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The clinical and virological features of two children's coinfections with human adenovirus type 7 and human coronavirus-229E virus

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Objective: Human adenovirus (HAdV) coinfection with other respiratory viruses is common, but adenovirus infection combined with human coronavirus-229E (HCoV-229E) is very rare.

Study design and setting: Clinical manifestations, laboratory examinations, and disease severity were compared between three groups: one coinfected with HAdV-Ad7 and HCoV-229E, one infected only with adenovirus (mono-adenovirus), and one infected only with HCoV-229E (mono-HCoV-229E).

Results: From July to August 2019, there were 24 hospitalized children: two were coinfected with HAdV-Ad7 and HCoV-229E, and 21 were infected with a single adenovirus infection. Finally, one 14-year-old boy presented with a high fever, but tested negative for HAdV-Ad7 and HCoV-229E. Additionally, three adult asymptotic cases with HCoV-229E were screened. No significant difference in age was found in the coinfection and mono-adenovirus groups (11 vs. 8 years, $p = 0.332$). Both groups had the same incubation period (2.5 vs. 3 days, $p = 0.8302$), fever duration (2.5 vs. 2.9 days, $p = 0.5062$), and length of hospital stay (7 vs. 6.76 days, $p = 0.640$). No obvious differences were found in viral loads between the coinfection and mono-adenovirus groups (25.4 vs. 23.7, $p = 0.570$), or in the coinfection and mono-HCoV-229E groups (32.9 vs. 30.06, $p = 0.067$). All cases recovered and were discharged from the hospital.

Conclusion: HAdV-Ad7 and HCoV-229E coinfection in healthy children may not increase the clinical severity or prolong the clinical course. The specific interaction mechanism between the viruses requires further study.

KEYWORDS

HAdV-Ad7, respiratory tract infections, coinfections, case-control study, children, human coronavirus-229E(HCoV-229E)

What is new?

This preliminary study is the first to investigate the differences between groups coinfected with HAdV-Ad7 and HCoV-229E versus a single adenovirus infection. This research for the extremely small sample size found that coinfection with HAdV-Ad7 and HCoV-229E in healthy children may not increase the clinical severity or prolong the clinical course.

Introduction

Human adenoviruses (HAdVs) are non-enveloped, double-stranded DNA viruses in the *adenoviridae* family (1). Since the first isolation of the adenovirus in 1953, seven species (A–G) have been recognized, including 113 known genotypes or serotypes of HAdV (2–6). More than 60 genotypes are known to cause human infection (7). Adenoviruses can cause illness in people of all ages at any time of the year (8). Most children have had at least one adenovirus infection by age 10 (9). Globally, 5–7% of respiratory tract infections in pediatric patients are ascribed to HAdV (10, 11).

Human coronaviruses (HCoVs) are enveloped, single-stranded RNA viruses (12, 13).

HCoV-229E is one of seven HCoVs: HCoV-229E, HCoV-NL63, HCoV-OC43, HCoV-HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (14–16). HCoV-229E usually causes mild to moderate upper-respiratory tract illness, similar to the common cold (17). People generally become infected with HCoV-229E in the fall and winter, but infection can occur at any time of the year (17).

Human adenovirus (HAdV) is commonly associated with acute respiratory illnesses (ARI) in children and is also frequently co-detected with other viral pathogens (18). HAdV co-detection with other respiratory viruses was associated with greater disease severity among children with ARI compared to HAdV detection alone (18). However, coinfection with HCoV is very rare and unusual. For example, national data from China indicate a rate of 7.2% for coinfection in hospitalized acute lower respiratory infections (ALRIs), with the most common pairs being adenovirus + respiratory syncytial virus (9.71%), followed by human parainfluenza viruses + adenovirus (3.86%); and influenza + adenovirus (3.45%) (19). Pathogen coinfection occurred only two times between adenovirus and hCoV (0.94%) (19). Additional research from Beijing indicated that, among 90 HAdV-positive children, 61.11% (55/90) were coinfected with other respiratory viruses, the most common of which were human respiratory syncytial virus (34.5%) and human rhinovirus (10.9%). The fewest cases of coinfection occurred with adenovirus and HCoV (only 1.8%) (20). To date, there are no documented adenovirus coinfections with HCoV-229E, so the clinical outcome of this pair of coinfections remains unknown.

An adenovirus outbreak was recognized in 2019 in Lihui city, Zhejiang Province, China. This outbreak involved 97 cases, including 24 admitted cases in the pediatric ward, with two confirmed coinfections of adenovirus and HCoV-229E. In order to compare clinical presentation and outcomes, and virological features in young children with HAdV detected alone vs co-detected with HCoV-229E, we explored a rare phenomenon in this study.

