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Seroprevalence of neutralizing antibodies to human mastadenovirus serotypes 3 and 7 in healthy children from guangdong province

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ABSTRACT

Severe adenovirus pneumonia is becoming more common in children infected with human mastadenovirus (HAdV)-3 and HAdV-7 than in those infected with other types of adenoviruses. Recently, there has been a trend toward an increasing prevalence of pneumonia caused by HAdV-7, an important viral pathogen in Pediatric Intensive Care Unit infections. Children infected with HAdV-7 have more serious symptoms of acute respiratory infections and other complications than those infected with HAdV-3. No specific anti-adenovirus drugs or vaccines are available for treatment or prevention. Therefore, we investigated the seroprevalence and titer levels of neutralizing antibodies (NAbs) against HAdV-3 and HAdV-7 in healthy children in Guangdong Province. We found that the seropositivity rates and antibody titers for HAdV-3 NAb were higher than those for HAdV-7 NAb. In children between 6 and 12 months of age, the seropositivity rates and titers were significantly low against HAdV-3 and HAdV-7. The HAdV-7-positive rate was significantly higher in the HAdV-3-positive samples than in the HAdV-3-negative samples. The HAdV-7 NAbs carried by the 0–6-month age group were dominated by low titers. These results reveal a low level of herd immunity against HAdV-3 and HAdV-7 in children, clarifying the importance of monitoring these two highly virulent adenoviruses, developing prophylactic vaccines, and predicting potential outbreaks.

1. Introduction

Human mastadenoviruses (HAdVs), belonging to *Adenoviridae*, members of the genus *Mastadenovirus*, are further divided into seven species (A to G) and more than 100 genotypes [1,2]. Different serotypes associate different pathogenicities and organ affinities [3,4]. Species B, C, and E are the main types that cause community-acquired pneumonia in infants and young children and include the HAdV-B (such as HAdV-3, -7, -11, −14, and −55), HAdV-C (such as HAdV-1, HAdV-2, and HAdV-5), and HAdV-E groups (such as HAdV-4).

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Globally, HAdV-3 and HAdV-7 are important serotypes in the community. They are found worldwide and cause epidemics and outbreaks [5–10]. Furthermore, they often lead to acute respiratory infections in children or patients with a weakened immune system. Severe cases can also result in respiratory failure, acute respiratory distress syndrome (ARDS), encephalitis, and other complications. Outbreaks of upper respiratory tract infections caused by HAdVs have occurred in China over the past decade. From 2006 to 2009, 78% of children with ARDS in Lanzhou were infected with HAdV-3 [11]. From 2010 to 2011, HAdV-3 was identified in 92.7% of respiratory samples from children suspected of adenovirus infection in the southern city of Guangzhou [12]. Recently, 37.5%–70% of HAdV respiratory tract infections in children were found to be related to HAdV-3, in central and southern China [13–16]. HAdV-7, which is in Group B with HAdV-3, has also been reported in global outbreaks and epidemics [7,8,10]. In mainland China, the outbreak of acute respiratory infection caused by HAdV-7 in 2009 was first reported in Shaanxi, followed by cases of HAdV-7 infection in Beijing, Shanghai, Jiangsu, and other provinces and cities, which accounted for 20.0%–51.5% [17–20] of outbreaks. In 2011, after a 21-year absence, HAdV-7 reappeared and became common in Guangzhou [21]. From 2012 to 2013, HAdV-3 was the main subtype of HAdV in Guangdong (70%), followed by HAdV-7 (28%) [15]. In recent years, there has been a trend toward the rising prevalence of pneumonia infections related to HAdV-7, an important viral pathogen associated with Pediatric Intensive Care Unit infections (52.9%) [22–26]. Several studies have found that in children with HAdV-7 pneumonia, the clinical manifestation, blood cell number index, biochemical index, proinflammatory cytokines, and imaging indicators are associated with more severe outcomes and higher mortality rates than in those with HAdV-3 pneumonia [6,13,27–29]. In 2019, we investigated the change from the predominance of HAdV-3 to that of HAdV-7 in the HAdV epidemic in Guangdong [30,31]. Because there are no specific anti-adenovirus drugs or vaccines for treatment or prevention, we investigated HAdV-3 and HAdV-7 seroprevalence and levels of neutralizing antibody (NAb) titers in healthy children in Guangdong Province. Monitoring NAbs against HAdV-3 and HAdV-7 is of great significance for assessing herd immunity, predicting potential outbreaks, and developing vaccines.

