

Research Article

Association of F40/41 Diagnostic Adenovirus with Diarrhea Disease in Children in Al-Diwaniyah City: A Case Study

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HSM performed the experiments and wrote the draft. RMS supervised the work and revised the manuscript.

Keywords

Adenovirus F40/41, Pediatric diarrhea, Diagnostic virology, Molecular detection, RT-PCR, Childhood infection



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Abstract | Acute diarrhea is a common infectious disease among children under 5 years old worldwide. The estimated mortality rate is 1.6–2.5 million cases annually, occurring in developing countries. Human adenovirus F40/41 is considered a major cause of epidemic diarrhea and one of the main factors causing severe gastroenteritis in infants and young children. The study aims to investigate the detection of adenovirus associated with acute gastroenteritis in children under 5 years old at the Maternity and Children's Hospital using rapid testing and diagnosis of enteric adenovirus types F40/41 in diluted stool samples causing acute diarrhea due to gastroenteritis using molecular techniques. Real-Time PCR. In a cross-sectional descriptive study over three months, 72 samples from children were collected and stool was tested using the rapid test. Subsequently, the virus's DNA was extracted, and all positive and negative samples were examined using Real-Time PCR. These results revealed using the rapid test that 16.66% of the positive samples were under two years old, all of whom were male. At the same time, the Real-Time PCR reaction showed that 40 (55.6%) out of 72 samples were positive in children with gastroenteritis. The results of this study revealed that adenovirus F40/41 is the causative agent of gastroenteritis in the city of Diwaniya and provides basic data on the potential role of adenovirus in causing diarrhea in children. There is a need for more extensive epidemiological surveys to better understand the disease burden and its clinical impact. While rapid testing and Real-Time PCR help us achieve early diagnosis and avoid the overuse of antibiotics.

Novelty Statement | This is the first study in Al-Diwaniyah City to identify adenovirus F40/41 as a major cause of diarrhea in children under five using both rapid testing and Real-Time PCR, providing valuable local data and highlighting the importance of molecular diagnostics in early detection.

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Introduction

Children younger than five years old die from diarrhea more than any other disease in the world (Rafie *et al.*, 2021). All age groups may have acute

gastroenteritis, the causative agents and clinical course could differ depending on age and causative agent. Children aged below five years have greater rates of the epidemics and fatalities linked to diarrhea connected to gastroenteritis, which results in around 1.5 million deaths annually, a serious public health concern (Bicer *et al.*, 2014).

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The digestive system, due to its continuous contact with the external environment primarily via the mouth is

a sensitive organ susceptible to infections. Gastroenteritis, which is also known as stomach flu, is a disease of the digestive tract. The symptoms and signs are abdominal pain, emesis, and diarrhea. Additionally, fever, fatigue, and dehydration may occur (Hussein *et al.*, 2018). Doctors warn about severe dehydration. Usually, there are no clinical signs until the child loses 5% or more of their body weight, and recent weight loss is a good indicator of the degree of dehydration (Elliott, 2007). The only two types, Adenovirus species F40\41 stand out as the only with a pronounced gastrointestinal tropism (Mahmood *et al.*, 2015). Prior studies indicate that alterations in the primary and secondary proteins responsible for regulating shell morphology, together with the presence of two fibrous proteins unique to non-intestinal species, may explain its stability and alignment with the digestive system (do Nascimento *et al.*, 2022). The F40/41 virus strains, prevalent in developing areas, account for 3.2% to 12.5% of acute diarrhea cases in children and infants worldwide. Children under two years old are often affected by these viral strains, which specifically target tiny intestine cells and induce watery diarrhea. If left untreated, it may lead to severe dehydration and could result in death, particularly in those with weakened immune systems and chronic conditions (AL-Nasrawy *et al.*, 2022). Children and infants living in remote areas are particularly susceptible to diarrhea, stunted growth, neurodevelopmental impairment, and increased mortality associated with recurrent episodes of moderate to severe diarrhea. Elevated infection rates resulting in gastrointestinal illnesses induce immune system impairment (Ali *et al.*, 2021). Adenovirus infections are found all year round, according to epidemiological research conducted in many parts of the globe; the winter season increases their frequency (Mohammadian and Kargar, 2011).

