

ORIGINAL ARTICLE

Study of Aichi Virus in Children with Acute Gastroenteritis: a Cross-Sectional Study

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ABSTRACT

Background: Acute gastroenteritis (AGE) is a leading cause of morbidity and mortality in children worldwide, particularly in low- and middle-income countries. Rotavirus and adenovirus are well-established viral etiologies, while Aichi virus (AiV) has emerged as a potential pathogen for AGE.

Methods: We conducted a cross-sectional study of 134 children ≤ 5 years old presenting with AGE to Mansoura Children's Hospital, Egypt, in 2024. Stool samples were tested for rotavirus and adenovirus antigens by ELISA and for AiV RNA by real-time PCR.

Results: Rotavirus was detected in 37.3% of cases, adenovirus in 25.4%, and AiV in 2.2%. All AiV-positive samples were co-detected with rotavirus ($p = 0.05$), while no significant association was observed with adenovirus ($p = 0.57$).

Conclusions: This is the first report of AiV detection in Egyptian children with AGE. Although prevalence was low, its consistent association with rotavirus suggests possible synergistic or opportunistic infection, warranting further epidemiologic and molecular investigations.

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KEYWORDS

Aichi virus, acute gastroenteritis, children, rotavirus,
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INTRODUCTION

Acute gastroenteritis (AGE) remains a significant cause of illness and death among children under five years of age, accounting for an estimated 2.5 million deaths annually [1]. Well-recognized viral pathogens include rotavirus (RVA), adenovirus (AdV), and norovirus (NoV) [2]. More recently, Aichi virus (AiV), a member of the Picornaviridae family and genus Kobuvirus, has been identified as an emerging cause of AGE in different regions worldwide [3,4].

The genus Kobuvirus includes six recognized species (A - F), with AiV type 1 infecting humans [5-10]. AiV is a non-enveloped, positive-sense RNA virus approximately 8.3 kb in size, resistant to chlorine, detergents, alcohol, heat, and acidic environments, facilitating persistence and transmission [11,14,15]. Transmission occurs primarily via contaminated food and water; asymptomatic carriers can contribute to environmental spread [16-18]. Clinical manifestations include fever, vomiting, nausea, abdominal pain, and diarrhea [5,19]. Although AiV has been detected in environmental samples from Egypt [20], it has not yet been investigated in clinical pediatric populations. Therefore, the present study used real-time PCR to determine the prevalence of AiV, rotavirus, and adenovirus in Egyptian children ≤ 5 years presenting with AGE.

MATERIALS AND METHODS

We conducted a retrospective cross-sectional study at Mansoura Children's Hospital, Egypt, between January and December 2024. 134 children ≤ 5 years old presenting with acute gastroenteritis (AGE) within 72 hours of onset were enrolled. AGE was defined as ≥ 3 episodes of watery diarrhea in 24 hours, with or without vomiting, abdominal pain, or fever. Children with secondary diarrhea due to therapy, concurrent respiratory or hepatic infections, or bacterial/protozoal causes confirmed by laboratory methods were excluded.

Sample size was based on non-probability convenience sampling of eligible patients presenting during the study [21].

Ethical approval was obtained from the Mansoura Faculty of Medicine Ethics Committee (R.24.11.2914). Parents or guardians provided written informed consent.

Clinical data collection

Each participant underwent complete clinical history taking and physical examination. Demographic data, clinical symptoms (diarrhea, vomiting, fever, abdominal pain), and residence (urban/rural) were recorded.

Stool collection and processing

Fresh stool samples were collected in sterile containers, immediately transported to the microbiology laboratory, and processed for viral detection.

Rotavirus and adenovirus detection

Antigens were detected using commercial ELISA kits (RIDASCREEN®, R-Biopharm AG, Darmstadt, Germany), following manufacturer instructions.

Aichi virus detection

The remaining stool was processed for RNA extraction and real-time PCR.

RNA Extraction

RNA was extracted from 20% stool suspensions in phosphate-buffered saline using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), per manufacturer's instructions. Extracted RNA was stored at -80°C until analysis.

Reverse transcription

Five microliters of extracted RNA were used for cDNA synthesis. Reactions contained buffer (Applied Biosystems, USA), 10 mM dNTPs, 50 U/ μL MuLV reverse transcriptase, 50 μM random primers, 40 U/ μL RNase inhibitor, and nuclease-free water, in a total volume of 50 μL . Incubation steps included 42°C for 15 minutes, 99°C for 5 minutes, and 5°C for 5 minutes [22].

Real-Time PCR for Aichi virus

AiV-specific primers and probes, previously described by Yamashita et al. [23], were used (Table 1). PCR amplification was performed in a 20 μL reaction containing 5 μL of cDNA, 200 nM forward primer, 900 nM reverse primer, 200 nM probe, and 1 $\times$ TaqMan PCR Master Mix (Applied Biosystems). Cycling conditions: 50°C for 2 minutes, 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Amplification was performed on an Applied Biosystems real-time PCR system.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA). Quantitative variables were tested for normality using the Shapiro-Wilk test and expressed as mean $\pm$ standard deviation (SD) or median (interquartile range [IQR]) as appropriate. Categorical variables were summarized as frequencies and percentages. Comparisons between groups were performed using the chi-square (χ^2) or Fisher's exact test for categorical data and the independent samples *t*-test or Mann-Whitney *U* test for continuous variables, depending on data distribution. A *p*-value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

The study included 134 children with AGE (86 males [64.2%], 48 females [35.8%]; mean age 37.36 ± 12.76 months). Most children were from rural areas (66.4%). The most common clinical manifestation was watery diarrhea (57.5%), followed by abdominal pain (42.5%) and fever (36.6%).

