA) to develop a predictive framework for assessing viral infection risks associated with domestic water use in Essien Udim Local Government Area in Akwa Ibom State, Nigeria. Surface water samples were analyzed for adenovirus and rotavirus using RT-qPCR to determine viral concentrations and spatial–temporal variability. Somatic coliphages were quantified as indicators of faecal contamination sources. Exposure scenarios encompassing drinking, bathing, and food preparation were modeled to estimate daily viral dose, while infection probabilities were calculated using exponential and Beta-Poisson dose–response models. Annual infection risks were derived and compared with WHO acceptable risk level of 10^{-4} infections/person/year. Sensitivity and uncertainty analyses were performed to identify dominant risk drivers. Results showed widespread detection of enteric viruses with significant variability across sites and seasons. Estimated annual infection risks exceeded acceptable thresholds (10^{-4} infections person⁻¹ year⁻¹) in multiple exposure scenarios, particularly for direct ingestion pathways. Source tracking indicated that human-derived faecal contamination was the dominant contributor to viral loads. Sensitivity analysis identified viral concentration and ingestion rate as the most influential parameters. This research highlights the value of combining environmental virology with predictive risk modeling to inform water safety management. The findings provide actionable insights for mitigating viral transmission risks and contribute to the development of sustainable water quality management strategies aligned with global health targets.

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1.0 Introduction

Access to safe water remains a major global challenge, particularly in developing regions where untreated surface water is frequently used for domestic activities (Okafor et al., 2024). Surface waters are highly susceptible to contamination from human and animal faecal inputs especially during swimming and heavy rainfall, serving as reservoirs for enteric viruses such as adenovirus and rotavirus (Fong & Lipp, 2005). These viruses are of significant public health concern due to their persistence in the environment, low infectious dose, and resistance to conventional water treatment methods (Chigor et al., 2014). The lack of social amenities in developing countries forces the people to use the water for domestic purposes despite knowing that it is contaminated (Galadima et al., 2011).

Traditional water quality monitoring relies heavily on bacterial indicators, which often fail to accurately represent viral contamination (Holcomb et al., 2020). This is because traditional water quality monitoring based on fecal indicator bacteria (e.g., *Escherichia coli*) often underestimates viral contamination because enteric viruses such as Adenovirus and Rotavirus persist longer in water bodies, resist treatment more effectively, and do not correlate reliably with bacterial indicators. Furthermore, viruses are much more resistant to environmental stress than bacterial, and they lack metabolic activities which makes them less susceptible to environment. Viruses persist longer in water and cannot replicate outside their host hence, advances in molecular techniques such as reverse transcription quantitative polymerase chain reaction (RT-qPCR) have improved the detection and quantification of viral pathogens in environmental matrices (Liu et al., 2023). Quantitative microbial risk assessment (QMRA) provides a structured framework for estimating the likelihood of infection based on pathogen concentration, exposure, and dose–response relationships

(Hamilton et al., 2024). However, there remains a gap in integrating molecular detection, source tracking, and predictive modeling for comprehensive risk evaluation (Zhang et al., 2019).

This study therefore aims to quantify adenovirus and rotavirus in surface waters using RT-qPCR, estimate exposure and infection risks using QMRA, apply exponential and Beta-Poisson dose–response models, and identify faecal pollution sources using somatic coliphages.

2.0 Materials and Methods

2.1 Sample collection

River water samples were collected from multiple locations in Essien Udim Local Government Area in Akwa Ibom State, Nigeria used for domestic purposes. Sampling was conducted across dry and wet seasons to capture variability. Water samples were collected aseptically in sterile 10 L polypropylene containers from designated sampling points. Samples were transported on ice (4 °C) to the laboratory and processed within 6–12 hours of collection to minimize viral degradation (Williams et al., 2024).

2.2 Viral concentration and extraction

Virus particles were concentrated using an electronegative membrane filtration method (Miao et al., 2019). 10 L of each water sample was filtered through a 0.45 µm pore-size, 47 mm diameter mixed cellulose ester membrane (Millipore). Prior to filtration, samples were acidified to pH 3.5 using 1 N HCl and supplemented with MgCl₂ to a final concentration of 25 mM to enhance viral adsorption onto the membrane. Following filtration, adsorbed viruses were eluted using 10 mL of 1.5% beef extract solution (pH 9.5) with gentle agitation for 10 minutes. The eluate was then neutralized to pH 7.0 using 1 N HCl. To further concentrate viral particles, the eluate was subjected to polyethylene glycol (PEG 8000) precipitation (final concentration: 10% w/v PEG and 0.3 M NaCl) and incubated at 4 °C

