



RESEARCH ARTICLE



Seroprevalence of Neutralizing Antibodies against Six Human Adenovirus Types Indicates the Low Level of Herd Immunity in Young Children from Guangzhou, China

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Abstract

Human adenoviruses (HAdVs) commonly cause many diseases such as respiratory diseases, gastroenteritis, cystitis worldwide. HAdV-3, -7, -4 and emergent HAdV-55 and HAdV-14 are the most important types causing severe respiratory diseases. There is no effective drug available for clinical treatment, and no vaccine available for the general population. Therefore, it is important to investigate the seroprevalence against HAdV for developing novel vaccines and vectors. In this study, we investigated the seroprevalence and titer levels of neutralizing antibodies (NAb) against HAdV-3, -4, -7, -14, -55, and -11 in total 278 healthy populations between 0 months and 49 years of age (228 children and 50 adults) from Guangzhou. In children under the age of 18 years, the seropositive rates were significantly increased against HAdV-3 at 12.07%, 33.96%, and 64.29% and against HAdV-7 at 0%, 18.87%, and 19.05% in age groups of 1–2, 3–5, and 6–17 years, respectively. The seroprevalence was very low (0% ~ 8.1%) for all other four types. In adults aged between 18 and 49 years, HAdV-3, -4, and -7 (> 50.00%) were the most common types, followed by HAdV-14 (38.00%), -55 (34.00%), and -11 (24.00%). Adults tended to have high NAb titers against HAdV-4 and -55. HAdV-55-seropositive donors tended to be HAdV-11- and HAdV-14-seropositive. These results indicated the low level of herd immunity against all six HAdV types in young children, and HAdV-14, -55, -11 in adults from Guangzhou City. Our findings demonstrate the importance of monitoring HAdV types and developing vaccines against HAdV for children and adults.

Keywords Human adenovirus (HAdV) · Seroprevalence · Neutralizing antibodies (NAb) · Vaccination · Vector · Acute respiratory disease

Introduction

Human adenoviruses (HAdVs) are non-enveloped, double-stranded DNA viruses and are divided into seven species (A–G). To date, approximately 100 types have been recognized according to genomic sequences (<https://hadvwg.gmu.edu/>) (Seto *et al.* 2011; Dehghan *et al.* 2019). HAdVs are highly contagious pathogens that exhibit a variety of tissue tropisms, and cause many diseases, such as acute respiratory disease (ARD), gastroenteritis, cystitis, keratoconjunctivitis, carditis, meningoencephalitis, depending on the infection type. The susceptible populations of HAdV infection include infants, immunocompromised patients (Sandkovsky *et al.* 2014).

Many HAdV types are relevant to ARD, including HAdV species B (type 3, 7, 14, 55, 11, 16, 21, 50), species C (type 1, 2, 5, 6, 57), and species E (type 4) (Lynch and

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Kajon 2016). Of these HAdVs, HAdV3, -4, -7, -14, and -55 have been reported to be associated with severe ARD outbreaks in military and civilian populations, and have caused severe and even fatal infections in both children and adults (Chen *et al.* 2015; Lin *et al.* 2017; Kajon *et al.* 2018; Li *et al.* 2018; Yao *et al.* 2019; Bautista-Gogel *et al.* 2020). HAdV-3 and -7 are the most common types in pediatric patients with ARD, and HAdV-7 is more likely to cause life-threatening pneumonia (Deng *et al.* 2013; Lin *et al.* 2017; Fu *et al.* 2019). Recently, a re-emergent genome type HAdV-7d associated with fatal pneumonia has been reported in Asia and the United States (Scott *et al.* 2016; Yu *et al.* 2016). HAdV-4 and -7 are the most prevalent types of HAdVs that have caused febrile ARD outbreaks in the military (Kajon *et al.* 2007; Cheng *et al.* 2016; Zhang *et al.* 2019). HAdV-14 and -55 are two re-emerging types that have caused numerous outbreaks among both civilian and military populations since 2006. HAdV-14 infection outbreaks have been reported in America (Kajon *et al.* 2010), Europe (Carr *et al.* 2011), and China (Huang *et al.* 2013). HAdV-55 is an inter-typic recombinant of HAdV-11 and HAdV-14, and HAdV-11 is the predominant type observed in patients with hemorrhagic cystitis (Numazaki *et al.* 1973). HAdV-55 has become a common pathogen causing severe pneumonia in northern China (Deng *et al.* 2013; Lu *et al.* 2014; Tan *et al.* 2016).

