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Molecular Epidemiology of Viral Gastroenteritis in Children under 5 Years: A Cross-sectional Study in Northeast Iran

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ABSTRACT

OBJECTIVE: To study the prevalence of the most important enteric viral infections causing acute diarrhea in children under five years of age in Northeast Iran.

METHODOLOGY: This cross-sectional study investigated the prevalence of viral infection using Real-Time PCR in children under five years of age with acute diarrhoea referred to Imam Reza Hospital, the main referral hospital in northeastern Iran, during early September to late December of 2024. Sixty-five stool samples were collected from patients who had symptoms of fever and diarrhea and no history of rotavirus vaccination. SPSS software version 26 was used to analyze the data.

RESULTS: A significant majority (60%) tested positive for Rotavirus. This highlights Rotavirus as a primary cause of gastroenteritis in this group. Adenovirus was detected in 10.8% of the samples, while Astrovirus and Sapovirus combined accounted for 13.8% of the positive cases. The highest prevalence of co-infection was reported for Rota/Astro/Sapo (12.3%), followed by 9.2% for Rota/Adeno, 3.1% for Adeno/Astro/Sapo, and 3.1% for Rota/Adeno/Astro/Sapo, respectively.

CONCLUSION: The results of our study show a significant prevalence of gastroenteritis infections, especially Rotavirus infection, among children, highlighting the importance of surveillance and vaccination.

KEYWORDS: Diarrhea, young Children, viral gastroenteritis, vaccination

INTRODUCTION

Acute gastroenteritis caused by viral infections remains a leading cause of death in children, especially in developing countries[1].

Rotavirus belongs to the *Reoviridae* family and infects mature enterocytes in the small intestine [2]. The *Rotavirus* genome consists of eleven discrete segments of double-stranded RNA (dsRNA). This segmented architecture is biologically significant, as it facilitates genetic reassortment during co-infection of a single host cell by different strains, potentially leading to the emergence of novel variants with altered antigenic properties. Each of the 11 segments typically encodes a single viral protein, which is categorized into two functional groups. First, the structural proteins (VP1–VP4, VP6, and VP7) form the architecture of the virion. Notably, VP4 and VP7 are critical for viral attachment and entry into mature enterocytes. Second, non-structural proteins (NSP1–NSP6) are essential for intracellular viral replication and pathogenesis[3].

The *Rotavirus* VP4 attaches to sialo-glycans or histo-blood group antigens and subsequently internalizes via endocytosis, forming viroplasm structures in the cytoplasm. On the other hand, NSP4, a viral enterotoxin, disrupts tight junctions between enterocytes, leading to increased intestinal permeability and fluid secretion. NSP4 also mediates calcium-dependent chloride secretion and stimulates the enteric nervous system, leading to watery diarrhea[4]. *Rotavirus* infection in the duodenal mucosa of infants disrupts normal cellular homeostasis, resulting in shortening and atrophy of villi, loss of microvilli(5), malabsorption, and osmotic diarrhea[4,6].

In addition to *Rotavirus*, which is the main cause of acute gastroenteritis in children, other viruses have recently been identified in acute diarrhoea samples more frequently than previously reported[7-10].

Adenovirus infections usually have a longer incubation period and cause more prolonged diarrhoea. Two serotypes, 40 and 41, are the most important enteric *Adenoviruses*[11]. *Adenovirus* is capable of infecting epithelial cells throughout the digestive tract, from the stomach to the large intestine. The viral replication cycle within host cells leads to cellular damage and inflammation[12]. *Adenovirus* can persist in intestinal cells, leading to chronic shedding and long-term symptoms[13]. In general, *Adenovirus*-induced inflammation and direct cytopathic effects on enterocytes impair fluid absorption, leading to diarrhoea. The slow onset and long-lasting nature of *Adenovirus*-associated gastroenteritis is attributed to its relatively slower replication cycle compared to *Rotavirus*[14].

