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Epidemiology of Acute Infectious Diarrhea-associated Viruses in Children in Hangzhou, China, 2023

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ABSTRACT

Objective: To investigate the epidemiological characteristics of children infected with diarrheal virus in Hangzhou, China, 2023.

Methods: From January 2023 to December 2023, 20,939 stool samples from children with acute infectious diarrhea were collected for detection of rotavirus A and human adenovirus, using Latex agglutination detection kits; 7584 stool samples were collected for detection of norovirus, using real-time PCR reagent.

Results: 639 (3.0%, 639/20,939) tested positive for rotavirus A, and 1,201 (5.7%, 1,201/20,939) positive samples were detected by Colloidal gold method. The positive rates of norovirus were 42.2% (3,203/7,585). Among all age groups, the rotavirus A positive detection rate was the highest in participants 3 to 6 years old (6.46%, 260/4,024). The monthly distribution of patients with rotavirus A showed that the number of cases was the lowest in October (0.45%, 8/1,779) and reached the peak in April (7.97%, 144/1,806). The highest and lowest positive rates among all age groups for human adenovirus were 3 to 6 years old (8.27%, 333/4,024) and 0 to 6 months (2.21%, 60/2,717). The monthly positivity rates of human adenovirus spanning from January to November 2023 were 1.38%, 1.44%, 2.34%, 3.65%, 6.64%, 7.71%, 7.54%, 7.13%, 6.82%, 4.15%, 6.50%, and reach the peak in December (8.17%). For norovirus, children aged 1 to 3 years had the highest positive detection rate (57.95%, 1,349/2,328), while infants aged 0 to 6 months had the lowest positive detection rate (19.60%, 205/1,046). The results show that January had the lowest number of cases (14.63%, 6/41), while September had the highest at 50.51% (545/1,079).

Conclusions: The detection rate of rotavirus A and human adenovirus was highest among participants aged 3 to 6 years, whereas the detection rate of norovirus was highest among those aged 1 to 3 years. The monthly distribution peaks for the three enteric viruses varied significantly.

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Introduction

Acute diarrhea remains a leading cause of morbidity and mortality in young children globally. More than 50% of acute diarrhea has been confirmed as being caused by viral pathogens.[1] Acute infectious diarrhea is generally defined as a decrease in the consistency of stools (loose or liquid) and/or an increase in the frequency of bowel movements. Clinically, it can be diagnosed with or without vomiting, nausea, fever, abdominal pain, and other symptoms. In children, it is commonly associated with viruses and bacterial infections.[2] Rotavirus, adenovirus, and norovirus are the most common viruses that cause acute diarrhea in children. The rotavirus genus, which belongs to the *Reoviridae* family, is an envelope-free icosahedral-shaped double-stranded RNA virus with spikes and an average diameter of 100 nm.[3] Rotavirus is classified into seven serotypes (A-G) according to the difference in VP6 serotypes, with A, B, and C being infectious to humans. Norovirus is an enteric, non-enveloped virus belonging to the *Caliciviridae* family and *Norovirus* genus.[4,5] It is estimated to be 27–35 nm in diameter, and its virion is highly stable under extremely hostile conditions, including a range of pHs (pH 3 to 7) and temperature as high as 60 °C, indicating the difficulty of eradicating and controlling its spread using routine methods.[6] Human adenovirus (HAdV), as a member of the genus *Mastadenovirus* in the family of *Adenoviridae*, is characterized as a double-stranded, non-enveloped DNA virus.[7] Since it was first isolated in 1953 by Rowe et al.,[8] over one hundred serotypes have been identified and grouped into seven species (HAdV A-G) based on the biological and genetic characteristics with the development of phylogenetic and bioinformatic technology.[9,10] Human adenovirus infections predominantly affect children under five due to their developing immunity. HAdV F species called enteric adenovirus was confirmed to be associated with acute gastroenteritis, in which HAdV-40 and HAdV-41 were the most common serotypes accounting for 1-20% acute diarrhea.[11,12]