Vaccination may be the most effective way to prevent viral infection and establish herd immunity. However, there is no vaccine currently available for use in children and adults in the general population, although an oral vaccine comprising live HAdV-4 and -7 has been approved and used in the United States military for 40 years (Chen and Tian 2018; Gray 2020). This prompted us to develop a licensed vaccine to prevent HAdV infection outbreaks in susceptible populations (Qiu *et al.* 2012; Tian *et al.* 2014, 2015, 2018, 2019; Chen and Tian 2018; Liu *et al.* 2018). Therefore, it is important to investigate the predominant epidemic types and pre-existing immunity levels among populations against these types. Herd immunity is defined as the proportion of persons with immunity in a given population. The vaccination objective for public health is to increase the level of herd immunity to that affording indirect protection to unimmunized persons in a given population. The level of herd immunity can be assessed by antibody surveys (sero-epidemiology). Although many recent surveys have investigated the epidemiology of HAdV types in both children and adults with ARD, the pre-existing antibody levels have rarely been reported. HAdV infection induces type-specific, long-term neutralizing antibody (NAb) responses. NAbs against HAdV is indicative of protective antibody levels and previous infection among populations. It is helpful to investigate the prevalence of NAbs against HAdV-3, -4, -7, -14,

-55, and -11 in the general population for understanding human immunity against these HAdV types and for guiding vaccine development. Although we previously investigated the seroprevalence of NAbs against HAdV-3, -4, -7, -14, and -55 in healthy adults in southern China (Tian *et al.* 2016; Zheng *et al.* 2017; Ye *et al.* 2018), the prevalence of NAbs in children remains unclear.

In this study, we aimed to investigate the seroprevalence of NAbs against HAdV-3, -4, -7, -14, -55, and -11 in healthy populations aged between 0 months and 49 years in Guangzhou, southern China.

Materials and Methods

Human Serum Samples

Serum samples were randomly collected from 228 healthy children who received health examinations from February to June 2017 at Guangzhou Women and Children's Medical Center (n = 228, 82.01%), and 50 healthy adult blood donors at Guangzhou Blood Center from February to June 2017 (n = 50, 17.99%) in Guangzhou, southern China. The age of donors ranged from 0 months to 49 years, which were divided into six groups: 0–5 and 6–11 months old, 1–2, 3–5, 6–17, and 18–49 years old. There were 37–58 donors in each age group. The data were analyzed anonymously. However, no other detail about the donors was available.

Virus Microneutralization (MN) Assays

Wild-type HAdV-11 Slobitski strain (GenBank No. AF532578.1) was purchased from American type culture collection (ATCC) and kept in State Key Laboratory of Respiratory Disease. HAdV-3 GZ01 (GenBank No. DQ099432), HAdV-4 GZ01 (GenBank No. KF006344.1), HAdV-7 GZ08 (GenBank No. GQ478341.1), and HAdV-55 Shanxi-Y16 (GenBank No. KF911353.1) strains were maintained at State Key Laboratory of Respiratory Disease. HAdV-14 GZ01 strain (GenBank No. JQ824845.1) was kindly provided by Prof. Qiwei Zhang (Southern Medical University, China). All HAdVs were cultured in HEp-2 or AD293 cells. The cells, maintained in our laboratory, were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA), supplemented with penicillin (100 IU/mL), streptomycin (100 µg/mL), and 10% fetal bovine serum (Gibco, USA).

The NAb titers against the human adenoviruses were calculated using standard *in vitro* MN assays. In brief, the AD293 cells were seeded into 96-well plates at the density of 2×10^4 cells per well and cultured overnight. Further, human sera were heated at 56 °C for 30 min and diluted

with DMEM at 1:18. Then the sera were serially two-fold diluted (from 36- to 1152-fold), and incubated with 100 TCID₅₀ (50% tissue culture infectious dose) viruses at 37 °C for 1 h. Then, the mixtures were added to the 96-well plates and incubated at 37 °C for 48 h. The neutralization titers from triplicate wells were defined as the highest dilution of sera that inhibited the adenovirus growth without visible cytopathic effect.

