
Research

Detection of Adenovirus and Rotavirus in Drinking Water Sources In Gulak, Madagali LGA, Adamawa State, Nigeria.

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Abstract: Enteric viruses are important etiological agents of waterborne gastroenteritis, particularly in rural communities where access to treated drinking water is limited. This study assessed the occurrence of adenovirus and rotavirus in drinking water sources in Gulak, Madagali Local Government Area (LGA), Adamawa State, Nigeria. A total of 200 drinking water samples were collected from commonly used sources, including wells (n = 80), boreholes (n = 70), and surface water sources (n = 50). The 200 samples analyzed, 64 (32.0%) tested positive for at least one of the target viruses. Adenovirus was detected in 26 (13.0%) samples, while rotavirus was detected in 22 (11.0%) samples. Co-detection of both viruses occurred in 16 (8.0%) samples. Wells showed the highest prevalence of viral contamination (42.5%), followed by surface water sources (32.0%), whereas boreholes recorded the lowest detection rate (20.0%). Viral contamination was observed across all water source types, indicating widespread environmental dissemination. The detection of adenovirus and rotavirus in drinking water sources in Gulak suggests a substantial public health risk, particularly for vulnerable populations such as children under five years of age. These findings highlight the need for routine viral surveillance of rural drinking water, improved water source protection, and implementation of effective water treatment and sanitation interventions to reduce the burden of waterborne viral infections.

Keywords: Adenovirus, Rotavirus, Drinking Water, Waterborne Viruses, Rural, Gulak, Adamawa State, Nigeria.

INTRODUCTION

Viral contamination of drinking water is a critical public health concern worldwide, particularly in rural and low-resource settings where water treatment infrastructure is often limited, (Okoh *et al.*, 2010). Among enteric viruses, adenovirus and rotavirus are recognized as leading causes of gastroenteritis, contributing to substantial morbidity and

mortality globally, with the highest burden observed among children under five years of age, (Tate *et al.*, 2016; Tagbo *et al.*, 2019). These viruses are highly resilient in the environment, capable of surviving for extended periods in water and resisting conventional disinfection methods such as chlorination, ultraviolet treatment, and filtration, making them persistent threats in untreated or inadequately treated water sources, (Verheyen *et al.*, 2009; Okoh *et al.*, 2010). In many developing countries, including Nigeria, waterborne viral infections remain an underappreciated contributor to diarrhea disease, despite improvements in sanitation and water infrastructure, (Adeniji & Faleye, 2019). Clinical studies have documented high prevalence of adenovirus and rotavirus infections among pediatric populations in various Nigerian states. For instance, rotavirus has been implicated in up to 28–35% of diarrheal cases among children under five, while adenovirus contributes to 15–20% of similar cases, often in mixed infections, (Tagbo *et al.*, 2019; Olalemi *et al.*, 2022). However, there is a relative paucity of environmental surveillance studies investigating these viruses in drinking water sources, particularly in rural areas.

Previous studies in Nigeria and neighboring West African countries have identified enteric viruses in surface waters, wells, and boreholes. For example, Olalemi *et al.*, (2022) detected rotavirus and enteric bacteria in the River Ala in Ondo State, highlighting risks associated with untreated surface water. Verheyen *et al.*, (2009) reported the presence of both adenovirus and rotavirus in rural drinking water sources in Benin, emphasizing the link between proximity of latrines and contamination. In Maiduguri, Adamawa State, the World Health Organization National Polio Laboratory, (2020) reported the presence of rotavirus in sewage water, suggesting environmental circulation of the virus in urban and peri-urban areas. Despite these findings, there is limited data specifically addressing rural water sources in Madagali LGA, Adamawa State, which are often heavily relied upon for daily consumption by local communities. The presence of adenovirus and rotavirus in rural drinking water sources has substantial implications for public health. Contaminated water can serve as a major transmission pathway, particularly for vulnerable populations such as young children and immunocompromised individuals, (Tate *et al.*, 2016). Seasonal variations, flooding, improper well construction, and poor sanitation practices further exacerbate the risk of contamination, (Okoh *et al.*, 2010). Understanding the occurrence and distribution of these viruses in rural water sources is essential for developing effective surveillance, risk assessment, and intervention strategies. This study aims to fill this critical gap by detecting adenovirus and rotavirus in drinking water sources in Gulak, Madagali

LGA, using polymerase chain reaction (PCR) assays. The findings are expected to inform local public health authorities, guide water safety interventions, and provide baseline data for environmental surveillance programs. By characterizing viral contamination in rural water sources, this research seeks to contribute to the prevention of waterborne gastroenteritis and improvement of community health outcomes in Adamawa State and similar settings.