Healthcare for this infection is provided by treating the symptoms and complications. Whereas this infection can be fatal in young children and hosts with compromised immunity, it is rarely seen in healthy individuals (Zhang *et al.*, 2023). Diarrhea ranks as a main cause of disease and mortality in Iraq. Many studies have so shown the rotavirus presence as well as viral infections in youngsters. So, the present work investigated the association of adeno virus with arrehea and prevalence in the children of Al-Diwaniyah city of Iraq.

Materials and Methods

Sample collection

Stool samples were obtained from 72 children aged between less than one month and 5 years at the Women's and Children's Hospital in Diwaniya City, from November 2024 to February 2025.

Sample inclusion criteria

The study included children and infants suffering from diarrhea due to gastroenteritis. The data considered in this study were age, gender, type of nutrition, as well as the extent of dehydration and nutritional condition evaluated during the assessment. Each child diagnosed with acute gastroenteritis had 5 ml of stool collected. The sample was then divided into two parts, with the first part (2 ml) immediately used for general direct stool examination and detection of enteric viruses using a rapid test.

Real time PCR detection of adenovirus

The second part of the stool (3 ml) was stored at -20 °C until it was used to detect enteric viruses by real-time PCR technique. A particular kit (Adenovirus combination quick test cassette CTK, USA) was used to test the samples for adenovirus antigen. Colored immunochromatographic technique for concurrent qualitative identification of viral antigen in fecal specimens, this one-step lateral flow tests the maker of this product established a process for conducting this test, which followed. These actions were followed: Before the test started, the specimen, buffer, and cassette were placed at room temperature. The specimen collection tube was filled with 50 microliters of feces, and the mixture was stirred gently to mix with the extraction buffer. In the meanwhile, the specimen collection tube stood upright and the tip cap was removed. In a specimen well inside the test cassette, two drops of the extracted material were placed after the specimen collecting tube was inverted. Ten minutes of specimen dispersion produced the desired outcome. While the test area (T) has neither pink nor red lines, nor the control region (C) has a single-colored line, indicating that the answer is zero. One colored line in the test line zone and another in the control line region, however, indicate a favorable result. Following manufacturer guidelines, viral DNA was isolated from feces samples using the Presto stool DNA extraction kit. The concentration of the isolated DNA was quantified using the Nano Drop spectrophotometer (Thermo, USA). Using primers and a TaqMan probe specific for human adenovirus, real time PCR was used to find human adenovirus from patient stool samples. Following company guidelines, the main mix was made using the GoTaq® Probe qPCR Master Mix and this method was executed. The major PCR mixture's components were placed in the Exispin vortex centrifuge for three minutes at 3000 rpm. After that, it was placed in the BioRad, USA Real-time PCR Thermocycler. The BioRad real-time polymerase chain reaction tools set the settings for the real-time polymerase chain reaction cycle based on the original heating temperature and the directions that came with the TaqMan kit.

Statistical analysis

Data collection and analysis were aided by SPSS (Statistical Package for Social Sciences, version 20, IBM, Armonk, New York). The quantitative data was summarized

using the mean and standard deviation. Qualitative data were presented as numbers (n) and percentages (%). One may determine correlations using Spearman correlation, chi square, and P-values.

Results

Rapid immunochromatographic of diagnostic adenovirus

The results of our current study through detection of Adenovirus using the method of rapid immunochromatographic analysis as shown in [Figure 1](#).