Materials and methods

Study design and participants

China's national surveillance system for influenza-like illness (ILI), severe acute respiratory illness, and pneumonia of unexplained origin indicated an outbreak of 97 adenovirus infections during July and August of 2019. Twenty-four children were hospitalized in the pediatric ward of the Second Hospital of JinYun. Two cases were confirmed to be HCoV-229E and adenovirus coinfections, and these children were admitted to different rooms (Rooms 830, 845) in the same pediatric ward. Twenty-one cases were identified as mono-adenovirus. One case involved a 14-year boy with a high fever who tested negative for Ad7 and HCoV-229E; this case was not included in our research.

Through extensive symptom surveillance, we found an additional three people (the parents of one of the coinfections and one doctor) who were confirmed as HCoV-229E positive. HAdV patients coinfected with HCoV-229E were categorized into the research group. For each patient in the research group, we matched three cases with a single HCoV-229E infection and 21 with a single adenovirus infection as controls.

The diagnosis of adenovirus and HCoV-229E followed the Protocol for Adenovirus Pneumonia Diagnosis and Treatment issued by the National Health Commission of the People's Republic of China in 2018.

Clinical information collection

The patients' clinical manifestations, laboratory examinations, imaging characteristics, disease severity, and clinical progress were collected from electronic medical records in the Second Hospital of JinYun's hospital information system (HIS). Radiologic abnormalities were determined according to descriptions in the clinical charts. All variables were compared between the three groups, including coinfections with adenovirus, mono-adenovirus, and HCoV-229E respectively.

Three methods were used to evaluate the severity and clinical progress of the disease. First, according to the national guidelines for pediatric CAP (community-acquired pneumonia)

in China, we divided cases of pneumonia into mild and severe (10). Second, the extrapulmonary manifestations involved in our study included kidney manifestations, myocardial damage, liver damage, and coagulation function. Third, we observed the number of days with fever, the highest degree of fever, and the median days from illness onset to discharge.

Laboratory testing

We collected specimens from the upper respiratory tract using pharyngeal swabs. All of the patients were laboratory confirmed using real-time reverse transcriptase polymerase chain reaction (RT-PCR) or PCR assays. Laboratory confirmation was also performed on other common respiratory pathogens, including influenza-A virus (H1N1, H3N2), influenza B virus, respiratory syncytial virus (RSV), parainfluenza virus, adenovirus, SARS-associated coronavirus (SARS-CoV), and human coronavirus HCoV-229E. Any coinfections were included from this study. Genetic sequences of viruses were obtained directly from positive clinical specimens or from virus isolates using an MiSeq desktop sequencer (Illumina, Inc., San Diego, CA, USA), as described (21).

Statistical analysis

Quantitative measurements were presented as medians; qualitative measurements were presented as counts and percentages. Abnormally high or low levels of laboratory findings were defined using age-specific or otherwise universal reference ranges. The differences between groups were analyzed using the Wilcoxon signed-ranks test (for continuous data) or McNemar's chi-square test (for binary data), with a p-value of less than 0.05 considered statistically significant. All analyses were conducted with R (version 3.4.6; The R Foundation for Statistical Computing, Vienna, Austria).

Results

Demographic characteristics and exposure history

We collected a total of two cases of coinfection with adenovirus and HCoV-229E, three cases with single HCoV-229E infection, and 21 cases with single adenovirus infection. None of the patients had underlying diseases. Patients' median ages were 11, 38, and eight years in the coinfection group with adenovirus and HCoV-229E, the mono-infection group with HCoV-229E, and the mono-infection group with adenovirus, respectively ($p = 0.332$) (Table 1). The male to female ratio was 0:2 in the coinfection group, 1:2 in the mono-infection with HCoV-229E group, and 2.5:1 in the mono-infection with adenovirus group ($p = 0.111$) (Table 1). All of the children with coinfections and adenovirus mono-infection had been to a swimming pool and had been exposed to someone with a fever. However, the three individuals with mono-infections of HCoV-229E had provided bedside and medical care for the two children with coinfections, as shown in Table 1.