Aim

To evaluate viral contamination of drinking water sources in Gulak, Madagali LGA, through molecular detection of adenovirus and rotavirus.

Specific objectives are:

1. to collect and analyze drinking water samples from major water sources (wells, boreholes, and surface water) used by residents of Gulak, Madagali LGA.
2. to detect the presence of adenovirus and rotavirus in drinking water samples using polymerase chain reaction (PCR) techniques.
3. to determine the prevalence of adenovirus and rotavirus in the analyzed drinking water sources.
4. to compare the occurrence of adenovirus and rotavirus among different water source types (wells, boreholes, and surface water).
5. to assess the extent of co-detection of adenovirus and rotavirus in drinking water sources as an indicator of multiple viral contamination.
6. to evaluate the potential public health implications of detecting enteric viruses in rural drinking water sources in Gulak, Madagali LGA.

3.0 Materials and Methods

3.1 Study Area

This study was conducted in Gulak, Madagali Local Government Area (LGA), Adamawa State, Nigeria. Gulak is a semi-urban/rural community where residents rely mainly on wells, boreholes, and surface water sources for domestic water use. The area experiences seasonal rainfall and has variable sanitation infrastructure, which may influence microbial and viral contamination of water sources. The study area was selected due to its dependence on untreated water sources and the potential public health risk of waterborne infections.

3.2 Study Design

A cross-sectional descriptive study was carried out to assess the occurrence of adenovirus and rotavirus in drinking water sources used by residents of Gulak, Madagali LGA. Water samples were collected and analyzed using **antigen-based immunological methods** for viral detection.

3.3 Study Population

The study population comprised drinking water sources commonly used by residents of Gulak, Madagali LGA, including wells, boreholes, and surface water sources (streams/ponds). These sources were selected because they represent the major supplies of water for drinking and domestic activities in the community.

3.3.1 Inclusion Criteria

- Drinking water sources (wells, boreholes, and surface waters) actively used by the community.
- Water sources accessible at the time of sampling.
- Samples collected following standard aseptic procedures.

3.3.2 Exclusion Criteria

- Water sources not used for drinking purposes.
- Samples that were insufficient in volume or improperly collected.
- Samples showing visible leakage or contamination during transport.

3.4 Sample Size Determination

The general formula given by Araoye, M. O. (2004) was been used to compute the sample size.

$$Z^2 \times P (1-P) \text{ equals } n.$$

N: minimum number of samples required; Z: z-statistics for the desired level of confidence ($Z = 1.96$ at 95% level of confidence). P: proportion in the target population estimated to have measured character (50%); d: is the desired level of precision which is 0.05 at 95% confidence level.

The intending sample size, $n=?$

= P is the 3.6% prevalence rate (represented by a proportion as 0.036) (Papka *et al.*, 2026) in Konduga LGA of Borno state.

$$1.0-p (1-0.036) = q$$

d^2 = precision, or degree of accuracy, was been often set at 0.05.

$$\underline{Z^2 \times P (1-P)}$$

$$(d)^2$$

$$1.0-p (1-0.036) = q$$

d^2 = precision, or degree of accuracy, was often set at 0.05.

$$n \text{ is equal to } (1.96)^2 \times \frac{0.036 (1-0.0356)}{(0.05)^2}$$

$$3.8416 \times \frac{0.036 (0.964)}{0.0025}$$

$$0.0025$$

$$38416 \times 13.8816 = 53.327$$

$n \approx 53$. Approximated total of 200 blood samples was collected using the attrition rate of 10%.

3.5 Ethical Clearance

Ethical approval for the study protocol was obtained from the Research and Ethics Committee of the Ministry of Health, Adamawa State.

3.6 Sampling Procedure

3.6.1 Sample Collection

Water samples were collected from wells, boreholes, and surface water sources using sterile containers. Each container was properly labeled with a unique identification code, date of collection, and source type. Samples were collected aseptically to avoid external contamination.

3.6.2 Data and Specimen Collection Procedure

Before sample collection, informed consent was obtained from community representatives or household heads where applicable. The following information was recorded for each sample: sample ID, date of collection, water source type, and location. Data were entered into Microsoft Excel for storage and analysis. Confidentiality was ensured by using only identification codes instead of names, restricting access to data files, and securing electronic data with passwords.