2. Materials and methods

2.1. Serum samples from children

Samples were obtained from children (0 months–16 years of age) who underwent health examinations at the Outpatient Health Department of Guangzhou Women and Children's Medical Center from August to December 2019. Healthy children were assessed by children's health care doctors, and sampling was done according to the following criteria: no respiratory symptoms, no disease symptoms, and no antibiotic exposure for one month. The use of serum samples for this study was approved by the institutional review boards, and informed consent was obtained from the guardians. Serum samples were heat-inactivated at 56 °C for 45 min before storage at −20 °C. Samples were divided into seven groups according to age: 0–6 m, 6–12 m, 12–24 m, 24–36 m, 36–48 m, 48–60 m, and >60 m, with 50 serum samples in each group and 350 in total.

2.2. Microneutralization assays

Recombinant HAdV-3 and HAdV-7 expressing enhanced green fluorescent protein (EGFP) were kindly provided by Dr. Xingui Tian (Guangzhou Medical University). The reporter genes were efficiently expressed in cells that were infected by these viruses [32]. The titers of NAbs against HAdV-3 and HAdV-7 were measured using microneutralization assays based on HAdV-3 EGFP and HAdV-7 EGFP [33–36]. Briefly, A549 cells were plated at a density of 2×10^4 cells/well in 96-well plates and maintained overnight. Sera were diluted serially four-fold (from 1:8 to 1:512) in Dulbecco's modified Eagle's medium (DMEM) and incubated with 100 $TCID_{50}$ (median tissue culture infective dose) virus for 2 h at 37 °C, and each sample was tested in triplicate. Subsequently, the mixtures were added to 96-well A549 cell plates. After 72 h of incubation, cytopathic effects were observed and recorded. Neutralization titers were obtained by observing GFP-expressing cells using a fluorescence microscope in comparison with the parallel virus control and cell control in triplicate. EGFP-expressing cells have an excitation peak at 488 nm and emit light maximally at 507 nm, which can be easily monitored using fluorescence microscopy. The neutralization titers were expressed as the reciprocal of the dilution of antibodies that completely

Table 1
Distribution of NAb titers against HAdV-3 and HAdV-7 in different group in healthy children.

	All age groups ($n = 350$)			0–6 m group ($n = 50$)		
NAb titers	HAdV-3 NAb	HAdV-7 NAb	p Value	HAdV-3 NAb	HAdV-7 NAb	p Value
<1:8	233 (66.57)	291 (83.14)	$p < 0.0001$	30 (60.00)	35 (70.00)	$p > 0.05$
≥1:8	117 (33.43)	59 (16.86)		20 (40.00)	15 (30.00)	
1:8	42 (12.00)	39 (11.14)	$p = 0.0009$	15 (30.00)	11 (22.00)	$p > 0.05$
1:32	15 (4.29)	7 (2.00)		4 (8.00)	3 (6.00)	
1:128	28 (8.00)	8 (2.29)		1 (2.00)	1 (2.00)	
1:512	32 (9.14)	5 (1.43)		0 (0)	0 (0)	

Abbreviations: HAdV, human adenovirus; NAb, neutralizing antibody.
Reported as n (%).
Statistics were performed by Chi-square test. There was a significant difference in seroprevalence against HAdV-3 and HAdV-7 in all age groups, $p < 0.05$; there was no significant difference in seroprevalence against HAdV-3 and HAdV-7 in 0–6 m group, $p > 0.05$.

blocked the development of cytopathic effects.