Comparison of clinical characteristics

Coinfection-case 1

The first coinfection patient was a 13-year-old girl (a student from Jinyun) who had no underlying diseases. She had taken swimming classes at a swimming center on July 25, 2019, where an adenovirus outbreak was identified, as seen in Figure 1. She was admitted to room 830 of the pediatric ward on July 30 (see Figure 2), presenting with a dry cough, a continual fever for two days with an initial temperature of 38.6°C and a high temperature of 40.2°C. She had no gastrointestinal symptoms. On physical examination, she had a body mass index of 23.5 kg/m^2, and her vital signs were as follows: a heart rate of 120 beats per minute, 28 breaths per minute, an oxygen saturation of 95% at ambiance, and pharyngeal congestion oozing. Further examination revealed a decreased neutrophil absolute count and percentage, but normal liver, heart, and kidney functions and C-reactive protein (CRP) levels, as seen in Table 2. Chest radiography, performed on August 2 following disease onset, indicated slight thickening of the wall of the anterior and posterior segments of the right upper lobe, with the lumen slightly narrowed and the lower right lung slightly inflamed (Figure 3). Symptomatic treatment was started on the day of illness onset, without any oxygen or glucocorticoid therapy. The fever and cough resolved by August 1 and August 4, respectively (Figure 1). A throat swab sample collected on August 2 was positive for adenovirus (ct value: 28.8) and HCoV-229E (ct value: 32.5), as seen in Table 1. On August 5, the patient was discharged and recovered completely (Table 1). The period from illness onset to discharge was eight days (see Table 1 and Figure 1).

Coinfection-case 2

The second co-case patient was a healthy nine-year-old girl who had visited the same swimming center as the first patient on July 28, 2019. She was admitted to room 845 of the pediatric ward on August 1 because of a history of high fever, 38.6°C for one day (Figures 1, 2). The fever continued for four days, reaching a high of 39.5°C. Upon physical examination, the patient had pharyngeal congestion oozing. The lab test indicated that the patient had a normal

TABLE 1 Comparison of the characteristics of two children coinfected with HCoV-229E and adenovirus admitted to different rooms of the same pediatric ward and mono-infections with HCoV-229E and Adenovirus in Lishui, Zhejiang Province, July–August 2019.

Characteristic	Coinfection withHCoV-229E and adenovirus ($n = 2$)		Mono-infection with HCoV-229E ($n = 3$)			Mono-infection with adenovirus ($n = 21$)	p-value
	Co-case 1	Co-case 2	Case 1	Case 2	Case 3		
Demographics							
Age (in years)	13	9	54	59	43	8 (2–14)	0.332
Gender	Female	Female	Female	Male	Female	Male/female = 2.5:1.0	0.111
Occupation	Student	Student	Farmer	Farmer	Doctor	Preschool children or students	/
Basic situation							
History of alcohol use	No	No	No	No	No	No	/
History of smoking	No	No	No	Yes	No	No	/
Underlying conditions	No	No	No	No	No	No	/
Exposure history							
Visit to the swimming center	Yes	Yes	No	No	No	100%	/
Exposure to a febrile person	Yes	Yes	Yes	Yes	Yes	100%	/
Provided bedside care	No	No	Yes	Yes	No	No	/
Medical care services	No	No	No	No	Yes	No	/
Laboratory results							
Specimen collection date	August 2	August 2	August 2	August 2	August 2	August 2	/
Specimen collection type	Throat swab	Throat swab	Throat swab	Throat swab	Throat swab	Throat swab	/
Diagnostic method	rRT-PCR and sequencing	rRT-PCR and sequencing	rRT-PCR and sequencing	rRT-PCR and sequencing	rRT-PCR and sequencing	rRT-PCR and sequencing	/
Date of confirmation	August 5	August 5	August 7	August 7	August 7	August 5	/
viral load (ct value)	Adenovirus (28.8)	Adenovirus (22)	Adenovirus (Negative)	Adenovirus (Negative)	Adenovirus (Negative)	Adenovirus (Average: 23.78)	0.570
	HCoV-229E (32.5)	HCoV-229E (33.3)	HCoV-229E (28.4)	HCoV-229E (30.5)	HCoV-229E (31.3)	HCoV-229E (Negative)	0.067
Clinical features							
Max temperature (°C)	40.2	39.8	/	/	/	39.3	0.1431
Fever duration (days)	3	2	/	/	/	2.90	0.5062
Exposure to onset (days)	2	3	No symptoms	No symptoms	No symptoms	3 (0–7)	0.8302
Onset to admission (days)	2	1	/	/	/	2.9 (0–6)	0.332
Hospital stays (days)	6	8	/	/	/	6.76 (3–13)	0.640
Days from onset to be discharged (days)	8	9	/	/	/	9.67 (3–17)	0.285

CT, cycle threshold. In this table, all of the statistical analyses were performed using the statistical package for the social sciences (SPSS; version 26.0). Categorical variables were compared using Fisher's exact test. The Mann–Whitney test was used to compare continuous variables. All statistical tests were two-sided, and a p-value of 0.05 was considered statistically significant. The symbol / indicates that data are not available.
P-value notes the comparison of the coinfection group and the mono-adenovirus group.