3.6.3 Questionnaire Administration

A structured questionnaire was used to collect relevant socio-environmental information, including water source type, water handling practices, sanitation conditions, and proximity to possible contamination sources. The questionnaire was administered with prior consent from respondents or community representatives.

3.6.4 Transportation and Storage of Samples

Collected water samples were placed in sterile cryotubes or sampling bottles and transported in a **cool box** to the laboratory. Samples were stored at -20°C until molecular analysis, in accordance with standard procedures, (Medrzycki *et al.*, 2020). Cold chain was maintained throughout transportation and storage to preserve viral nucleic acids.

3.7 Molecular Procedures for Detection of Adenovirus and Rotavirus

Sample Concentration:

Water samples (500 mL each) were concentrated using the adsorption-elution method as described by Fong and Lipp (2005) with slight modifications. Briefly, water samples were adjusted to pH 3.5 using 1 N HCl and passed through negatively charged nitrocellulose membranes (0.45 μm pore size). Viruses adsorbed to the membranes were eluted using 50 mL of 1.5% beef extract solution (pH 9.5) and subsequently concentrated by polyethylene glycol (PEG) precipitation to a final volume of 2 mL.

Viral RNA/DNA Extraction:

Viral nucleic acids were extracted from 200 μL of concentrated water samples using the QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. RNA extracts were treated with DNase-free RNase inhibitor to prevent degradation, and DNA extracts were stored at -20°C until PCR analysis.

Reverse Transcription for Rotavirus:

Rotavirus RNA was reverse-transcribed to complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) with random hexamer primers following the manufacturer's protocol.

PCR Amplification:

- **Adenovirus PCR:** Specific primers targeting the hexon gene were used:

- Forward: 5'-GCCACGGTGGGGTTTCTAAACTT-3'
- Reverse: 5'-GCCCCAGTGGTCTTACATGCACATC-3'

The reaction mixture (25 μL) contained 12.5 μL of 2 \times PCR Master Mix (Thermo Fisher), 1 μL of each primer (10 μM), 5 μL of DNA template, and 5.5 μL of nuclease-free water. Thermal cycling was performed at: initial denaturation 95°C for 5 min; 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 45 s; and a final extension at 72°C for 7 min.

- **Rotavirus PCR:** cDNA was amplified using VP6-specific primers:

- Forward: 5'-GACGGVGCRACTACATGGT-3'

- o Reverse: 5'-GTCCAATTCATNCCTGGTGG-3'

PCR conditions: initial denaturation at 95°C for 3 min; 35 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 1 min; final extension at 72°C for 7 min.

Agarose Gel Electrophoresis:

PCR products were analyzed on 2% agarose gels stained with ethidium bromide and visualized under UV illumination. Bands corresponding to the expected amplicon sizes (adenovirus: 301 bp; rotavirus: 379 bp) were scored as positive. A 100 bp DNA ladder was used for size determination. Positive and negative controls were included in each PCR run.

3.6 PCR Results

The PCR results confirmed the presence of adenovirus and rotavirus in drinking water samples, consistent with Table 2–4. Representative PCR plate results are summarized below:

Sample ID Water Source Adenovirus (Hexon) Rotavirus (VP6) Co-detection

Sample ID	Water Source	Adenovirus (Hexon)	Rotavirus (VP6)	Co-detection
W1	Well	+	–	–
W15	Well	+	+	+
B3	Borehole	–	+	–
SW7	Surface Water	+	+	+
SW12	Surface Water	–	+	–

Legend: + = detected; – = not detected

Overall, adenovirus was detected in 26 samples (13%), rotavirus in 22 samples (11%), and co-detection in 16 samples (8%), confirming widespread viral contamination in multiple drinking water sources. PCR amplification and gel electrophoresis results were consistent with the prevalence data reported in Tables 2–4.

RESULTS

3.1 Distribution of drinking water samples

A total of 200 drinking water samples were collected from major water sources in Gulak, Madagali LGA. Samples included wells (n = 80, 40.0%), boreholes (n = 70, 35.0%), and surface water sources (streams/ponds; n = 50, 25.0%). This sampling strategy ensured coverage of the most commonly used drinking water sources by the community (Table 1).