Over the past few years, the global pandemic (COVID-19) has significantly decreased reported cases of diarrhea-associated viruses and other infections. It is possibly due to overburdened health services, which can lead to unchecked surveillance and testing,[13] or the implementation of non-pharmaceutical interventions under the dynamic zero-COVID policy, including measures such as social distancing, home isolation and improved personal hygiene practices, which has subtly influenced the occurrence of gastrointestinal infections in children and adolescents.[14] However, since the relaxation of COVID-19 restrictions and measures, there has been a sudden increase in reported cases of COVID-19.[15] The positive rate of diarrhea-associated virus infection began to rise again. To explore the epidemiological characteristics of children infected with diarrheal virus in Hangzhou during the post-epidemic era, this study conducted diagnostic testing and statistical analysis on stool samples from children with acute infectious diarrhea in 2023. This study's results can provide valuable information on how to improve children's hygiene environment and carry out effective disease prevention.

Materials and Methods

The study was conducted from January 2023 to December 2023, and the participants were children from the inpatient wards and outpatient departments of the Children's Hospital of Zhejiang University School of Medicine. The inclusion criteria were: (1) all patients who visited the Children's Hospital of Zhejiang University School of Medicine between January 2023 and December 2023, (2) aged under 18 years with suspected acute diarrhea infection, (3) Fecal characteristics were watery or mushy, without blood; (4) The number of stool increased, the number of times > 3 /24 h; （5） With or without fever and vomiting, the duration of the disease was less than 14 days; （6） Suspected illness children with toxic acute diarrhea; (7) complete clinical data. Exclusion criteria: （1） presence of red and white blood cells in stool specimen, suspected bacterial infection, (2) with other systemic infections and long-term use of antibiotics.

The study was approved by the Ethics Committee of the Children's Hospital Affiliated to Zhejiang University School of Medicine, with informed consent from patients (2023-IRB-0242-P-01).

Latex agglutination test kits (Abon Biopharm Co., Ltd.) were used, as reported in our previous study. [16] About 50 mg of stool samples were mixed with 1 mL sample extraction reagent. The well plate was composed of four major parts: (a) sample well plate, (b) control line, (c) rotavirus test line, and (d) adenovirus test line. Two drops (about 80 μL) of the mixture were also added to a sample well plate for 10-20 minutes. If both the control line and the test