2.3. Statistical analysis and figure development

SPSS 16.0, Excel, and GraphPad Prism 8 were used for statistical analysis, constructing spreadsheets, and making figures. The chi-square test was used to compare the seroprevalence. The Mann–Whitney or Kruskal–Wallis test was used to compare NAb titers between groups, and $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Seroprevalence and distribution of titers in the population

We measured the seroprevalence against HAdV-3 and HAdV-7 in the 350 serum samples. NAb titers were divided into three levels: negative (<1:8), low titer (1:8, 1:32), and high titer (1:128, 1:512). As shown in Table 1, a significantly higher seropositive rate for HAdV-3 NAb (33.43%) was observed than for HAdV-7 NAb (16.86%), overall ($p < 0.001$). NAb response at <1:8, 1:8, 1:32, 1:128, and 1:512 against HAdV-3 and HAdV-7 was 66.57%, 12.00%, 4.29%, 8.00%, and 9.14% and 83.14%, 11.14%, 2.00%, 2.29%, and 1.43%, respectively. A significant proportion of NAb-positive samples had low NAb titers (1:8) against HAdV-3 and HAdV-7. A comparison of NAb titers against HAdV-3 and HAdV-7 showed a significant difference ($p = 0.009$) between the two groups.

Briefly, in all age groups, children showed a higher seroprevalence against HAdV-3 than against HAdV-7. Moreover, the NAb titers against HAdV-3 in healthy children were significantly higher than those against HAdV-7.

3.2. Seroprevalence and titer distribution against HAdV-3 and HAdV-7 in different age groups

As shown in Fig. 1A, the trends in the HAdV-3 and HAdV-7 seropositivity rates followed a similar pattern in this study; relatively high in the 0–6-month age group initially, both decreased in the 12–24-month age group and then increased with age. The number of cases for NAbs against HAdV-7 was very low (3/50–4/50) in those aged between 12 and 36 months. We suggest that children between the age of 12 and 36 months had a low rate of herd immunity against HAdV-7. In Fig. 1B and C, there were very few samples with high titer NAbs against HAdV-3 and HAdV-7 in the 0–6- and 6–12-month age groups. Children in the 0–6-, 6–12-, and >60-month age groups showed a high rate of low NAb titers against HAdV-3. Children in the 12–24-month age group had notably high NAb titers against HAdV-3, which decreased with age. In contrast, high NAb titers against HAdV-7 were rare, and most of the NAb-positive samples against HAdV-7 were quite low in all age groups.

3.3. Relevance of seroprevalence for HAdV-3 and HAdV-7 NAbs

We analyzed the frequency of HAdV-7-seropositive donors among HAdV-3-seropositive or seronegative individuals. The HAdV-7-positivity rate was significantly higher in HAdV-3-positive samples than in HAdV-3-negative samples. Similarly, the HAdV-3-positivity rate was significantly higher in HAdV-7-positive samples than in HAdV-7-negative samples (χ^2-test, $p < 0.001$; Table 2).

A total of 41 samples were NAb-positive against HAdV-3 and HAdV-7 (Fig. 2). In general, titers of NAb against HAdV-3 were higher than (23/41, 56.10%) or equal to (12/41, 29.27%) those against HAdV-7 (Fig. 2), and only six samples showed a contrary result (one in the 0–6 month age group, two in the 24–36-month age group, and three in the 48–60-month age group).

As shown in Fig. 2, there were significantly more high-titer cases of NAb positivity against HAdV-3 than against HAdV-7 (21/41 vs.