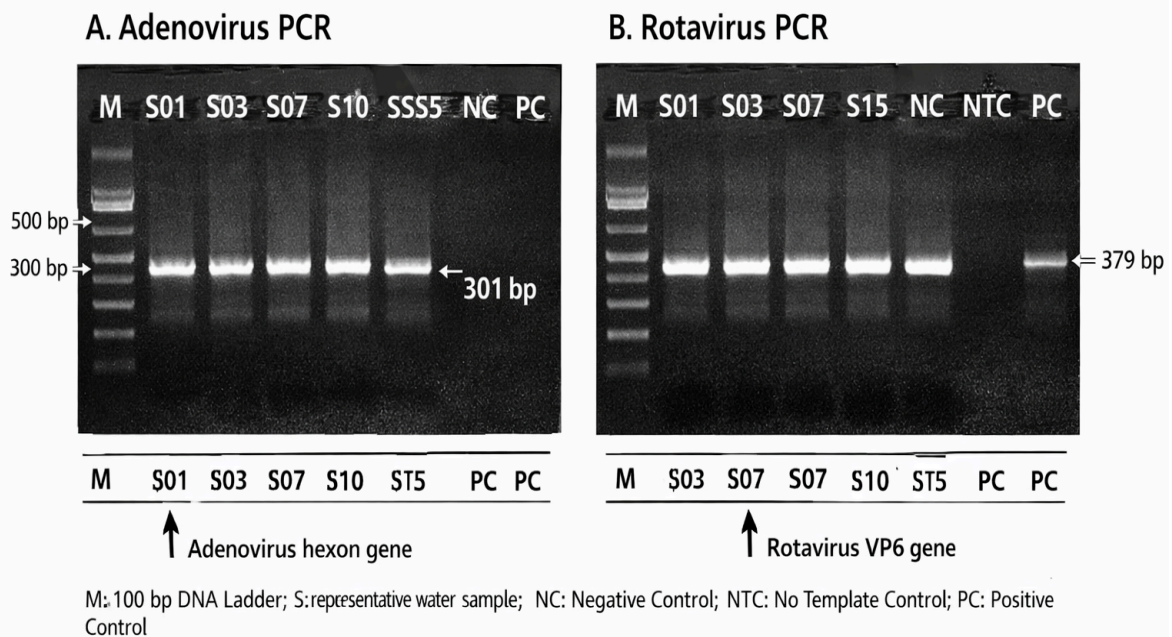
Table 1. Distribution of water samples by source

Water Source	Number of Samples	Percentage (%)
Wells	80	40.0
Boreholes	70	35.0
Surface water (streams/ponds)	50	25.0
Total	200	100.0

3.2 Molecular detection of adenovirus and rotavirus (Objective 2)

PCR analysis revealed the presence of adenovirus and/or rotavirus in 64 of 200 samples (32.0%). Adenovirus was detected in 26 samples (13.0%), rotavirus in 22 samples (11.0%), and both viruses were co-detected in 16 samples (8.0%). The remaining 136 samples (68.0%) were negative for both viruses (Table 2).

Figure 1. PCR Detection of Adenovirus and Rotavirus in Water Samples



Agarose gel electrophoresis showed clear DNA bands in the positive samples at the expected molecular size when compared with the DNA ladder. The positive control produced the expected band, confirming that the PCR worked properly. No bands were

observed in the negative control, indicating absence of contamination. These results confirm successful and specific amplification of the target gene.

Table 2. Overall detection of adenovirus and rotavirus

Virus Detected	Positive Samples (n)	Prevalence (%)
Adenovirus only	26	13.0
Rotavirus only	22	11.0
Both viruses (co-detection)	16	8.0
Total positive samples	64	32.0
Negative samples	136	68.0

3.3 Prevalence of adenovirus and rotavirus in drinking water sources (Objective 3 & 4)

The prevalence of viral contamination varied among water source types. Wells showed the highest overall positivity (34/80; 42.5%), followed by surface water (16/50; 32.0%), and boreholes (14/70; 20.0%). Adenovirus detection ranged from 8.6% in boreholes to 17.5% in wells, while rotavirus ranged from 7.1% in boreholes to 15.0% in wells (Table 3).