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FIGURE 1
Symptoms and results of rtPCR testing and antigen rapid test for the two children coinfected with HCoV-229E and adenovirus; they were admitted to different rooms in the same pediatric ward in Lishui, Zhejiang Province, July–August 2019. rtPCR = real-time PCR. ADV = adenovirus. The red cross indicates that the pathogens (adenovirus or 229E) were detected by PCR or rapid antigen detection. The gray – indicates an HCoV-229E negative test.

blood count, liver function, and kidney function, but slightly increased lactate dehydrogenase (Table 2). A throat swab sample collected on August 2 was positive for adenovirus (ct value: 22) and HCoV-229E (ct value: 33.3), as shown in Table 1. Symptomatic treatment and support therapy was started, without any glucocorticoid therapy. The patient was discharged eight days after admission (see Table 1 and Figure 1).

Mono-infection with HCoV-229E

The parents of coinfection-case 1 (mother: 54 years old, father: 59 years old, both farmers) undertook bedside care and remained asymptomatic (Table 1). A 43-year-old female doctor without any symptoms was in charge of the two co-cases. Three throat samples collected on August 6 showed that the parents (ct values: 30.5, 28.4, respectively) and doctor had HCoV-229E (ct value: 31.3) (Table 1).

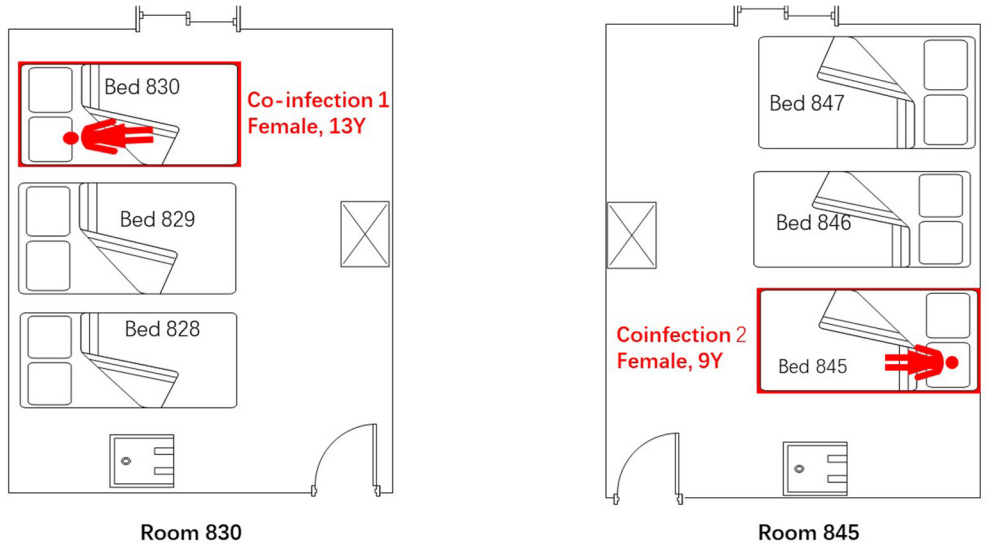

FIGURE 2
Spatial distribution of the two admitted children who were coinfected with adenovirus and HCoV-229E in the pediatric ward of Lishui, Zhejiang Province, July–August 2019.

TABLE 2 Hematological and blood biochemical measurements of two children coinfected with HCoV-229E and adenovirus admitted to different rooms of the same pediatric ward and mono-infections with adenovirus in Lishui, Zhejiang Province, July–August 2019.

Variables	Coinfection-case 1	Coinfection-case 2	Mono-infections with adenovirus ($n = 21$)	Normal range	p-value
Blood routine					
WBC ($\times 10^9$ per L)	2.1	4.7	5.66	4.0–10.0	0.347
Lymphocyte absolute count ($\times 10^9$ per L)	1.22	1.02	2.61	0.8–4	0.06
Neutrophil absolute count ($\times 10^9$ per L)	0.63	3.17	2.11	2–7.7	0.764
Lymphocyte percentage (%)	59.54	21.90	50.57	20–40	0.648
Neutrophil percentage (%)	30.74	68.00	42.10	50–70	0.876
Platelet count ($\times 10^9$ per L)	210	201	234.91	100–300	0.465
Hemoglobin (g/L)	115	127	119.32	110–150	0.917
Blood biochemistry					
Albumin (g/L)	39.7	36.5	39.48	35.0–55.0	0.958
ALT (U/L)	7	13	15.64	0–40	0.295
AST (U/L)	24	28	26.77	0–40	0.793
Urea (mmol/l)	3.60	3.14	3.84	2.50–7.20	0.497
Creatinine(μmol/L)	50	38	42.41	44–132	0.754
C reactive protein (mg/dl)	3.0	0.5	5.61	0–10	0.825
Lactate dehydrogenase (UI/liter)	186	229	240.82	65–220	0.230

AST, alanine aminotransferase; *ALT*, aspartate aminotransferase.