Astroviruses are known to be a prevalent cause of mild to moderate gastroenteritis, particularly in children[15]. These viruses replicate within the enterocytes of the small intestine, primarily in the apical regions of the villi. *Astrovirus* infection results in subtle changes in intestinal morphology, including mild villous atrophy and epithelial vacuolation. The pathogenesis is believed to involve a combination of direct cytopathic effects on infected cells and an inflammatory response[16]. *Astroviruses* are thought to disrupt the integrity of the intestinal epithelium, impairing nutrient and fluid absorption. Although symptoms are generally milder than those caused by *Rotavirus*, its mechanisms of action include disruption of normal intestinal function, leading to watery diarrhea and vomiting. The specific viral proteins involved in inducing fluid secretion are under investigation, but they likely interfere with ion transport mechanisms in intestinal cells[17].

Sapoviruses, belonging to the *family Caliciviridae,* are increasingly recognized as another important cause of gastroenteritis in children[18]; their pathogenesis shares similarities with other *Caliciviruses*, such as *Norovirus*. *Sapoviruses* primarily infect the enterocytes of the small

intestine. The hallmark of *Calicivirus* infection is the rapid onset of symptoms and the highly contagious nature of the virus, often resulting in outbreaks. *Sapoviruses* are thought to induce gastroenteritis by causing transient malabsorption and increased fluid secretion[19]. This is achieved through direct damage to the villi and alterations in the enzymes of the brush border of enterocytes, leading to impaired digestion and absorption of nutrients. The inflammatory response induced by *Sapovirus* replication leads to altered intestinal permeability and diarrhoea. Although the duration of *Sapovirus* infection is relatively short compared to *Adenovirus*, the intestinal dysfunction is more severe [20].

Although several studies from Iran and neighboring countries have investigated viral gastroenteritis in children, most have focused on single pathogens or limited panels and have been conducted in central or southern regions. There is a paucity of molecular epidemiological data on *Rotavirus*, *Adenovirus*, *Astrovirus*, and *Sapovirus* in combination in northeastern Iran, particularly in the context of the recent implementation of *Rotavirus* vaccination programs. Moreover, co-infection patterns involving these viruses remain underreported. Therefore, this study aimed to determine the prevalence and co-infection profiles of major enteric viruses among hospitalized children under five years of age in northeastern Iran during the autumn-winter period of 2024, providing baseline data that may inform future post-vaccination surveillance and public health interventions.

METHODOLOGY

Study design and participants

This cross-sectional study examined the frequency of enteric viral infections among children under 5 years of age hospitalized at Imam Reza Hospital, a central children's hospital in northeastern Iran, from early September to late December 2024.

The symptoms included diarrhea, with or without vomiting, fever, nausea, abdominal pain, and cramping. Exclusion criteria included blood in stool samples, prolonged or persistent diarrhea lasting more than 7 and 14 days, respectively, fever above 40°C, absence of vomiting before the onset of diarrhea, and biliary diarrhea. Children with a history of rotavirus vaccination were also excluded from the study.

Genome Extraction and Real-Time PCR

Among hospitalized patients, stool samples from 65 who tested negative for bacterial infections were studied to investigate enteric viral infections. The stool samples were transported on ice to the Reference Molecular Laboratory of North Khorasan University of Medical Sciences. The fecal specimens were diluted with 10% PBS and then centrifuged to obtain a clear liquid. This clarified liquid was stored at 4-8°C for short-term use and at –70°C for long-term storage.

Viral genomes were extracted from all stool samples using the QIAGEN (QIAamp® MinElute® Virus Spin), according to the manufacturer's instructions. Subsequently, Real-Time PCR testing was conducted using the Geneva kit (GA Gastro5G OneStep RT PCR Kit). The Real-Time PCR thermal profile consisted of a reverse transcription step at 50°C for 15 minutes, followed by initial denaturation at 95°C for 2 minutes, and 45 cycles of denaturation (95°C for 15 seconds) and annealing/extension (60°C for 1 minute). Fluorescent signals were captured in specific channels (FAM, HEX, ROX, and CY5) to differentiate between the viral target.