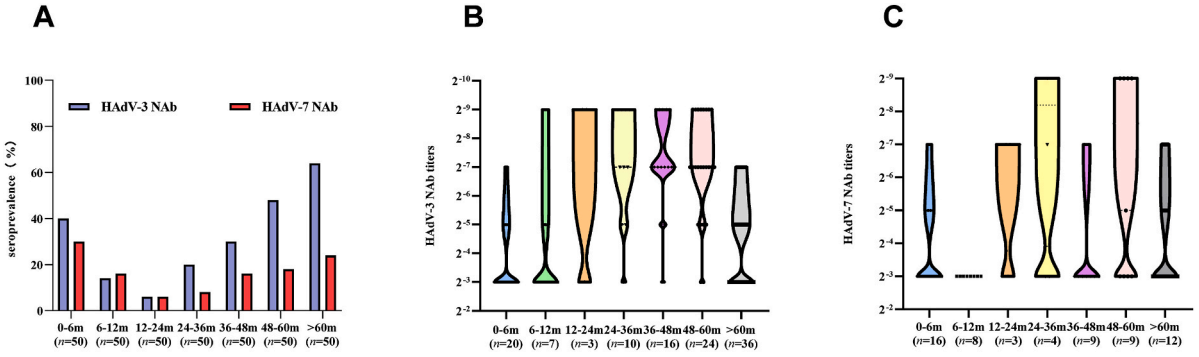

Fig. 1. Seroprevalence and titers distribution against HAdV-3 and HAdV-7 in different age groups. (**A**)Seroprevalence distribution against HAdV-3 and HAdV-7 in different age groups ($n = 350$). (**B, C**) The titers distributions of HAdV-3 NAb (**B**) and HAdV-7 NAb (**C**) in different age groups in seropositive samples. Data are presented as violin plot with median and quartiles. The data was analyzed with the Kruskal-Wallis test. The different for the seroprevalence population distribution and titer between HAdV-3 NAb and HAdV-7 NAb was analyzed by Chi-test. Abbreviations: HAdV, human adenovirus; NAb, neutralizing antibody; m, month.

Table 2
Relevance of HAdV-3 and HAdV-7 seropositive cases.

	HAdV-3 NAb$^+$	HAdV-3 NAb$^-$	HAdV-3 NAb + rate (%)	*p* Value
HAdV-7 NAb$^+$	41	18	69.491*	0.0000
HAdV-7 NAb$^-$	76	215	27.052*	
HAdV-7 NAb$^+$ rate (%)	35.04[3#]	7.73[4#]		
p Value	0.0000			

Abbreviations: HAdV, human adenovirus; NAb$^+$, neutralizing antibody titer ≥1:8; NAb$^-$, neutralizing antibody titer <1:8.
Cases are reported as *n*; the rates are reported as percentage.
*, #, Statistics were performed by Chi-square test.
NAb: neutralizing antibody.
1 the rate of NAb against HAdV-3 in HAdV-7 NAb$^+$ cases.
2 the rate of NAb against HAdV-3 in HAdV-7 NAb$^-$ cases.
3 the rate of NAb against HAdV-7 in HAdV-3 NAb$^+$ cases.
4 the rate of NAb against HAdV-7 in HAdV-3 NAb$^-$ cases.

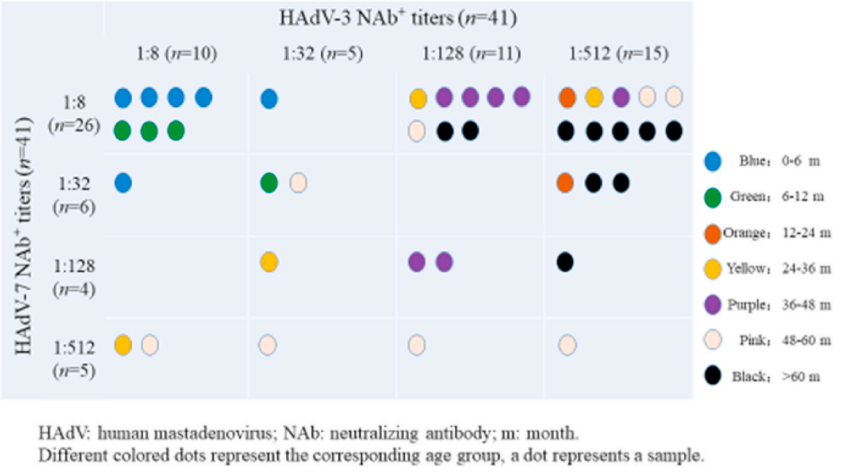

Fig. 2. NAb-positive titers again HAdV-3 and HAdV-7 in Double-Positive cases.