overnight. The precipitate was recovered by centrifugation at $10,000 \times g$ for 30 minutes, resuspended in 1 mL of sterile phosphate-buffered saline (PBS), and stored at -80°C until nucleic acid extraction. Viral nucleic acids were extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer's instructions with slight modifications to improve yield. For each sample, 140 μL of concentrated viral suspension was lysed using AVL buffer containing carrier RNA to enhance nucleic acid recovery. After lysis, ethanol was added, and the mixture was transferred to silica spin columns for binding. Sequential washing steps with AW1 and AW2 buffers were performed to remove impurities and PCR inhibitors. RNA/DNA was eluted in 60 μL of RNase-free water and immediately stored at -80°C . Extraction efficiency was monitored using an external process control virus (e.g., murine norovirus).

Quantitative detection was then performed targeting Adenovirus (hexon gene) and Rotavirus VP6 gene and previously validated primer-probe sets were used to ensure high specificity and sensitivity. RT-qPCR assays were carried out using the QuantiTect Probe RT-PCR Kit (Qiagen, Germany) in a 25 μL reaction volume (12.5 μL 2 \times RT-PCR Master Mix, 0.5 μL reverse transcriptase enzyme, 400 nM of each primer, 200 nM probe, 5 μL template RNA/DNA). Thermal cycling was performed on a Applied Biosystems 7500 Real-Time PCR System under the following conditions (reverse transcription 50°C for 30 min, Initial denaturation 95°C for 15 min and 40 cycles of denaturation 94°C for 15 s and annealing/extension: 60°C for 60 s). All reactions were performed in triplicate, including no-template controls (NTCs) and positive controls.

2.3 Standard Curve

Quantification was achieved using standard curves generated from tenfold serial dilutions (10^1 – 10^7 copies/ μL) of plasmid DNA containing

target gene inserts. Amplification efficiency (90–110%) and correlation coefficients ($R^2 \geq 0.99$) were considered acceptable. Viral concentrations were expressed as genome copies per liter (gc/L)

2.4 Somatic Coliphage Enumeration

Somatic coliphages were enumerated using the standardized double agar layer (DAL) method, adapted from International Organization for Standardization (ISO 10705-2) protocols (Mooijman et al., 2001). The bacterial host strain, *Escherichia coli* WG5 (ATCC 700078), was used due to its high sensitivity to somatic coliphages (Yildirim et al., 2021). A fresh overnight culture was grown in tryptic soy broth (TSB) at 37°C to the logarithmic phase ($\text{OD}_{600} \approx 0.5$). 10 mL of water samples was used after appropriate dilution in sterile phosphate-buffered saline (PBS). Each aliquot was mixed with 1 mL of the host *E. coli* WG5 culture and 3 mL of molten soft agar (0.7% agar), maintained at 45°C . The mixture was immediately poured onto pre-solidified bottom agar plates (1.5% agar) and allowed to solidify. Plates were incubated inverted at 37°C for 16–18 hours. After incubation, clear zones (plaques) representing lytic phage activity were counted manually. Plates with 20–200 plaques were considered valid for enumeration. All samples were analyzed in duplicate to ensure accuracy and reproducibility. Host culture viability and media sterility were verified using spiked samples

2.5 Exposure Assessment

Exposure assessment was conducted to estimate human ingestion of viral pathogens through multiple domestic water-use pathways. The assessment incorporated measured concentrations of enteric viruses (genome copies per liter, gc/L) and somatic coliphages (PFU/100 mL) with pathway-specific ingestion rates and exposure frequencies. A deterministic and probabilistic framework was applied to capture variability in human behavior and environmental conditions (Chiu et al., 2015). Virus-specific parameterization was applied to account

for differences in recovery and infectivity between adenovirus and rotavirus. Recovery efficiency (R) was modeled using triangular distributions of 0.40–0.60 (mode = 0.50) for adenovirus and 0.20–0.50 (mode = 0.35) for rotavirus, reflecting variability in electronegative filtration and PEG concentration performance reported in previous studies (Miao et al., 2019; Chigor et al., 2014). Since the presence of the virus's nucleic acid does not indicate the infectivity of the virus, the proportion of infectious viruses was calculated using infective viral ratios previously calculated. The proportion of 1:2 was used for adenovirus (Van Heerden *et al.* 2005), and rotavirus, we have 1:40,000 (Sibanda and Okoh 2013) to account for differences in environmental persistence and viability. These parameters were treated as stochastic variables in the Monte Carlo simulation to propagate uncertainty in virus-specific risk estimates. This distribution was propagated through 10,000 Monte Carlo iterations to capture variability and uncertainty in risk estimates. The exposure analysis was calculated using equation 1.

$$d = C \times 1/R \times I \times 10^{-DR} \times V \quad (1)$$

where d = daily exposure; C = enteric viral concentration in water; R = recovery technique efficiency; I = infectivity of the virus; DR = removal efficiency of the treatment process, it is usually zero for untreated surface water); V = amount of water consumed by an individual (Van Heerden *et al.* 2005; Venter *et al.* 2007; Chigor et al., Okoh 2014).