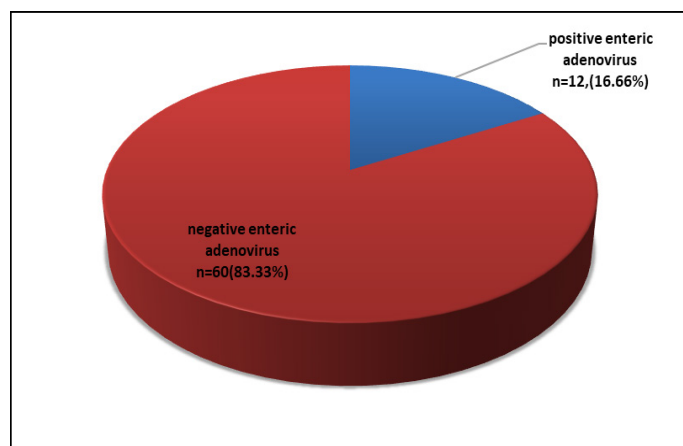


Figure 1: Pie chart displaying fast test for viral antigens detection results in gut samples of gastrointestinal disease patients.

Classification of adenovirus infection between male and female by real-time PCR, Adenovirus infection according to types of gender, feeding, adenovirus and infection by Real-Time PCR

[Table 1](#) showed the presence of 72 children with diarrhea during the period from November, 2024, to February 2025, at the Women's and Children's Hospital in Diwaniya. The male category had the highest incidence, with 24 (60.0%). In terms of nutrition, breastfeeding constituted the highest percentage at 18 (45.0%), and regarding the type of infection, the secondary infection rate was higher at 22 (55.0%).

P-values < 0.05 indicated that there was statistically significant variation in the data. The demographic characteristics and clinical features are shown in [Table 1](#). In this investigation, age matching was accomplished to avoid variations in parameter outcomes that could arise from the substantial age variance.

Adenovirus detection with RT-PCR

Total cases of adenovirus infection: Out of 72 stool samples tested using the polymerase chain reaction (PCR) for adenovirus are capable of providing rapid results but lack sensitivity when compared with PCR tests. A study in Ireland showed that the sensitivity and specificity of RAT were 88 and 98.5%, respectively, for adenovirus compared to PCR ([Barsoum, 2020](#)). This diminished sensitivity can also result in false negative test results, especially if viral loads are low or infection is early in the disease. On the other hand, the real-time PCR assay described by [Buckwalter et al. \(2012\)](#) showed 100.0 % sensitivity and 99.0 % specificity for all 57 known human adenovirus genotypes from different specimen sources using real-time PCR, which indicated that our method had high diagnosis accuracy. From a clinical standpoint, the implementation of PCR-diagnosis can also greatly improve patient care. Rapid, sensitive, and specific diagnosis of viral pathogens by PCR not only enables timely and appropriate antiviral therapy, but also contributes to antimicrobial stewardship. A report that analyzed children who were hospitalized and presented with adenovirus respiratory tract infection, observed that higher C-reactive protein and the presence of seizures were predictors to a bigger prescription of antibiotics independent of the viral nature of the condition ([Buonsenso et al., 2024](#)). Rapid and accurate PCR diagnostics can help clinicians differentiate between viral and bacterial infections, which not only reduces the unnecessary use of antibiotics, but can also help fight antibiotic resistance. In a reaction technique, the results indicated that 40 (55.6%) were positive ([Figures 2 and 3](#)).

Table 1: Classification of adenovirus infection by real-time PCR.

Test	Gender	Adenovirus +ve	Adenovirus -ve	X ²	P value
Real-Time PCR	Male, n (%)	24 (60.0%)	18 (56.2%)	0.103	0.748
	Female, n (%)	16 (40.0%)	14 (43.8%)		
	Types of feeding	Adenovirus +ve	Adenovirus -ve	1.553	0.670
	Breast, n (%)	18 (45.0%)	12 (37.5%)		
	Artificial, n (%)	8 (20.0%)	10 (31.2%)		
	Mixed, n (%)	4 (10.0%)	4 (12.5%)		
	Food, n (%)	10 (25.0%)	6 (18.8%)	13.918	0.001*
	Type of infection	Adenovirus +ve	Adenovirus -ve		
	Primary, n (%)	18 (45.0%)	28 (87.5%)		
	Secondary, n (%)	22 (55.0%)	4 (12.5%)		

¥, Chi-square test; *, significant at P ≤ 0.05.