Mono-infection with adenovirus

All patients infected with a single adenovirus showed mild illness, with no serious illness found (Table 3). The most common clinical symptoms or signs were fever (100%, 22/22), fatigue (40.91%, 9/22), sore throat (40.91%, 9/22), and vomiting (36.36%, 8/22) (Supplementary Figure 1). The highest fever temperature (40 vs. 39.3°C, $p = 0.1431$) and fever duration (2.5 vs. 2.9 days, $p = 0.5062$) were the same between the coinfection

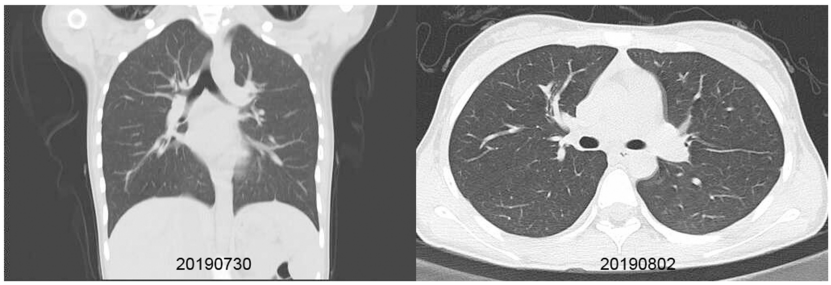

FIGURE 3
Chest X-ray and CT scan taken for a 13-year-old girl with HCoV-229E and adenovirus coinfection-induced fever on July 27, 2019. Chest CT scan and chest X-ray indicated slight thickening of the wall of the anterior and posterior segments of the right upper lobe, a slightly narrower lumen, and slight inflammation of the lower right lung. The bilateral hilum was normal, and no pleural effusion was observed. No obvious swollen lymph node shadow was found in the mediastinum. **(A)** Left, Chest X-ray taken on July 30, 2019. **(B)** Right, Chest CT scan taken on August 2, 2019.

TABLE 3 Treatment and disease severity of two children coinfected with HCoV-229E and adenovirus admitted to different rooms of the same pediatric ward and mono-infections with adenovirus in Lishui, Zhejiang Province, July–August 2019.

Variables	Co-case 1	Co-case 2	Mono-infections with adenovirus ($n = 21$)	p-value
Mild CAP	Yes	No	One case	Not available
Severe CAP	No	No	No	Not available
Extremely severe pneumonia	No	No	No	Not available
PICU admission	No	No	No	Not available
Immune global protein	No	No	No	Not available
Glucocorticoids therapy	No	No	One two-year-old child	Not available
NCPAP	No	No	No	Not available
Invasive mechanical ventilation	No	No	No	Not available
Oxygen therapy	No	No	No	Not available
Clinical outcome	Survival	Survival	Survival	Not available

CAP, community-acquired pneumonia; *NCPAP*, nasal continuous positive airway pressure; *PICU*, the pediatric intensive care unit.

and mono-infection with adenovirus groups (Table 1). We compared the clinical progress among the 21 mono-adenovirus infections with the HAdV-7 group and two coinfections with HAdV-7. Between the coinfection group and mono-HAdV-7 group, the results indicated no differences in average days from exposure to onset (2.5 vs. 3 days, $p = 0.8302$), onset to admission (1.50 vs. 2.90 days, $p = 0.332$), hospital stay (7 vs. 6.76 days, $p = 0.640$), or onset to discharge (8.50 vs. 9.67 days, $p = 0.285$) (see Table 1).

One coinfection with adenovirus mycoplasma pneumoniae

The case patient was a healthy two-year-old boy who had visited a JinYun swimming center on July 27, 2019. On July 31, he developed a constant high fever of 40.2°C and a cough, leading to admission to room 840 of the pediatric ward on August 4 (Figure 4). The lab test indicated that the patient had an elevated white blood cell count, an increased lymphocyte percentage, a decreased neutrophil percentage, and a low level of hemoglobin (113 g/L). A chest X-ray was taken on August 4, 2019, depicting exudation and small patchy shadows in the upper lobe of the right lung. In addition, the right hilum shadow was slightly enlarged (Figure 5A). A chest CT (Computed Tomography) scan on August 5 showed signs of an air bronchogram in the consolidation tissue, accompanied by segmental consolidation in the dorsal segment of the right lower lobe (see Figure 5B). On August 5, the mycoplasma DNA loads by rt-PCR were 3.60×10^4 copies/ml (normal level: <400) (Figure 4). A throat swab sample collected on August 2 was positive for adenovirus (ct value: 21.4) (Figure 4). A chest CT plain scan taken on August 11 indicated that the upper lobe of the right lung was redilated (Figure 5C). The patient was discharged on August 17 after the use of glucocorticoids (Figure 4 and Table 3). None of the above patients were treated with invasive mechanical ventilation, transferred to the