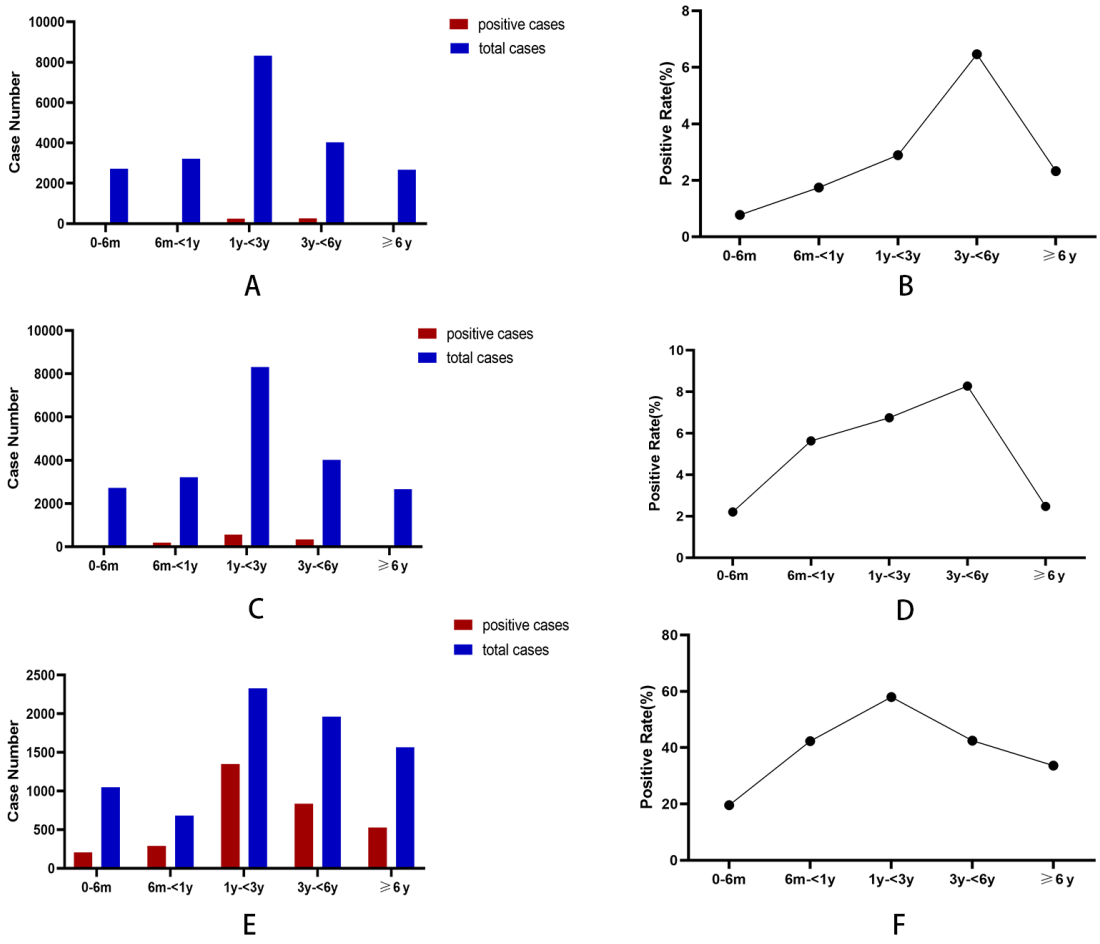

Figure 1. Age distribution of the case number and positive rates in children with rotavirus A, adenovirus and norovirus

line for rotavirus appeared blue, the sample was considered positive for rotavirus. Conversely, if the control line was blue and the test line for adenovirus appeared red, the sample was determined to be positive for adenovirus.

Approximately 1 mL of normal saline was added to each child's collected stool sample. The mixtures were centrifuged at 4000g at 20°C for 30 s. A 200μL supernatant was separated, and RNA was extracted by SSNP-2000A nucleic acid automatic extraction instrument with magnetic bead method. A commercial real-time PCR reagent (Land Medical Limited Company) was utilized to detect the norovirus RNA.[17]

Statistical analysis was performed using the Chi-square (χ^2) test, and the statistical significance was calculated using SPSS 26.0 version (SPSS Inc., Chicago, IL, USA), with a significance level set at $P < 0.05$, indicating statistically significant differences.

Results

From January 2023 to December 2023, a total of 20,939 children aged from 1 day to 17 years with acute infectious diarrhea diseases were tested by Colloidal gold method in this study, including 12,420 (59.3%) boys and 8,519 (40.7%) girls. Overall, 639 (3.0%, 639/20,939) tested positive for rotavirus A, with 387 (3.1%, 720/12,420) males and 252 (2.9%, 252/8,519) females, which were not significantly different (χ^2=0.426, P=0.514). According to age distribution (**Figure 1A, Figure 1B**), the positive rates of rotavirus A in 0 to 6 months, six months to 1-year-old, 1 to 3 years old, 3 to 6 years old, and > six years old groups were 0.77% (21/2,717), 1.74% (56/3,217), 2.89% (240/8,313), 6.46% (260/4,024), and 2.32% (62/2,668), respectively, which were significantly different(χ^2=230.026, $P<0.001$). Among all age groups, the positive detection rate was the highest in participants 3 to 6. Conversely, the positive detection rate of children 0 to 6 months was the lowest. The

positive rates of rotavirus A from January 2023 to December 2023 were 2.00%, 3.93%, 6.58%, 7.97%, 5.85%, 1.70%, 1.25%, 1.19%, 1.17%, 0.45%, 1.69%, and 3.28%, respectively (**Figure 2A, Figure 2B**). The monthly distribution of patients with rotavirus A showed that the number of cases was the lowest in October (0.45%, 8/1,779) and reached the peak in April (7.97%, 144/1,806).