2.6 Dose Response Modelling

Dose–response modeling was conducted to estimate the probability of infection associated with ingestion of enteric viruses detected in surface waters. The models relate the ingested viral dose (derived from exposure assessment) to the likelihood of infection in exposed individuals. Two widely accepted models - the exponential model and the beta-Poisson model were applied to capture variability in host–

pathogen interactions and to ensure robustness of risk estimates. The ingested dose calculated for each exposure pathway (drinking, recreation, domestic use, and food preparation) was used as input into both models to estimate daily probability of infection and the annual infection risk using equations 2 – 4 (Van Heerden et al., 2005; Sibanda & Okoh 2013; Chigor et al., 2014).

$$HAdV: P_{infection} / day = 1 - \exp(-rd) \quad (2)$$

$$RoV: P_{infection} / day = 1 - [1 + d/\beta]^{-\alpha} \quad (3)$$

HAdV, RoV:

$$P_{infection} / year = 1 - (1 - P_{infection} / day)^{365} \quad (4)$$

where $\alpha = 0.2531$, $\beta = 0.4265$, $r = 0.4172$ according to Sibanda and Okoh (2013), *HAdV* stands for Human Adenovirus and *RoV* stands for Rotavirus.

2.7 Monte Carlo Simulation

Monte Carlo simulation (10,000 iterations) was performed using Microsoft Excel with the @RISK add-in. Convergence was assessed by monitoring the stabilization of mean and percentile infection risk estimates and by evaluating cumulative mean plots across iterations. The simulation was considered converged when changes in output statistics were less than 1% with increasing iterations.

3.0 Result

The quantified concentrations of Adenovirus and Rotavirus across the sampling sites (10^3 – 10^4 genome copies/L) clearly indicate substantial viral contamination of surface waters used for domestic purposes as shown in table 1. With site B exhibiting the highest viral load for both viruses and site C showed the lowest concentrations, although still significant. Both viruses followed a similar spatial pattern, suggesting common contamination sources. Table 2 showed that the estimated daily and annual infection risks across exposure pathways reveal extremely elevated health risks. The result showed that out of the three exposure

pathways, drinking water pathway had the highest annual risk (~ 0.98). Water used for food preparation (~ 0.89) followed because some foods are often consumed raw like vegetables and fruits which are often prepared using this water directly and finally bathing (~ 0.72) also show substantial risks because during the process of bathing ingestion of water is believed to occur. However, all estimated values far exceeded the acceptable benchmark of 10^{-4} infections/person/year. Figure 1 illustrates spatial variability in adenovirus contamination with clear variation between sites, peak concentration at Site B and noticeable decline at Site C. Rotavirus distribution Figure 2 closely mirrors that of adenovirus, reinforcing consistency in contamination patterns. However,

showing similar ranking of sites ($B > A > C$) and comparable magnitude of concentrations among the research areas. Figure 3 compared estimated annual risks with acceptable health benchmarks and the result showed that all exposure pathways exceeded the WHO benchmark (10^{-4}) and the drinking pathway approaches maximum risk (~ 1) and finally even the lowest pathway (bathing) remains critically high. The Monte Carlo risk distribution histogram (Figure 4) represents the probability distribution of daily infection risk, capturing uncertainty and variability. The figure showed wide spread of risk values, right-skewed distribution (presence of high-risk scenarios) and no clustering around "safe" risk levels.

Table 1: Viral concentration in surface water

Site	Adenovirus (gc/L)	Rotavirus (gc/L)
A	2.5×10^3	1.8×10^3
B	4.2×10^3	3.1×10^3
C	1.7×10^3	1.2×10^3

Table 2: Estimated infection risk

Exposure Pathway	Daily Risk	Annual Risk
Drinking	1.2×10^{-2}	0.98
Bathing	3.5×10^{-3}	0.72
Food Prep	6.1×10^{-3}	0.89