Statistical methods

Data were analyzed using SPSS software version 26. Statistical tests (such as the Chi-square or Fisher's exact test) were used to assess the association between viral prevalence (including single and co-infections) and demographic variables, such as age and gender. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Demographic characteristics of participants:
Out of the 65 participating patients, 61.5% (n=40) were male, and 38.5% (n=25) were female. Also, children under one year old constituted the largest group, accounting for 35.4% (n=23) of the participants, as detailed in **Table I**.

Table I: Gender and age distribution of participating patients

		Frequency	Percent	Valid Percent
Gender	Male	40	61.5	61.5
	Female	25	38.5	38.5
Months	Under12	23	35.4	35.4
	13-24	19	29.2	29.2
	25-36	12	18.5	18.5
	36-48	8	12.3	12.3
	49-60	3	4.6	4.6

Frequency of single enteric viruses:
Rotavirus was the most frequently detected virus (60%), followed by Adenovirus (10.8%). Comprehensive data on single viral frequencies are summarized in **Table II**.

Table II: Frequency of diarrhea samples associated with rotavirus and adenovirus

		Frequency	Percent	Valid Percent
Rotavirus	Negative	26	40.0	40.0
	Positive	39	60.0	60.0
Adenovirus	Negative	58	89.2	89.2
	Positive	7	10.8	10.8

Frequency of enteric viral Co-infection: Co-infection patterns were notably diverse, with the Rotavirus, Astrovirus, and Sapovirus combination being the most frequent (12.3%). Other profiles are presented in **Table III**.

Table III: Frequency of enteric viral co-infection in Participants

		Frequency	Percent	Valid Percent
Astro/Sapo	Negative	56	86.2	86.2
	Positive	9	13.8	13.8
Adeno/ Astro/Sapo	Negative	63	96.9	96.9
	Positive	2	3.1	3.1
Rota/Adeno	Negative	59	90.8	90.8
	Positive	6	9.2	9.2
Rota/ Astro/Sapo	Negative	57	87.7	87.7
	Positive	8	12.3	12.3
Rota/Adeno/Astro/Sapo	Negative	63	96.9	96.9
	Positive	2	3.1	3.1

DISCUSSION

The findings of this study highlight the high prevalence of enteric viral infections among children under 5 years in Northeastern Iran. The most important finding was the high prevalence of *Rotavirus* (60%), confirming its role as the major etiological agent of diarrhea in this age group. This result is in line with the latest systematic review and meta-analysis studies in Iran, which found that rotavirus is the most prevalent cause of acute diarrhea in children under 5 years of age[21].

Moreover, our result was higher than a recent study by Monavari SHR et al. [22], which found that the prevalence of *Rotavirus* infection in Iranian children ranged from 6.4% to 79.3%, with an average rate of 39.9%.

Hamkar et al. reported the presence of *Rotavirus* in 62% of samples, a rate similar to the high prevalence rates observed elsewhere[23]. In another study, Jadali F et al.[24] surveyed five major cities in Iran (Tehran, Tabriz, Mashhad, Shiraz, and Bandar Abbas) and reported positive Rotavirus result*s* in 55.48% of total cases. Similar high prevalence rates have been reported in neighboring countries, including India25 and Pakistan26, where detection ranged from 36.7% to 47%, consistent with our findings.

Taken together, these findings suggest that the prevalence of *Rotavirus* infection in northeastern Iran is at the upper end of the range previously reported from other Iranian provinces. Differences between studies may reflect variations in study period, climatic conditions, population characteristics, and vaccination coverage, as well as methodological differences such as sample size and diagnostic assays used.