Statistical Analyses

The statistical significance of seroprevalence between different groups was tested using the chi-squared test, and Fisher's exact method when appropriate. The correlation between serum titer levels of HAdVs and age groups was assessed using Goodman–Kruskal Gamma method. All statistical analyses were computed with SPSS software (ver. 17.0) and *P* values of < 0.05 were considered significant.

Results

Seroprevalence of NAbs against HAdVs

The titers of NAbs against several HAdV types were measured in the sera from 278 healthy donors aged between 0 months and 49 years. As shown in Table 1, among the 278 serum samples, 122 (43.89%) were positive for at least one of the six HAdV types. We observed that a high proportion of samples (34.21%) from age group of 0–5-months-old had NAbs for at least one of the six HAdV types, which was higher than that in age groups of 6–11 months old (13.51%) and 1–2 years old (15.52%). In donors aged between 6 months and 2 years, the percentage of samples with NAbs was very low. Above the age of

1 year, the percentage of samples that was positive for HAdV NAbs (titers > 18) increased with the age of the donors (Fig. 1). The group of 18–49 years old had the highest proportion of positive samples (90%) (*P* < 0.001).

The seroprevalence of NAbs against different HAdV types (HAdV-3, -4, -7, -11, -14, and -55) in different age groups was investigated to illustrate the impact of age on HAdV infection. As shown in Table 1 and Fig. 1, the seroprevalence of the adult group (18–49 years old) was the highest among all HAdV types. In adults aged between 18 and 49 years, HAdV-3 (78%), -4 (56%), and -7 (54%) were the most common types, followed by HAdV-14 (38.00%) and -55 (34.00%). HAdV-11 (24.00%) was the rarest type. There was a significant difference between the prevalence of different types in 6–17-year-old group and 18–49-year-old group (*P* < 0.001). It is noteworthy that the percentage of positive samples from donors less than 3 years old was much lower for all six HAdV types. Children under 18 years of age showed significantly increased seropositive rates against HAdV-3 from 12.07%, 33.96% to 64.29%, HAdV-7 from 0%, 18.87% to 19.05% at ages of 1–3, 4–6, and 7–18 years, respectively, and the seroprevalence was very low (0% ~ 8.1%) for all other four types. These results indicated high infection rates of HAdV-3 and -7 in children, high infection rates of HAdV-4 and -7 in adults, and the low rate of herd immunity against HAdV-14, -55, and -11 in all ages.

Neutralizing Antibody Titers against Different HAdV Types in Healthy Children and Adults

NAbs titers against different HAdV types was performed (Table 2). The NAbs titers were divided into four levels: negative (< 18), low titer (18–144), medium titer (145–576), and high titer (> 576). As shown in Table 2, 38.13%, 18.71%, 14.39%, 7.55%, 8.99%, and 5.40% of the

Table 1 Seroprevalence of neutralizing antibodies against human adenovirus (HAdV) in different age groups.

Age	Total		Different genotypes, n (%)						<i>P</i> value
	Samples tested	HAdV-positive, n (%)	HAdV-3	HAdV-7	HAdV-4	HAdV-55	HAdV-14	HAdV-11	
0–5 m	38	13 (34.21)	10 (26.32)	4 (10.53)	2 (5.26)	2 (5.26)	1 (2.63)	0 (0)	0.915
6–11 m	37	5 (13.51)	5 (13.51)	3 (8.11)	3 (8.11)	2 (5.41)	2 (5.41)	1 (2.70)	0.909
1–2 y	58	9 (15.52)	7 (12.07)	0 (0)	2 (3.45)	0 (0)	0 (0)	0 (0)	0.375
3–5 y	53	20 (37.74)	18 (33.96)	10 (18.87)	2 (3.77)	0 (0)	3 (5.66)	1 (1.89)	0.109
6–17 y	42	29 (69.05)	27 (64.29)	8 (19.05)	3 (7.14)	0 (0)	0 (0)	1 (2.38)	0.001
18–49 y	50	45 (90.00)	39 (78.00)	27 (54.00)	28 (56.00)	17 (34.00)	19 (38.00)	12 (24.00)	0.000
Total	278	122 (43.89)	106 (38.13)	52 (18.71)	40 (14.39)	21 (7.55)	25 (8.99)	15 (5.40)	< 0.001
<i>P</i> value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	

P value was assessed by Fisher's exact test.