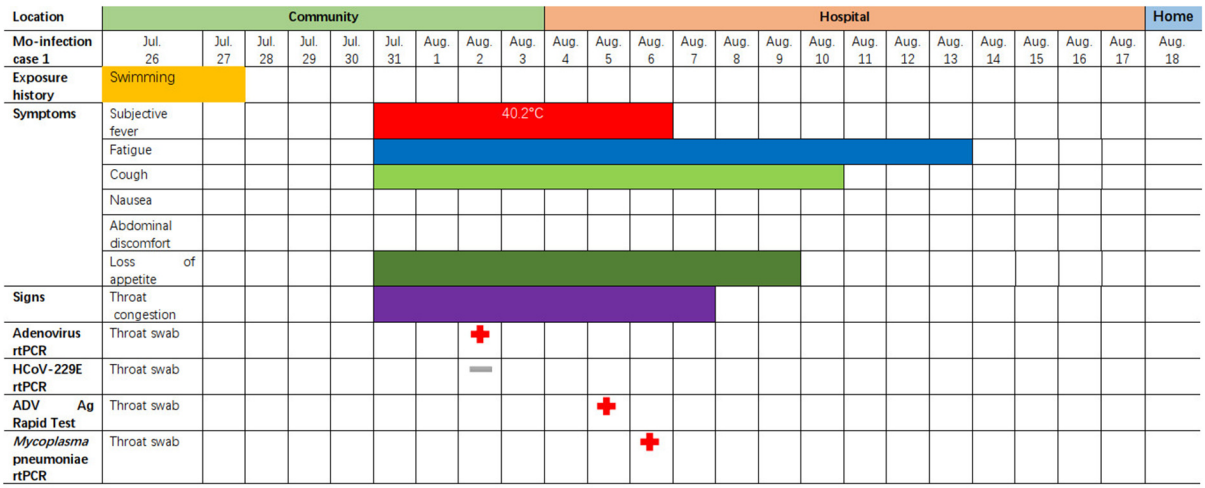

FIGURE 4
Symptoms and results of rtPCR testing and antigen rapid test for a two-year-old child coinfected with adenovirus and *single M. pneumoniae* who was admitted to the pediatric ward in Lishui, Zhejiang Province, August 4–17, 2019. rtPCR = real-time PCR. ADV = adenovirus. The red cross indicates that the pathogen (adenovirus or single *M. pneumoniae*) was detected by PCR or rapid antigen detection. The gray—indicates an HCoV-229E negative result.

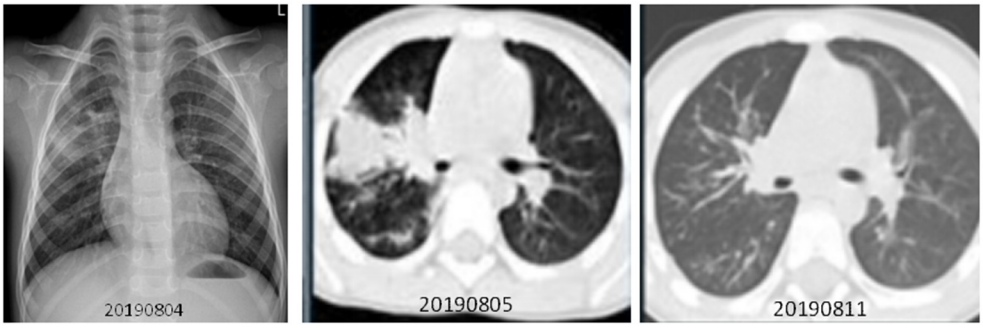

FIGURE 5
Chest X-ray and CT scan were taken for a two-year-old boy with adenovirus and *mycoplasma pneumoniae* coinfection-induced fever on July 31, 2019. (1) **(A)** Left, Chest X-ray taken on August 4, 2019. Exudation and small patchy shadows in the upper lobe of the right lung. The right hilum shadow was slightly enlarged, and the left hilum was normal. No bilateral pleural effusion was observed. (2) **(B)** Middle, Chest CT plain scan was taken on August 5, 2019. Incomplete, segmental lung consolidation and exudation lesions were observed. Signs of air bronchogram were seen in the consolidation tissue, accompanied by segmental consolidation in the dorsal segment of the right lower lobe. The right hilar shadow was enlarged, and the left hilar was normal. There was no bilateral pleural effusion and no obvious enlarged lymph nodes in the mediastinum. **(C)** Right, Chest CT plain scan taken on August 11. The upper lobe of the right lung was redilated, showing upper, middle, and lower lobe bronchial wall thickening in the right lung as well as a weak patchy shadow distributed along the airway. There was no thickening of the lobular septum or pleura, and no pleural effusion was observed. The hilar had a normal morphology, and there was no significantly enlarged lymph node shadow in the mediastinum. Compared with the CT scans from the week prior, the right lung lesion had been significantly absorbed.