Colloidal gold assay was performed on the same specimens as above to detect adenovirus. For human adenovirus, 1,201 (5.7%, 1201/20,939) positive samples were detected, with 720 (5.8%, 720/12,420) males and 481 (5.6%, 481/8,519) females. However, the distinction was not statistically significant (χ^2=0.213, P=0.645). For age distribution of positive human adenovirus cases (**Figure 1C, Figure 1D**), the positive rates of human adenovirus in 0 to 6 months, six months to 1 year, 1 to 3 years, 3 to 6 years, and those exceeding six years were as follows: 2.21% (60/2,717), 5.63% (181/3,217), 6.75% (561/8,313), 8.27% (333/4,024), and 2.47% (66/2,668), which were significantly different (χ^2=178.876, P＜0.001). The highest and lowest positive rates among all age groups were 3 to 6 years old (8.27%, 333/4,024) and 0 to 6 months (2.21%, 60/2,717). Additionally, the monthly positivity rates of human adenovirus spanning from January to November 2023 were 1.38%, 1.44%, 2.34%, 3.65%, 6.64%, 7.71%, 7.54%, 7.13%, 6.82%, 4.15%, 6.50% (**Figure 2C, Figure 2D**), and reach the peak in December (8.17%).

A total of 7584 children aged from 1 day to 17 years with diarrhea were detected, using real-time reverse transcriptase PCR (RT-PCR) between January and December 2023, among which 59.4% (4,507/7,584) of the participants were boys, while 40.6% (3,077/7,584) were girls. The positive rates of norovirus were 42.2% (3,203/7,585). According to all test results, though positive rates were higher in males (42.2%, 2,590/4,507) than in females (41.8%,1791/3,077), the difference is not statistically significant (χ^2=0.410, P=0.522). From the perspective of age distribution (**Figure 1E, Figure 1F**), the norovirus positivity rates of each age group from 0 to 6 months to over six years old are 19.60% (205/1,046), 42.29% (288/681), 57.95% (1349/2,328), 42.48% (834/1,963) and 33.65% (527/1,566) which were significantly different (χ^2=496.356, P＜0.001). Among all age groups, children aged 1 to 3 years had the highest positive detection rate, while infants 0 to 6 months had the lowest positive detection rate. Between January 2023 and December 2023, the positive rate of each month was 14.63%, 37.28%, 36.70%, 38.37%, 43.10%, 32.62%, 26.76%, 45.76%, 50.51%, 46.50%, 48.49% and 39.94% respectively (**Figure 2E, Figure 2F**). Monthly statistics on the number of norovirus cases show that January had the lowest number of cases (14.63%, 6/41), while September had the highest number of cases at 50.51% (545/1,079).

Discussion

Virus infection is often the cause of acute diarrhea in children.[18] Rotavirus A, norovirus, and human adenovirus are common pathogens that cause viral diarrhea in children. As the COVID-19 restrictions and measures begin to ease, the positive infection rate of diarrhea-related viruses that declined during the pandemic seems to be rising again. This is a trend that we need to be concerned and vigilant about. Therefore, we need to investigate this trend in-depth and understand the prevalence of diarrhea-related viruses to be fully prepared for the potential rebound of viral infections after the pandemic.

We need to adopt some proactive preventive measures against enteric virus infections. Firstly, parents should encourage their children to maintain good hygiene by reminding them to wash their hands before meals and after using the toilet, and by teaching them to cover their mouths and noses with clean handkerchiefs or tissues when coughing or sneezing and to clean any nasal secretions properly. Parents should also frequently change their children's clothes and bedding and use hygiene products in daily life. If needed, items can be exposed to sunlight for disinfection. To ensure that the child's living environment has sufficient fresh air circulation, parents should open windows more frequently for ventilation.

Parents should also pay greater attention to the child's diet and drinking water hygiene. The food and water that children consume must be boiled. Do not let the child eat unclean food or drink cold water or beverages. Reduce the possibility of your child being infected by taking them to crowded places less frequently. Regarding how to mitigate the sequelae of enteric virus infection, we should commence by reducing its prevalence, encompassing early prevention and vaccination, as well as providing more science education for the parents of children and, for instance, teaching them how to prevent

dehydration when children fall ill and paying attention to the mental state of children.