The bold values indicating statistical significance were obtained from compared groups. m: month; y: year.

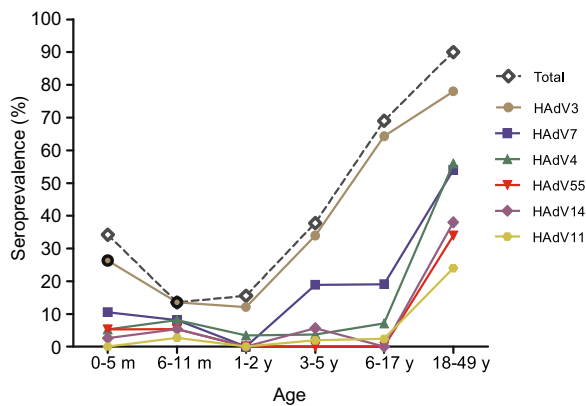


Fig. 1 Seroprevalence against different human adenovirus (HAdV) types in different age groups. m: month; y: year.

278 samples were positive for HAdV-3, -7, -4, -55, -14, and -11, respectively; and 8.63%, 6.12%, 2.52%, and 2.88% of the samples had notably high NAb titers (> 576) against HAdV-3, -4, -7, and -55, respectively. There was a significant difference in the seroprevalence against different HAdV types ($P < 0.001$). As shown in Fig. 2, a significantly higher proportion of NAb-positive samples had high titers of NAb (> 576) against HAdV-4 and -55 ($P < 0.001$). Most of the NAb-positive samples against HAdV-14 were in the group of low titers. There was no sample in the group of high titers against HAdV-11.

Further comprehensive analyses were performed on the distribution of NAb titers against the six HAdV types in different age groups. As shown in Fig. 3, 0%, 2.7%, 3.4%, 9.4%, 26.2%, and 10% of samples from age groups of 0–5 and 6–11 months, 1–2, 3–5, 6–17, and 18–49 years had notably high NAb titers (> 576) against HAdV-3. Children in the age group of 6–17 years showed the highest rate of high NAb titer against HAdV-3. There was no sample with high-titer NABs against HAdV-7 in the age groups of 0–5 and 6–11 months and 1–2 years; 3.77%, 4.76%, and 6% of samples from age groups of 3–5, 6–17, and 18–49 years had notably high NAb titers (> 576) against HAdV-7. The samples from children aged under 18 years showed only

low-titer NABs (18–144) against all other four HAdV types. In adults, the samples showed a relatively high proportion of high-titer NABs against HAdV-4 (34%) and -55 (16%). Only 2% of samples from adults had high NAB titers against HAdV-14. There was no sample with high-titer NABs against HAdV-11 in all age groups.

Correlation of NAb Seropositive Rates for HAdV-55, HAdV-11 and HAdV-14

Many serum samples were positive to two (total 27 samples) or more (total 35 samples) HAdV types, of which four samples (all from the age group of 18–49 years old) were positive to all six HAdV types. The HAdV-7-positive rate was significantly higher in HAdV-3-positive samples than in HAdV-3-negative ones and *vice versa*; The HAdV-7-positive rate was significantly higher in HAdV-4-positive samples than in HAdV-4-negative ones and *vice versa* (data not shown). These results may be attributed to the cross-reaction of one HAdV type NAB with other HAdV types, and another possibility is that some individuals might be susceptible to infection by multiple HAdV types and thus generated NABs to them.