5/41). Low titers of NAb against both HAdV-3 and HAdV-7 (11/41) were mainly observed in the 0–6 month age group, and there were few samples in the 36–48-, 48–60-, and >60-month age groups that had high titers against both HAdV-3 and HAdV-7 (5/41). Samples with high-titer HAdV-3 NAb and low-titer HAdV-7 NAb (21/41) were from children 12–>60-month age group; samples with a low titer against HAdV-3 and high titer against HAdV-7 (4/41) were rare (two cases each in the 24–36- and 48–60-month age groups).

3.4. HAdV-3 and HAdV-7 NAbs in the 0–6-month age group

A total of 58.00% of samples (29 of 50) in the 0–6-month age group were NAb-positive for HAdV-3 or HAdV-7 (40.00% [20/50] and 30.00% [15/50], respectively). Single-positive NAbs against HAdV-3 were observed in 14 samples; nine samples carried NAbs positive against HAdV-7, and six samples were positive for NAbs against HAdV-3 and HAdV-7. The distribution of titers was similar to that of HAdV-3 NAb and HAdV-7 NAb populations. There were no samples with a 1:512 titer against HAdV-3 or HAdV-7 (Table 1). The seroprevalence and distribution of NAb titers against HAdV-3 was slightly higher than those against HAdV-7, but these differences did not reach statistical significance ($p > 0.05$). However, most NAb-positive samples against HAdV-3 or HAdV-7 were in the low-titer group (1:8).

4. Discussion

HAdV-3 and HAdV-7 are important human mastadenovirus types worldwide, and they often cause acute respiratory infections in children. In severe cases, HAdV-3 and HAdV-7 lead to respiratory failure, ARDS, encephalitis, and other complications. Currently, there is inadequate monitoring for HAdV-3 NAb and HAdV-7 NAb in children; therefore, we assessed the seroprevalence and titer levels of NAbs against HAdV-3 and HAdV-7 in 350 healthy children. In this study, we detected NAbs against HAdV-3 and HAdV-7 using an EGFP-expressing recombinant adenovirus. This assay is faster and more sensitive than the conventional serum neutralization test [37], facilitating epidemiological monitoring during outbreak investigations. A limitation of this assay is the particularly onerous requirement for manual observation. Optimization of the assay using automated analysis is necessary.

Several clinical studies have indicated that severe adenovirus pneumonia in children is closely related to HAdV-7, which mainly affects children under 2 years of age [13,23,28,38,39]. Studies indicate that HAdV-7 infection can cause more severe pneumonia, unspecified hydrocephalus, and respiratory failure than HAdV-3 infection [27–29,40]. A survey of hospitalized pediatric patients with adenovirus infection showed that there was an adenovirus-related outbreak in 2019, and a trend toward severe adenovirus pneumonia was more common in patients infected with HAdV-7 than in those infected with HAdV-3 [41]. An epidemiological study by our institution that included 1203 cases of HAdV infection in children in 2019 showed that the dominant HAdV subtype was HAdV-7 (46.30%), followed by HAdV-3 (40.57%), and the adenovirus infection rate of HAdV-7 (50.87%) was higher than that of HAdV-3 (35.22%) in children aged between 0 and 3 years [30]. In this study, we found that the seroprevalence of HAdV-3 NAb and HAdV-7 NAb in the pediatric cohort was low (31.71% and 16.86%, respectively) (Table 1), and the seroprevalence of HAdV-7 NAb was lower than that of HAdV-3 NAb in all age groups. We also found that the distributions of different NAb titers in the entire cohort showed that the lowest titer was observed in the 1- to 2-year-old age group, and low NAb titers were frequently detected in HAdV-7-seropositive donors (Fig. 2C). These results probably explain why there was a significant negative association between attack rate and herd immunity. Therefore, we speculate that the HAdV-7 NAb protection rate in children is far less than the HAdV-3 NAb protection rate, which could cause HAdV-7 reinfection in the short term and is consistent with clinical studies [30,31]. To sum up, NAbs against HAdV-3 and HAdV-7 showed low seroprevalence and titers in children; however, their implications for susceptibility to HAdV-3/-7 infection remain unclear. In the future, we aim to determine the relationship between waning antibody levels and infection risk.