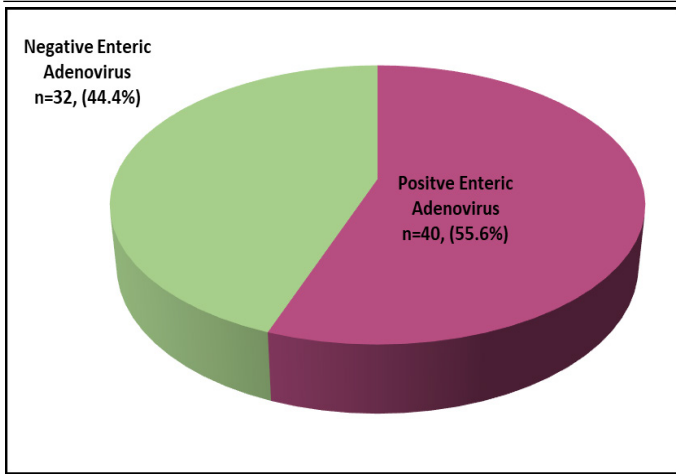


Figure 2: Percentages of enteric adenoviruses by real-time PCR diagnosis of stool samples.

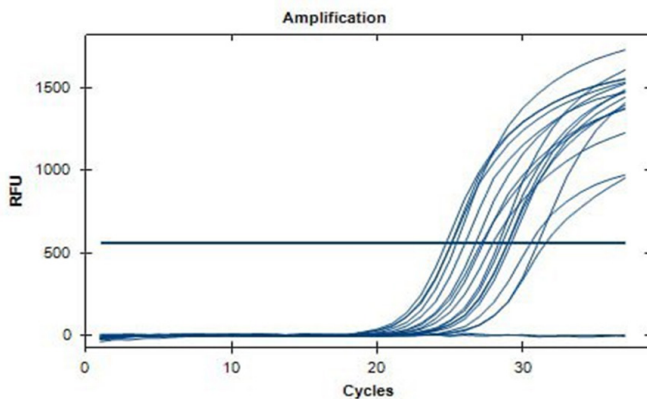


Figure 3: Blue curve shows the expression of Enteric Adenoviruses (20–30 Ct) by the RT-PCR amplification of them.

Discussion

Samples of the 72 stool samples examined in this investigation, 12 (16.66%) were positive for adenovirus. The rotavirus infection rate was greater than the viral infection rate. Viruses caused gastroenteritis at rates comparable to those reported in prior studies of viral diarrhea in children in Iraq and other countries, according to previous research conducted in that country. This was accomplished by evaluating stool samples rapidly. According to research conducted in Baghdad, the prevalence of rotavirus infection was greater than that of adenovirus infection in newborns and young children (Mahmood *et al.*, 2015) that used rapid tests to detect adenovirus infection in stool samples from children and infants, where the vast majority of cases were rotavirus, while adenovirus showed an 8% rate in the samples. A study (Al-Sayidi *et al.*, 2014) also showed that out of 450 diarrhea cases, 2.8% were infected with adenovirus, especially in children aged between 3 and 4 years. While the results differed from those obtained by Al-Ali *et al.* (2011) and Al-Shuwaikh (2016). Which were identified by this immunological test, adenoviruses were 0% in the exit samples. Furthermore, research

conducted in Sulaymaniyah city by Jaff *et al.* (2015) revealed that 3% of three out of one hundred exit samples had positive results for this virus. In southern Iran, the results conducted by Sharifi-Rad *et al.* (2015), indicated that the prevalence of the virus among children with diarrhea was 20.30%. In Turkey (Rad and Gözalan, 2010) the result was 2.6% in 77 positive samples out of a total of 2962 samples from discharge samples. Using the fast cassette test kit for rotavirus/ Adenovirus (Acro Biotech), adenovirus antigens were investigated also in Turkey (Öner *et al.*, 2022). Winter had the greatest incidence of adenovirus infection; 180 samples (2.2%) tested positive for the virus. In situations of gastroenteritis, particularly in children, winter and spring should not be taken for granted regarding adenovirus and rotavirus. In 2022, the frequency of adenovirus declined. The results of the current study for detecting adenovirus also approached the result obtained in America (Rodriguez-Baez *et al.*, 2002), which is (0.8%). This difference in viral infection rates in Iraq and other countries is attributed to variations in sample collection methods and conditions, as well as differences in the areas from which samples are collected. In some previous studies, sample collection was limited to children in hospitals, while in other studies, the collection process focused on children in outpatient clinics and hospitalized patients. Some people chose to collect from hospitals in the center of the province. In this study, adenovirus was found to be the second virus that causes vomiting in kids, after rotavirus. Children's stool samples commonly show adenovirus antigen, particularly in fall and winter utilizing fast test. Still, because quick tests are cheap and simple to administer, we advise using them in diagnosis.