Then we analyzed the frequency of double-seropositive donors and single-seropositive donors against HAdV-55 and HAdV-11 in detail. The HAdV-55-positive rate was significantly higher in HAdV-11-positive samples than in HAdV-11-negative ones; Similarly, HAdV-11-positive rate was significantly higher in HAdV-55-positive samples than in HAdV-55-negative ones (χ^2 -test, $P < 0.0001$; Table 3). Similar trends were detected for HAdV-55 and HAdV-14, or HAdV-11 and HAdV-14 (data not shown). The numbers of single-positive, double-positive and tri-positive donors against HAdV-55, HAdV-11, and HAdV-14 were shown in Fig. 4A. Of total 278 samples, 6 samples were tri-positive to HAdV-55, HAdV-11, and HAdV-14, all of which had high titers of HAdV-55 NAB (Fig. 4B). In contrast, only one sample in double-negative against HAdV-14 and HAdV-11 had high titer of HAdV-55 NAB (χ^2 -test,

Table 2 Distribution of neutralizing antibody titers against human adenovirus (HAdV) types in health donors from Guangzhou.

Neutralizing titer	Viruses, n (%)						P value
	HAdV-3	HAdV-7	HAdV-4	HAdV-55	HAdV-14	HAdV-11	
< 18	172 (61.87)	226 (81.30)	238 (85.61)	257 (92.45)	253 (91.01)	263 (94.60)	< 0.001
18–144	64 (23.02)	33 (11.87)	16 (5.76)	10 (3.60)	20 (7.19)	13 (4.68)	< 0.001
145–576	18 (6.48)	12 (4.32)	7 (2.52)	3 (1.08)	4 (1.44)	2 (0.72)	< 0.001
> 576	24 (8.63)	7 (2.52)	17 (6.12)	8 (2.88)	1 (0.36)	0 (0)	< 0.001
≥ 18	106 (38.13)	52 (18.71)	40 (14.39)	21 (7.55)	25 (8.99)	15 (5.40)	< 0.001

P value was assessed by Pearson's chi-squared test

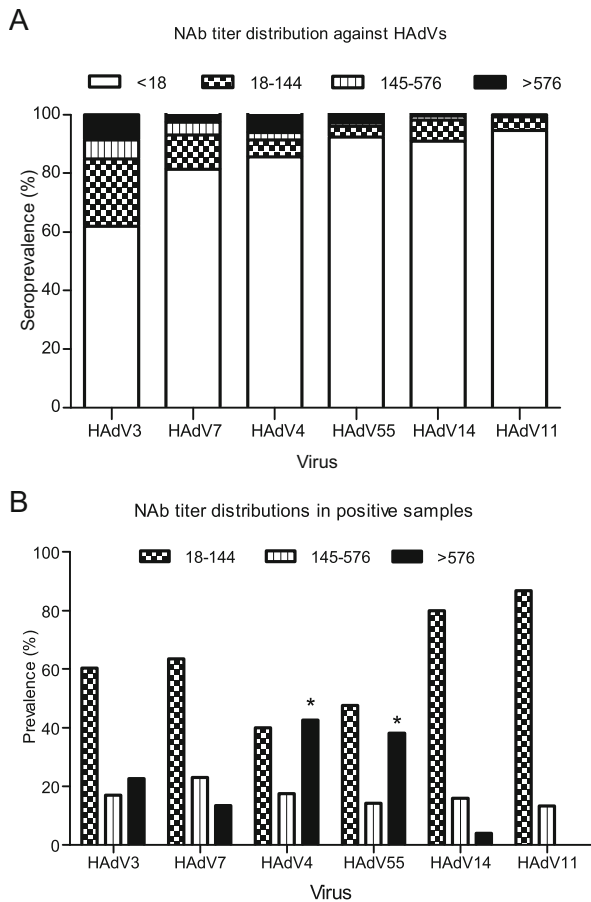


Fig. 2 Distribution of NAb titer levels against HAdV types in healthy populations. **A** NAb titer levels against human adenovirus types. **B** Distribution of NAb titers against HAdV types in positive samples. The NAb titers of 278 sera samples were determined using neutralization tests *in vitro* against six HAdV types. NAb, neutralizing antibody. * $P < 0.05$ for the difference in prevalence between titer groups.