Multiple studies in the past have shown that rotavirus A has the highest positive infection rate among viral pathogens in children with acute diarrhea.[19,20] The positive rates of the three viruses, ranked from high to low, were norovirus 42.2% (3,203/7,585), human adenovirus 5.7% (1201/20,939), and rotavirus A 3.1% (639/2,0939). attention to norovirus. The positivity rates for the other two viruses in this study were relatively low compared to norovirus. In our preliminary studies before and under the outbreak of the COVID-19 epidemic, we monitored the epidemiology of rotavirus A and human adenovirus in Hangzhou from 2017 to 2018 and 2020.[22, 23] After comparison, it was found that the positive rate of rotavirus A in this study decreased compared with the previous

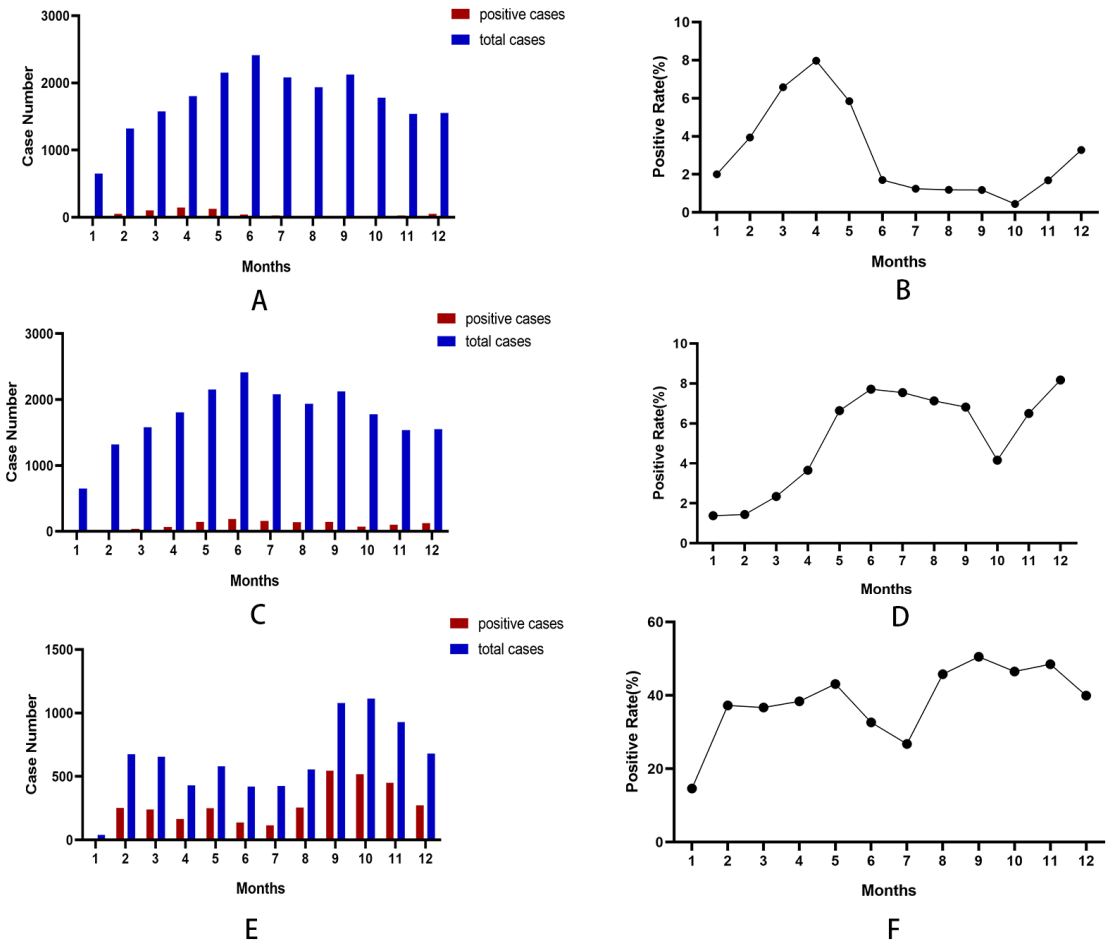

Figure 2. Monthly distribution of the case number and positive rates in children with rotavirus A, adenovirus and norovirus