PICU (Pediatric Intensive Care Unit), or given oxygen therapy (Table 3).

Comparison of viral loads and genetic identity

The average viral ct was 25.4 and 23.7 in the coinfection and single adenovirus groups ($p = 0.570$), while the average viral ct was 32.9 and 30.06 in the coinfection and single HCoV-229E groups ($p = 0.067$), respectively (Table 1). The difference in viral loads was not statistically significant between the two groups (Table 1).

Regarding the alignment of the penton, fiber, and hexon of the adenovirus isolated from the coinfection group ($n = 2$) and single adenovirus group ($n = 21$), these adenovirus sequences shared 99.5 and 100% of the nucleic acid sequence identity. The HCoV-229E S1 gene segments for the coinfection group ($n = 2$) and single HCoV-229E group ($n = 3$) were 97.5–99.9% identical.

Discussion

We report on the rare phenomenon of HAdV-Ad-7 and adenoviral HCoV-229E coinfection in children. Compared with HAdV-Ad-7 mono-infection, there was no increase in clinical severity for children coinfected with HCoV-229E. There were also no differences in terms of incubation, fever duration, high temperature, length of hospital stays, or viral load between the two groups. The three adults infected with HCoV-229E did not develop any symptoms. No patients with coinfections or mono-infections of HAdV-Ad-7 or HCoV-229E had severe complications; they fully recovered after early and adequate supportive and symptomatic treatment.

Several studies have reported on HAdV-Ad-7 coinfections with other pathogens (19). One study indicated that 4% of pediatric pneumonia admissions were associated with endemic HCoVs, with a high proportion of cases co-occurring with another respiratory virus (22, 23). However, coinfection with HCoV-229E and adenovirus has not been documented in the literature. In this study, our joint investigation team continuously sampled the outbreak cases (symptomatic and asymptomatic patients) and used a more sensitive method (Multiplex PCR method for simultaneous tests of 14 different pathogens). Finally, two co-infections were isolated from the 24 admitted cases of human adenovirus genotype 7, accounting for 8.33% (2/24) of the total number of cases. This number was far lower than adenovirus coinfected with other pathogens, such as respiratory syncytial virus (19/37, 34.5%) and influenza virus (6/37, 10.9%] (20). The exact reasons for the coinfections remain unclear, but several factors may have played a role. Most importantly, the children infected with adenovirus were weakened and susceptible to other pathogens after the persistent high fever. Secondly, there are no vaccines against adenovirus or HCoV-229E virus in China. Meanwhile, HCoV-229E has a low prevalence in this area. As a result, most of population has no immunity to these two viruses (24). Thirdly, adenoviruses are non-enveloped viruses that are unusually resistant to physical and chemical agents, which gives them prolonged survival capacity in various environments (25).The incubation period for mono- or coinfection was 2–3 days, which aligns with previous estimates of adenovirus incubation periods (26). However, mono-adenovirus infection, mono-HCoV-229E infection, and coinfection with HAdV-Ad-7 and HCoV-229E involve non-specific signs and symptoms. This lack of specific symptoms contributes to delayed diagnosis and treatment (27–29).

Furthermore, the present study showed that both groups had similar clinical signs and laboratory test results, such as white blood cell (WBC), AST and ALT, lactate dehydrogenase (LDH), and C-reaction protein (CRP) counts. Therefore, it is difficult to distinguish whether or not patients have a mono-infection with adenovirus or coinfection with two viruses based on clinical signs and routine blood tests. Although coinfection is not common, in cases where the disease is not explained by a single pathogen, additional studies, such as a nested PCR for multi-respiratory panel pathogens, are required to detect potentially treatable pathogens, such as COVID-19 mycoplasma, influenza virus, and 229E (30, 31).

Fever is a manifestation of the body's resistance to inflammation, often used to judge the progress or outcomes of a disease (32). In this study, both groups developed acute illness, characterized by a constant high fever; they also had similar fever durations (3–5 days), which was not consistent with previous reports (33). Chen et al., for instance, indicated that 6/103 of the children in their study had HAdV coinfected with *mycoplasma pneumoniae,* and the proportion of fever duration >10 days (40.8%) in the mixed infection group was significantly higher than in the mono-infection group (24.5%, $p = 0.014$) (34). The present study found no significant difference in patient age and sex between the mono- and coinfection groups. The median duration of hospital stay in the two groups was generally shorter than eight days, which aligns with information on single adenovirus infections reported from Beijing (20). This limited data suggest that coinfection with two viruses does not prolong the clearance time of the pathogen, aggravate the host immune response, lead to organ damage, or show more internal and exogenous pyrogen.