Viral detection

Rotavirus was the most frequently detected pathogen (50/134, 37.3%), followed by adenovirus (34/134, 25.4%). AiV was detected in 3 cases (2.2%).

Table 1. Primer and probe sequences for Aichi virus real-time PCR.

Target	Sequence
Forward primer	CCCAGTGTGCGTAACCTTCT
Reverse primer	GTACCTGCCTGGCATYCCTA
Probe	HEX-ACGCCCTGTGCGGGATGAAA-BHQ

Table 2. Demographic and clinical characteristics of the study population (n = 134).

Age, months (mean $\pm$ SD)	37.36 $\pm$ 12.76
Male sex	86 (64.2%)
Female sex	48 (35.8%)
Watery diarrhea	77 (57.5%)
Abdominal pain	57 (42.5%)
Fever	49 (36.6%)
Rural residence	89 (66.4%)
Urban residence	45 (33.6%)

Table 3. Prevalence of enteric viruses in children with AGE.

Virus	n (%)
Rotavirus	50 (37.3%)
Adenovirus	34 (25.4%)
Aichi virus	3 (2.2%)

Table 4. Association of Aichi virus with rotavirus and adenovirus.

Virus	AiV-positive (n = 3)	AiV-negative (n = 131)	p-value
Rotavirus	3 (100%)	47 (35.9%)	0.05
Adenovirus	0 (0%)	34 (25.9%)	0.57

Coinfections

All AiV-positive samples (n = 3) were co-detected with rotavirus (p = 0.05). No co-detection with adenovirus was observed (p = 0.57).

DISCUSSION

Acute gastroenteritis (AGE) in children remains a significant cause of morbidity and mortality worldwide, particularly in low- and middle-income countries [24,25].

In the present study, rotavirus was the most frequently identified viral pathogen (37.3%), consistent with earlier Egyptian studies that reported prevalence rates ranging from 24% to 39.1% [26-28]. Differences in detection rates across studies may reflect variations in diagnostic methods, geographic regions, and whether participants were hospitalized or recruited from outpatient clinics [28].

Adenovirus was detected in 25.4% of cases, higher than previously reported in Egyptian children with AGE (6.7 - 10.4%) [29,30]. This variation may be related to dif-

ferences in sample size, diagnostic assays, or study population.

Aichi virus (AiV) was identified in 2.2% of children. Although prevalence was low, this is the first report of AiV detection in clinical pediatric samples in Egypt. Previous studies from Europe, Asia, and Africa have documented AiV prevalence ranging from 0.9% to 4.1% in children with AGE [31-38]. The detection of AiV in Egyptian wastewater samples [20], but not previously in patients, suggests environmental circulation with potential spillover to vulnerable pediatric populations.

We also found a significant association between AiV and rotavirus infection ($p = 0.05$), as all three AiV-positive samples were co-detected with rotavirus. However, given the small number of AiV-positive cases, this observation should be interpreted cautiously. Similar findings have been reported in India, China, France, and Australia, where AiV was frequently identified in mixed infections with rotavirus and other enteric viruses [38, 43]. This pattern may reflect shared fecal-oral transmission routes rather than a specific biological interaction between the two viruses. While some studies have suggested that viral coinfections could exacerbate disease severity or prolong viral shedding, the limited data available do not allow firm conclusions about the clinical relevance of AiV-rotavirus coinfection. Further studies with larger sample sizes and detailed clinical characterization are needed to clarify whether AiV plays an active role as a co-pathogen or is merely a bystander in mixed infections.

Due to sample size limitations, this study's relatively low detection rate of AiV may be underestimated. Larger, multicenter studies with molecular characterization of AiV strains are needed to clarify its epidemiology, genotype distribution, and clinical significance in Egyptian children.

Limitations

This study has several limitations. It was retrospective in nature and conducted at a single tertiary care center, which may limit the generalizability of the findings to the broader pediatric population. Furthermore, bacterial and protozoal entomopathogens were not included in the analysis, precluding evaluation of potential mixed infections involving these agents. The small number of AiV-positive cases ($n = 3$) also constrains statistical power. It limits the ability to draw definitive conclusions regarding the prevalence, epidemiological significance, or clinical relevance of Aichi virus infection. Accordingly, the results should be interpreted with caution, and future large-scale, prospective, multicenter investigations are required better to delineate the role of AiV in pediatric acute gastroenteritis.

CONCLUSION

This study provides the first clinical evidence of Aichi virus infection in Egyptian children with acute gastroenteritis. Although rare, AiV was consistently associated with rotavirus coinfection, highlighting the need for expanded surveillance and molecular studies. Understanding the role of emerging viruses such as AiV is essential for comprehensive control strategies against pediatric gastroenteritis in resource-limited settings.

Ethics Approval and Consent to Participate:

The study was approved by the Ethical Committee of Mansoura Faculty of Medicine (R.24.11.2914). Written informed consent was obtained from the parents of each child.

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Data Availability:

The dataset supporting this study is available at Figshare: <https://doi.org/10.6084/m9.figshare.27933381.v1>

Declaration of Interest:

The authors declare no conflicts of interest.

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