$P < 0.01$; Fig. 4B). Further, in HAdV-11-positive samples compared to HAdV-11-negative ones, the frequency of samples with high titers of HAdV-55 NAb was much higher (χ^2 -test, $P < 0.01$; Fig. 4C). Similarly, many more donors with high titers of HAdV-55 NAb were HAdV-14-positive (χ^2 -test, $P < 0.05$; Fig. 4D). However, the frequency of samples with high or moderate levels of HAdV-14 or HAdV-11 NAb was not significant different in HAdV-55-positive compared to HAdV-55-negative samples (Fig. 4C and 4D). Totally, HAdV-55-seropositive samples tended to be HAdV-11- or HAdV-14-seropositive.

Discussion

In the past decade, HAdV-3 and -7 have been the two predominant HAdV types causing ARD in children in southern China, sometimes leading to outbreaks. The most

common type was HAdV-3 for many years (Han *et al.* 2013; Chen *et al.* 2016; Xie *et al.* 2019; Yao *et al.* 2019). Most patients are younger than 5 years. Here we found the seroprevalence was very low in the age groups of 6–11 months and 1–2 years for all six HAdV types (Fig. 1). However, a notable proportion of samples from age groups of 0–5 and 6–11 months had NAbS as shown in Fig. 1 and Table 1, which should be inherit immunity (IgG) from mother. Therefore, children under 1 year old were rarely infected by HAdVs. Meanwhile, we observed notably high NAb titers (> 576) against HAdV-3 in 2.7% and 3.4% samples from age groups of 6–11-months and 1–2-years, respectively, which indicated that HAdV-3 infection was rare but do exist in these young children (Fig. 3). The seroprevalence against HAdV-3 and -7 increased significantly in age groups of 3–5 and 6–17 years (Fig. 1). For all other four types, the seroprevalence in the children groups was very low (0% ~ 8.1%). These results suggest that 3–5-year-old children are a high-risk population for HAdV-3 and HAdV-7 infections, and a novel vaccine against these two types, targeting pre-nursery children under the age of 3 years should be developed.

Since 2018, HAdV-7 has replaced HAdV-3 to be the most predominant type causing ARD in children in Guangzhou and has caused many severe infections (our unpublished data). This study enrolled 278 healthy donors in 2017, of which 228 were children aged between several days and 17 years. We found that children of 3–5 and 6–17 years of age had higher seropositive rates against HAdV-3 (33.96% and 64.29%, respectively) than that against HAdV-7 (18.87% and 19.05%, respectively). We also found that children of 3–5 and 6–17 years of age had a high rate of high-titer NAbS against HAdV-3 (9.4% and 26.2%, respectively). However, only 4.76% and 6% samples had notably high NAb titers against HAdV-7 from age groups of 6–17 and 18–49 years, respectively. Thus, nursery- and school-age children are high-risk groups. The lower seroprevalence and lower rate of high-titer NAbS against HAdV-7 in children reflects the lower-level of pre-existing immunity to HAdV-7, which may contribute to the change of HAdV-7 replacing HAdV-3 to be the most predominant type.

HAdV-4 and -7 caused febrile ARD outbreaks in the military, and are also circulating in civilian population. Patients infected with HAdV-4 and HAdV-7 were at increased risk for severe disease (Coleman *et al.* 2020). HAdV-14 and -55 are two re-emerging types. HAdV-14 has caused outbreaks among both civilian and military populations in America, Europe, and China since 2006, while HAdV-55 has become a common pathogen in northern China. However, these two types were never reported in Guangzhou, and rarely reported in southern China (Chen *et al.* 2016). In this study, a sizable proportion of the

Fig. 3 Neutralizing antibody titers against human adenovirus (HAdV) types in different age groups. m: month; y: year.