The positivity rate for norovirus is much higher than the other two viruses. In addition to the influence of the sensitivity difference between the Colloidal gold method and the RT-qPCR method, this is more likely to be caused by changes in the epidemiology of rotavirus A in the post-epidemic era. Norovirus is an RNA virus. The unstable nature of its nucleic acid structure makes recombination and antigenic drift quickly occur between different norovirus strains, leading to new virus strains.[21] Therefore, norovirus is more likely to cause outbreaks of disease. This indicates the necessity to reassess the role of viruses in childhood diarrhea cases and to pay greater study, while the positive rate of human adenovirus was consistent with the previous study. This may be because, with the gradual promotion of rotavirus vaccines in recent years, norovirus has gradually replaced the place of rotavirus as the predominance of children with acute diarrhea.[24,25]

From a gender perspective, there is no statistical significance between the positive rates of the three viruses and gender (P>0.05), indicating that lifestyle, environmental, and physiological differences between children of different genders do not affect viral infection. Another study also

supports this conclusion.[19]

In terms of age, the results show that infants aged 0-6 months have the lowest detection rate for all three viruses, primarily due to the presence of maternal antibodies. The age groups with the highest positive detection rates of the three viruses are primarily concentrated in 1-3 years old and 3-6 years old. A higher positive rate was observed in 3-6 years old children for rotavirus A (6.46%) and human adenovirus (8.27%), while the highest positive rate of norovirus occurred in 3-6 years old children (57.95%). These findings differ from previous studies, which suggested that higher incidences of infections for rotavirus A,[26,27] norovirus,[28] and human adenovirus 29,30 were all in the age group of 6 months to 2 years old.

Concerning the epidemic time, the three viruses can be detected throughout the year, and there are obvious differences in the epidemic months. Different from a nationwide study that suggested the peak of the rotavirus A epidemic in China is more likely to occur in winter,[29] our data showed that the peak prevalence of rotavirus A was from March to May, with the highest peak occurring in April (7.97%). Corresponding geographical differences in epidemic seasons can also be observed with other viruses. There are two peaks in the prevalence of human adenovirus, observed in July (7.71%) and December (8.17%). We reviewed recent studies and noted that the peak times for human adenovirus prevalence vary across different regions. For instance, in Chongqing, the peak of human adenovirus infection occurred in May.[30] Conversely, data from Shanghai indicated that human adenovirus infection mainly occurred during autumn and winter.[31] In Tianjin, the primary incidence of human adenovirus infection is in the summer.[32] Similarly, in our study, the prevalence of norovirus mainly persisted from August to October, with the highest peak in September (50.51%). Previous studies have illustrated that norovirus infection rates are highest from July to October in Hangzhou and Chongqing.[33] In contrast, most cases in Shanghai peak from October through March of the following year.[34] Although the reasons for the abovementioned differences remain unclear, which might be attributed to factors such as geography or climate, the result indicates that we should attach importance to the epidemiological situation across different seasons in Hangzhou to prevent potential virus outbreaks in hospitals.

Our study still has some limitations, such as the time span not being long enough and the fact that further analysis of virus genotypes has not been conducted. Due to the lack of commercialized testing reagents, we cannot monitor other acute diarrhea-related viruses and co-infections, such as astroviruses and sapovirus. Despite its limitations, this study certainly adds to our understanding of the new trends in rotavirus A, norovirus, and human adenovirus causing acute diarrhea in children after the COVID-19 pandemic. This provides us with valuable information to better comprehend the transmission mechanisms of these viruses, optimize children's hygiene environment, and develop more targeted disease prevention strategies.

The positive detection rate of rotavirus A and human adenovirus was highest in participants aged 3 to 6 years, while that of norovirus was highest in those aged 1 to 3 years. The monthly distribution peaks of the three viruses varied significantly.

Declarations

Authors' contribution

W.L. conceived the idea and supervised this work. Y.G. and L.L. wrote the manuscript. L.L. and Q.L. performed data processing and analysis. All authors reviewed and approved the manuscript.

Conflict of interest

None declared.

Acknowledgment

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