The current study concludes that the Adenovirus positive patients by gender showed 24 (60.0%) cases in men and 16 (40.0%) in women. The frequency distribution of Adenovirus negative patients by gender was 18 (56.2%) cases in men and 14 (43.8%) instances in women; the difference was non-significant at ($P = 0.748$). The results of our present research matched those of the researchers (Hamza and Mohammed, 2021) in Al-Diwaniyah city, where the proportion of men was 0.55% while the percentage of women was 0%. The current study also agrees with the results of the Egyptian researcher (Allayeh *et al.*, 2018) in Cairo. According to several research carried out both within and outside of Iraq, the infection rate was 7.2% for men and 6% for females who tested positive for this virus. These findings are consistent with each other. However, in Iran, (Sanaei-Dashti *et al.*, 2016) indicated in their study that the male-to-female ratio is 1.7 among children infected with adenoviral infection, suggesting a higher prevalence of adenoviral gastroenteritis among males compared to females in the under-five age group. These findings were comparable to the results of some studies in Turkey (Tüzüner *et al.*, 2016) that included 5,156 pediatric patients, 46.6% of the adenovirus-positive

cases were female, while 53.4% were male. The study recorded infection rates among patients with thyroid virus infection who were fed through natural, artificial, mixed, and dietary means. As shown in the [Table 1](#). That showed the percentage of children infected with Adenovirus infection who were fed through Breastfeeding was 18 (45.0%), 8 (20.0%) cases were with Artificial feeding, 4 (10.0%) cases with mixed feeding and 10 (25.0%) cases with food feeding. The frequency distribution of Adenovirus-negative patients by feeding technique was as follows: 6 cases (37.5%) were breastfed, 10 cases (31.2%) were artificially fed, 4 cases (12.5%) were mixed-fed, and 6 cases (18.8%) were food-fed; $P = 0.670$ indicates that the difference was not statistically significant. There are no notable differences between the two groups, according to statistical analysis ($p > 0.05$).

The current research observed that the incidence of infants with Adenovirus who are breastfed exceeds that of children using other feeding methods. The study by [Al-fatlawi and Mohammed \(2020\)](#) indicated that the prevalence of adenovirus infections in breastfed newborns in Diwaniya was 58.06%, whereas in children with mixed feeding, it was 59.32%. No statistically significant differences in adenovirus infection rates were observed across the various dietary types. As a study in southern Iran ([Ziaei et al., 2013](#)) clarified, the kind of diet made no bearing on the frequency of diarrhea among youngsters. This study ([Guga et al., 2022](#)) in low-resource countries agrees with our study that breastfeeding was protective against both adenovirus 40/41 95% (0.48-0.85). The study's findings indicated positive Adenovirus infections throughout both main and secondary phases, with substantial variations between these infections, as seen in [Table 1](#). The frequency distribution of Adenovirus-positive patients by infection type included 18 (45.0%) instances of original infection and 22 (55.0%) cases of secondary infection. The frequency distribution of Adenovirus-negative patients by residency included 28 cases (87.5%) with initial infection and 4 instances (12.5%) with secondary infection. The current study observed that the incidence of secondary infections in children with Adenovirus exceeds that of primary infections, and statistical analysis reveals significant differences between the two groups ($p = 0.001$). The study ([De Francesco et al., 2021](#)) indicates that adenovirus infections in hospitalized children can be primary, as they are found in 7.1% of stool samples. Additionally, 23.5% of the patients infected with this virus indicate the possibility of a secondary infection. Another study ([LeMay et al., 2023](#)) indicates that patients receiving hematopoietic stem cell transplants who have an adenovirus infection can be classified as a secondary infection due to immunocompromised status.