In our study, detection rates of *Adenovirus* (10.8%) and *Astrovirus*/*Sapovirus* (13.8%) also highlight their contribution to gastroenteritis in children in northeastern Iran. Previous Iranian studies reported lower prevalences, with Mousavi et al. reporting prevalences of 5%, 6.7%, and 2.5% for Adenoviruses, Astroviruses, and Sapoviruses, respectively[27]. Another study by Sanaei et al. showed the prevalence of Adenovirus to be 5.18% [28]. Hamkar R et al. [23] reported the prevalence of *Adenovirus*, *Astrovirus*, and *Sapovirus* in 2.3%, 3%, and 2.5% of samples, respectively. A systematic review on viral gastroenteritis in Iran indicated average prevalences of 5.7%, 2.7%, and 4.2% for enteric Adenoviruses, Astroviruses, and Sapovirus*es*, respectively[29].

As mentioned above, compared with previous Iranian studies, our detection rates for Adenovirus and for Astrovirus/Sapovirus were generally higher. This discrepancy may be due to regional variation in circulating strains, temporal differences in sampling, or differences in inclusion criteria. Another possibility is the use of sensitive Real-Time PCR assays in our study, which may have increased the detection of low-level viral shedding compared with conventional methods used in some earlier investigations.

One notable aspect of our findings is the occurrence of co-infections, particularly involving *Astrovirus*es and *Sapoviruses*. These viruses, although historically considered minor factors in the development of gastroenteritis, appear to play a more prominent role than previously thought. Their presence in co-infections may increase the severity or duration of illness, although our study did not observe any statistically significant association between symptoms and co-infection status. This finding warrants further investigation. Co-infections may interact synergistically or antagonistically in the host, potentially influencing clinical manifestations and disease outcomes in ways that are not yet well understood. The low rate of co-infection with all four viruses in this study (3.1%) suggests that although exposure to multiple viruses can occur, infection with a dominant viral agent, *Rotavirus*, is more common. The low rate of co-infection appears to be

influenced by seasonal or temporal dynamics in which certain viruses are dominant at certain times. More detailed data, such as the precise timing of infections and the genetic composition of viral strains, would provide greater clarity into the interactions between these pathogens.

CONCLUSION

Future studies with larger sample sizes to examine the prevalence of dominant genotypes could provide a more comprehensive picture for public health interventions such as vaccination. Our study emphasizes the important role of *Rotavirus* infection in pediatric gastroenteritis. It also highlights the key role of *Astrovirus* and *Sapovirus* infections in the development of gastroenteritis, more than previous studies suggest.

While clinical management of viral diarrhea remains supportive, the molecular detection of these viruses has a significant long-term impact. Specifically, our findings provide a baseline for the newly implemented rotavirus vaccination program in Iran, allowing health authorities to track changes in disease burden and the potential emergence of non-vaccine strains. Furthermore, understanding the prevalence of co-infections may explain variations in clinical severity that are not addressed by standard rehydration protocols alone.

Limitations

The small sample size is a major limitation of this study, partly due to the simultaneous implementation of rotavirus vaccination programs in northeastern Iran. Therefore, the generalizability of our findings to other studies may be limited. Furthermore, our study was conducted during a specific seasonal time frame, from autumn to winter. Because enteric virus circulation often follows distinct seasonal trends, our observed prevalence rates may not reflect annual patterns. For example, rotavirus peaks in colder months, whereas other viruses such as Astrovirus and Sapovirus may have distinct seasonal profiles.

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AUTHOR CONTRIBUTION

Baratian F: Performed DNA extraction, Real-Time PCR, and wrote the manuscript.

Khayat HJ: Participated in conceptualization, data analysis, and writing the manuscript, supervised the research and participated in conceptualization, data analysis, and writing and critical revision of the manuscript.

Shoraka HR: Contributed to data analysis.

Firouzeh N: Contributed to Real-Time PCR and writing the manuscript.

Abaspour A: Participated in conceptualization and critically revised the manuscript.

Fani M: Supervised the research and participated in conceptualization, data analysis, and writing and critical revision of the manuscript.

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