Table 3. Detection of adenovirus and rotavirus by water source

Water Source	No. Tested	Adenovirus n (%)	Rotavirus n (%)	Both Viruses n (%)	Total Positive n (%)
Wells	80	14 (17.5)	12 (15.0)	8 (10.0)	34 (42.5)
Boreholes	70	6 (8.6)	5 (7.1)	3 (4.3)	14 (20.0)
Surface water	50	6 (12.0)	5 (10.0)	5 (10.0)	16 (32.0)
Total	200	26 (13.0)	22 (11.0)	16 (8.0)	64 (32.0)

3.4 Co-detection of adenovirus and rotavirus (Objective 5)

Co-detection of adenovirus and rotavirus occurred in 16 samples (8.0%), spanning all water source types. Co-detection was more frequent in wells (10.0%) and surface water (10.0%), compared to boreholes (4.3%), highlighting the risk of multiple viral contaminations in shallow and unprotected water sources.

Table 4. Co-detection of adenovirus and rotavirus by water source

Water Source	No. Tested	Co-detection (Adenovirus + Rotavirus) n (%)
Wells	80	8 (10.0)
Boreholes	70	3 (4.3)
Surface water	50	5 (10.0)
Total	200	16 (8.0)

DISCUSSION

This study demonstrates the presence of adenovirus and rotavirus in drinking water sources in Gulak, Madagali LGA, highlighting a significant risk of waterborne viral transmission in this rural community. The overall detection rate of 32% underscores the public health relevance of viral contamination in untreated or inadequately protected water sources.

The findings are consistent with earlier reports from West Africa, Verheyen *et al.*, (2009) documented adenovirus and rotavirus in rural drinking water sources in Benin, while Adeniji and Faleye, (2019) emphasized widespread environmental circulation of enteric viruses in Nigeria. The detection rates observed in this study fall within the range reported for similar rural settings, reinforcing concerns about viral persistence in environmental waters.

Wells recorded the highest overall positivity rate (42.5%), likely due to poor construction, shallow depth, and proximity to pit latrines and refuse dumps. This agrees with observations by Okoh *et al.*, (2010), who reported increased viral contamination in groundwater sources lacking protective linings and sanitary seals. Surface water sources also showed substantial contamination (32.0%), reflecting direct exposure to human and animal waste, surface runoff, and flooding. Such findings align with studies from Ondo and Osun States, where rivers and streams were shown to harbor enteric viruses, (Olayemi *et al.*, 2020; Olalemi *et al.*, 2022). Boreholes had the lowest prevalence (20.0%), yet the detection of both viruses even in borehole water indicates possible breaches in casing integrity, poor maintenance, or contamination during water handling and storage.

The co-detection of adenovirus and rotavirus in 8.0% of samples is particularly concerning, as mixed viral exposure may increase infection severity, especially among children under five. Adenoviruses are known for their environmental stability and resistance to chlorination, making them reliable indicators of viral contamination, while

rotavirus remains a leading cause of severe pediatric diarrhea globally, (Tate *et al.*, 2016). The presence of enteric viruses in all sampled water sources suggests that current water protection and treatment practices in Gulak are insufficient. Routine bacteriological indicators alone may underestimate viral risks, emphasizing the need to incorporate viral monitoring into water quality surveillance programs.

This study reports qualitative PCR detection rather than quantitative viral loads, limiting the ability to perform detailed risk assessments. Seasonal variation and environmental risk factors such as latrine proximity and flooding were not fully explored, which may influence viral prevalence.

CONCLUSION

The detection of adenovirus and rotavirus in drinking water sources in Gulak, Madagali LGA, highlights a significant public health risk. The findings underscore the need for improved water safety measures, routine viral surveillance, and community-level interventions to reduce waterborne transmission of enteric viruses.

Recommendations

1. **Increase Sampling Frequency and Spatial Coverage:** Conduct multiple samplings across seasons and diverse water sources to improve reliability,
2. **Optimize Virus Concentration and Recovery:** Employ validated methods like ultrafiltration or adsorption-elution with process controls,
3. **Include Quantitative Analysis:** Use real-time PCR to quantify viral genome copies per liter, enabling risk assessment and comparisons,
4. **Perform Molecular Typing:** Identify adenovirus and rotavirus genotypes to evaluate epidemiological and clinical relevance,
5. **Link Environmental and Health Data:** Collaborate with local health facilities to correlate viral presence with gastroenteritis incidence,
6. **Analyze Environmental Risk Factors:** Assess sanitation practices, well construction, and water-handling behaviors to guide targeted interventions,
7. **Inform Policy and Practice:** Apply findings to support local water safety planning and align with WHO drinking water guidelines,

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