Adenovirus detection with real-time PCR, Human

Adenovirus F, including serotypes 40 and 41, sometimes referred to as Enteric Adenoviruses, is among the primary viral agents responsible for gastroenteritis in humans. Several studies were conducted to investigate the presence of Adenovirus and many intestinal viruses and their spread in the Iraqi Governorates because these viruses are of great importance as they cause the death of tens of children. Given that these viruses are often shed in great quantities into symptomatic individuals' stools. The molecular method (real-Time PCR) was used in the present study to detect the DNA of Enteric Adenoviruses in stool samples. The current study showed that 40 (55.6%) positive samples out of 72 samples were from children with acute diarrhea and acute gastroenteritis. These results are more clarified in [Figure 2](#). The adenovirus is the second most prevalent virus in the positive samples, according to the study's findings, after the rotavirus, as it constitutes the highest infection rate. The results of this study, performed by RT-PCR method for adenovirus, were compatible with those obtained in other molecular studies in the same region. For example, research in Baghdad that was done by [Al-Janabi et al. \(2022\)](#) found 14% adenovirus positives in stool from children with gastroenteritis, RT-PCR. This very closely corresponds to the 16.66% in the current study. Additionally, [Khan et al. \(2022\)](#) identified adenovirus DNA in 14.8% of stool samples analyzed from hospitalized Pakistani under-five children with community acquired GE, adding to the dependability of molecular diagnosis. These findings underscore the enhanced sensitivity of RT-PCR relative to immunochromatographic assays, which can be markedly more insensitive and which are more likely to underestimate true infection rates. Thus RT-PCR may be introduced in clinical diagnostics to increase the accuracy of diagnostic detection and develop more suitable treatment options. This finding differs from the results obtained from testing the samples using the immune chromatographic assay. This test has been adopted by many researchers, and they obtained different rates, some of which were low. Molecular methods such as PCR and microRNA applications have recently gained attention for the diagnosis and monitoring of infectious and cancerous cases in Iraq. For example, in a study in Baghdad ([Al-Janabi et al., 2022](#)) reported the results of detecting enteric adenovirus by RT-PCR, showing 7 (14%) positive samples. A study by ([Yilmaz et al., 2022](#)) found that 13 samples (13.8%) from Central Turkey tested positive for adenovirus. A separate study conducted in Pakistan ([Khan et al., 2022](#)) indicated that 14.85% of adolescents ($n = 57/384$) tested positive for adenovirus by RT-PCR. A global study in low-resource countries ([Guga et al., 2022](#)) detected adenovirus in 698 (10.3%) out of 6748 samples. While Research in Tanzania ([Moyo, 2014](#)) showed that the adenovirus rate is (3.5%) among children aged under five years.

Conclusion

This study provides basic data on the prevalence of adenoviral gastroenteritis in the city of Diwaniya, Iraq. In conclusion, our study highlights that this virus is significantly spreading in the community as an important causative agent of acute gastroenteritis in children under the age of five. Although this study does not represent the true picture of adenoviral gastroenteritis in Iraq, it draws the attention of healthcare professionals for future studies, aiding in understanding the true picture of adenoviral gastroenteritis in Iraq.

Declarations

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IRB approval

This study was reviewed and approved by the Institutional Review Board (IRB) of the University of Al-Qadisiyah, College of Education, under Reference No. 14864, dated 10/11/2024.

Ethics approval

The research conducted was approved by the Ethical Approval Committee (Reference No. 14864, dated 10/11/2024). The University of Al-Qadisiyah, College of Education, also approved this study.

Declaration of generative AI and AI-assisted technologies in the writing process

No Generative AI and AI-assisted technologies were used in the writing process.

Statement of conflict of interest

The authors have declared no conflict of interest.

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