We found that the HAdV-3 NAb positivity rate was higher in HAdV-7 NAb-positive cases than in HAdV-7 NAb-negative cases and vice versa (Table 2). We also found 41 double-positive cases in the entire cohort; HAdV-3 caused an earlier and stronger NAb response than HAdV-7 (Fig. 2). These results might be attributed to cross-reactions between these HAdV types. HAdV-3 and HAdV-7 are members of HAdV-B. Although the hexon epitope is the criterion for assigning different serotypes [42,43], fiber as a NAb protein has also been reported, and the difference in the gene sequence between HAdV-3 and HAdV-7 is mainly in the fiber protein [44–46]. The difference in NAb titers may explain the difference in the production intensity of NAbs elicited by HAdV-3 and HAdV-7 infections. Another possible explanation for these results is that individuals can be simultaneously or successively infected with HAdV-3 and HAdV-7 and produce the corresponding NAbs. When individuals were simultaneously infected with the two viruses at the same time, the immune response to the viruses increased with age. However, this needs to be confirmed by future research.

HAdV-3 and HAdV-7 infections in individuals with normal immunity are usually mild and self-restrictive. Infants and young children are a special group that is more vulnerable, and infection can be life threatening without the protection of antibodies. In our study, the seroprevalence of HAdV-7 NAb in the 0–6-month age group was 30.00%. Additionally, the majority of NAb-positive samples against HAdV-7 had low titer (Table 1). According to previous researches [12,36], individuals of fertile age, between 21 and 40 years, showed a 63.0% seroprevalence of HAdV-7 NAb, which suggests the limited maternal transfer of HAdV-7 NAb to neonates. In the limited period, small positive cases of HAdV-7 NAb in the 0–6-month age group were underpowered to show statistical differences between groups. However, this aspect needs to be confirmed in future studies using larger groups of cases.

Presently, there is no efficacious drug treatment and no vaccine available for the general population; consequently, a seroprevalence survey provides a more informed decision for pediatricians to proceed with the early diagnosis and treatment of HAdV-3/7 infection. Additionally, since HAdV-7 became the predominant subtype in 2019, it will be interesting to analyze whether seroprevalence and titers against HAdV-7 have increased.

It would be beneficial to improve the limitations of this study. For example, (1) a lack of detailed background information about the healthy children, such as community background status, nutritional status, and family history; (2) a small sample size of each age group would, (3) the small number of positive cases of NAb in the 0–6-month-old group, and (4) the technique of measurement for NAb titer. These limitations on the study would decrease the precision of the conclusions or were insufficient to show statistical differences between different titer levels.

In summary, we studied NAbs and titer levels against HAdV-3 and HAdV-7 in healthy children in 2019. The results showed that the NAb titer against HAdV-7 was lower than that against HAdV-3, which is possibly why the HAdV-7 infection rate was higher than that of HAdV-3 in 2019. The seroprevalence and titers against HAdV-7 are relatively low in healthy children; therefore, children in southern China are at a high risk of HAdV-7 infection. Thus, children aged 1–2 years are the most appropriate cohort to receive the vaccine.

Declarations

Ethics

This project was approved by the Ethics Committee of Guangzhou Women and Children's Medical Center, and the informed consent of the participants' parents or guardians was obtained.

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Author contribution statement

Lu Kuang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Changbing Wang: Conceived and designed the experiments; Performed the experiments.
Haiyang Chen: Yinghua Li: Tiantian Xu: Min Guo: Contributed reagents, materials, analysis tools or data.
Zhuofu Liang: Analyzed and interpreted the data.
Bing Zhu: Conceived and designed the experiments.

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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