Regarding the severity of disease, some research has indicated that HAdV has strong virulence that can cause high mortality in children (32). HAdV-7 in particular may cause severe infection (35, 36). One study indicates that HAdV coinfection with another pathogen aggravates the severity of pneumonia in children (10). Studying the risk factors of severe atypical pneumonia using multivariate logistic regression analysis, Huong et al. found that coinfection with a respiratory virus was a risk factor for severe atypical community-acquired pneumonia in children (OR = 4.36, $p = 0.008$) (37). Previous studies indicated that HAdV coinfection with another pathogen aggravates the severity of pneumonia in children (10). For example, Juan et al. reported that adenovirus and SARS-CoV-2 mixed infections are associated with adverse clinical outcomes, such as shock, lymphopenia, and thrombocytopenia. Patients with these mixed infections are also more likely to require ventilatory support and admission to intensive care units (38). However, these studies are not consistent with our findings, indicating that both mono- and coinfections typically resulted in mild upper respiratory tract infections. Furthermore, the cases in this study also did not show any liver damage, which differed from other studies that found the adenovirus 41 genotype to lead to hepatitis of unknown origin (39–44). Only one 13-year-old girl, coinfected with adenovirus and HCoV-229E, and one two-year-old boy with adenovirus and mycoplasma, developed mild pneumonia, without any severe

complications. Both patients were cured and discharged after the use of symptomatic support therapy and glucocorticoids. The severity of HAdV infection is affected by many factors, including the patient's age, immune status, diagnosis, viral load, and socioeconomic status (20). In this study, all cases presented mild illness regardless of whether they had a single infection (adenovirus or HCoV-229E) or coinfection with two viruses. Several factors may have contributed to this outcome. First, all coinfections and mono-infections were in young children who had no underlying diseases or need for regular medicine administration. By contrast, adenovirus has been recognized as a cause of severe illness among immunocompromised children (45). Second, diagnosis and therapy was provided early and in a timely fashion for these cases. Third, the viral loads in the two groups were at the low to middle level, without any mutations associated with viral reproduction. However, the acute mechanism of severity following HAdV-7 and HCoV-229E co-infections should be further investigated in the future.

In summary, this study explored the unusual phenomenon of HAdV-7 and HCoV-229E coinfection in healthy children. Compared with single adenovirus infections, coinfection with HAdV-Ad7 and HCoV-229E virus did not contribute to disease severity, a finding that may be attributed to the children's good underlying health and low viral loads. Other factors in these positive outcomes include early identification of a potential adenovirus coinfection and successful treatment. However, larger and better-designed prospective analytical studies are required to examine further risk factors as well as the interaction between adenoviruses and other respiratory pathogen coinfections.

This study has some shortcomings. First, as a retrospective study, data are inevitably missing; for example, the serum antibody counts against the adenovirus and HAdV or HCoV-229E and the systemic inflammatory cytokines were not available for the mono-infections or coinfections. Second, the number of ADV+ HAdV-229E coinfections was relatively small. The interpretation and extrapolation of these results should be conducted with caution. A multicenter prospective study with a larger sample is needed. Third, the epidemiology level of adenovirus coinfection with other pathogens in the general population was unclear in this research area.

The world is at a new stage of the COVID-19 pandemic. With non-pharmaceutical interventions (NPIs) having been released, HAdV and HCoVs, including SARS-CoV-2, are circulating endemically in human populations. The early signs and symptoms of infection with adenovirus and HCoV-229E are similar to those of COVID-19 (27). In the future, physicians and public health doctors should be alert to the possibility of HAdV coinfection during the COVID-19 pandemic.

Data availability statement

The original contributions presented in the study are included in the Supplementary material for this article. All sequences from mono-infections with adenovirus and the HCoV 229E, and coinfections with adenovirus and the HCoV-229E were submitted to the NIH genetic sequence database (GenBank, https://www.ncbi.nlm.nih.gov/genbank/). The number of the sequence GenBank are seen in Supplementary Table 1. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the study was approved by the Medical Ethics Committee of the Zhejiang Province Center for Disease Control and Prevention (No. 2019013). Written consent was obtained from all patients or their family. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

SL drafted the first version of the manuscript and obtained funding for the study. AZ, JP, LY, WS, and HW collected clinical data for the study. HZ and HL verified and analyzed data for the study. LW, SQ, ZY, JC, and EC conducted the investigation. YC analyzed all samples. All of the authors contributed data to the study and participated in data interpretation and critical review of the manuscript. All of the authors approved the final manuscript for submission.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1048108/full#supplementary-material

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