4.0 Discussion

The detection of adenovirus and rotavirus across all sampling sites confirms widespread viral contamination of surface waters used for domestic purposes as seen in table 1. The observed concentrations are consistent with previous studies in underdeveloped countries, highlighting the persistence and environmental stability of enteric viruses due to low income (Awere-Duodu & Donkor, 2024). The elevated viral loads reflect recent or continuous faecal contamination, which is likely from domestic sewage discharge into the river body or open defecation and surface runoff during rainfall events, washing off faeces in the environment

into the river. Poor drainage which empties into the surface water also serve as a source of contamination and waste disposal by some people into the water act as a source of contamination to the water body. The co-occurrence of adenovirus and rotavirus suggests shared transmission pathways, reinforcing the reliability of these viruses as indicators of faecal pollution. It is important to note that even the lowest recorded concentrations remain epidemiologically significant, given that low infectious dose of enteric viruses can cause disease. This means that even minimal exposure can result in infection.

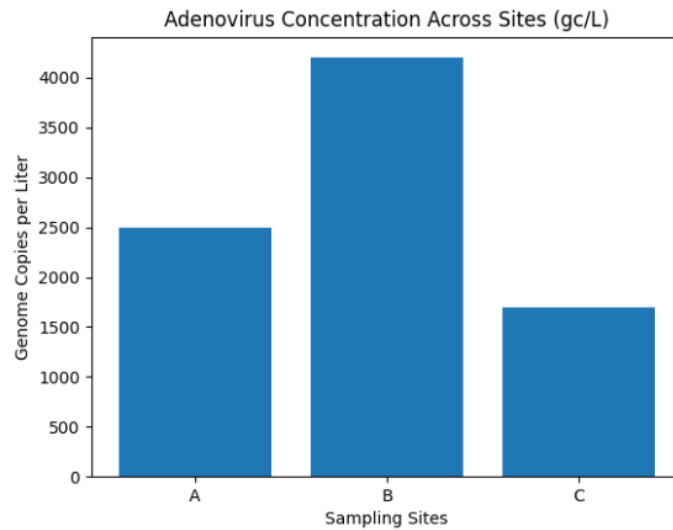


Figure 1: Adenovirus concentrations (genome copies/L) across sampling sites showing spatial variability in viral contamination

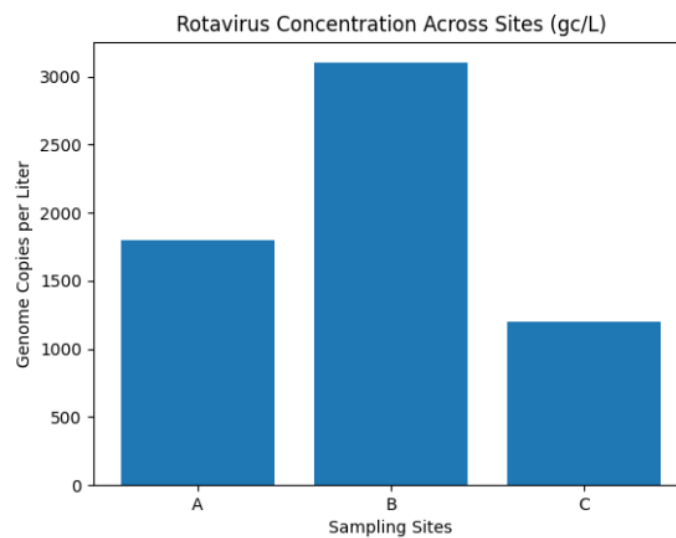


Figure 2: Rotavirus concentrations (genome copies/L) across sampling sites indicating similar contamination trends

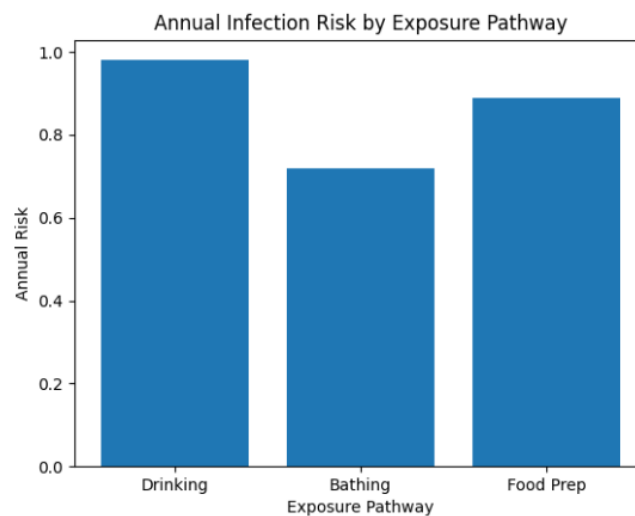


Figure 3: Estimated annual infection risk across domestic exposure pathways compared to acceptable health benchmarks

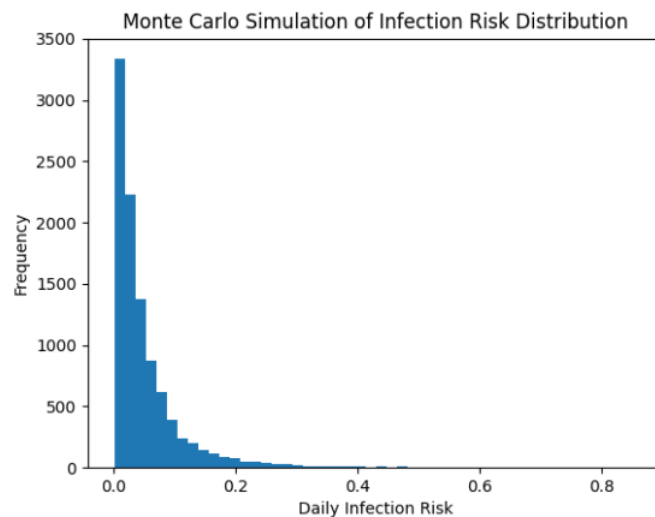


Figure 4: Probability distribution of daily infection risk derived from Monte Carlo simulation ($n = 10,000$), illustrating variability and uncertainty in exposure

The QMRA results indicate that infection risks exceed acceptable thresholds, particularly for drinking water exposure. This underscores the significant health risks associated with untreated water consumption and is in accordance with other research work in under developed countries (Bivins et al., 2017). The application of both exponential and Beta-Poisson models provides robustness in risk estimation, accounting for variability in host-pathogen interactions. The quantitative microbial risk results further indicated significant contribution of indirect exposure routes (food such as fruits and vegetables and bathing) to infection and showed that the high risk were driven by an elevated viral concentration in the water body, frequent exposure to the contaminated river water and a high ingestion rate (intentional or accidental) of the contaminated water. This research has proved that surface water use without treatment is unsafe, even for non-drinking purposes.

The use of somatic coliphages as indicators enabled the identification of faecal pollution sources, with evidence suggesting a predominance of human-derived contamination. This has important implications for targeted interventions such as sanitation improvements and waste management. As shown in Figure 1 and 2 both viruses show a common faecal origin with viral contamination pattern

been stable for both pathogens. The parallel trends strengthen confidence in the dataset and suggest that monitoring one virus could serve as a proxy for others in similar environments. The finding of this research has linked contamination directly to sanitation failure and provided a basis for targeted mitigation strategies, such as improved sewage management, control of open defecation and protection of water sources from human waste. Sensitivity analysis revealed that viral concentration and ingestion rate are the most critical factors influencing infection risk. This suggests that reducing exposure through water treatment and behavioral changes could significantly lower health risks. The variability in Monte-Carlo risk distribution reflects real-world fluctuations in the viral concentration, exposure frequency and individual behavior. The right-skewed nature of the graph indicates occasional extreme risk events and potential outbreak scenarios under certain conditions like in conditions of famine and scarcity of resources. This highlights that average risk values may underestimate true danger and risk management must consider worst-case scenarios, not just mean estimates. Overall, this study demonstrates the effectiveness of integrating molecular detection with QMRA and source tracking in providing a comprehensive assessment of waterborne viral risks. The

findings support the need for improved water quality management strategies and policy interventions to safeguard the health of the public.

5.0 Conclusion

This study demonstrates that surface waters used for domestic purposes are significantly contaminated with enteric viruses, posing substantial health risks to exposed populations. The integration of RT-qPCR detection, faecal source tracking, and quantitative microbial risk assessment revealed that infection risks far exceed internationally acceptable thresholds across multiple exposure pathways. Drinking water ingestion was identified as the most critical route of exposure; however, non-potable uses such as bathing and food preparation also contribute significantly to overall risk. The application of both exponential and Beta-Poisson dose–response models confirmed the high infectivity of enteric viruses, even at low doses. Monte Carlo simulation highlighted considerable variability and uncertainty in infection risk, with viral concentration and ingestion rate emerging as key determinants. Source tracking analysis further identified human faecal contamination as the dominant pollution source, emphasizing the role of inadequate sanitation infrastructure in driving waterborne disease transmission. Overall, this study underscores the urgent need for improved water quality management, including effective treatment interventions, sanitation upgrades, and public health education. The integrated QMRA framework presented herein provides a robust, evidence-based tool for guiding policy decisions and mitigating the burden of waterborne viral infections, particularly in resource-limited settings.

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