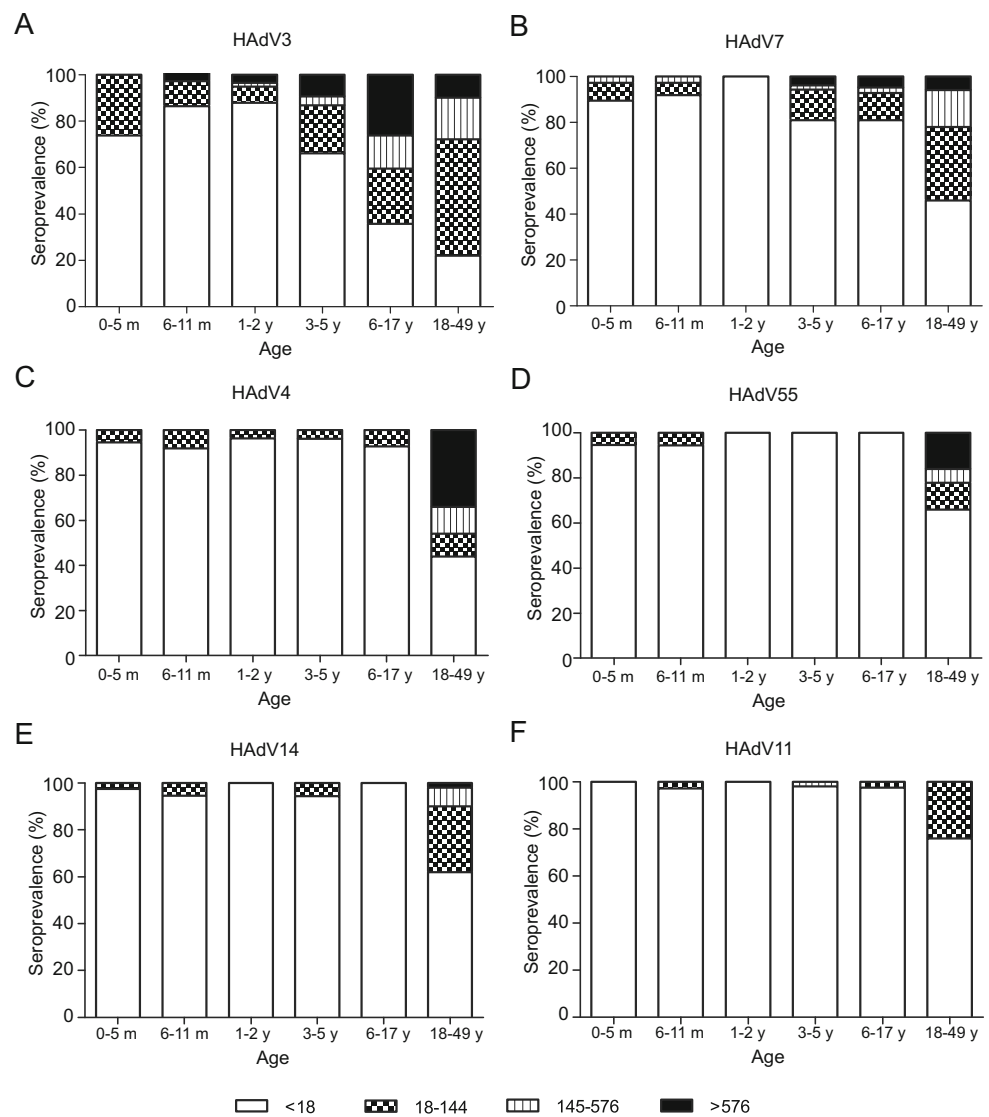


Table 3 Correlation of HAdV-11 and HAdV-55 NAb seropositive rates^a.

	HAdV-55 ⁺	HAdV-55 ⁻	Total	HAdV-55 positive rate ^b (%)
HAdV-11 ⁺	10	5	15	66.67
HAdV-11 ⁻	11	252	263	4.18
Total	21	257	278	
HAdV-11 positive rate ^c	47.62%	1.95%		

^aStatistics were performed by χ^2 -test, $P < 0.0001$.

^bThe rates of HAdV-55-seropositive sera in HAdV-11-seropositive or negative ones.

^cThe rates of HAdV-11-seropositive sera in HAdV-55-seropositive or negative ones.

samples in adults were positive for NAbs against HAdV-4 (56%), -55 (34.00%), -14 (38.00%), and -11 (24.00%). We also found that a relatively high proportion of adults had high titers of NAbs for HAdV-4 (34%) and -55 (16%) while only 2% of adults had high NAb titers against HAdV-14. However, there was no sample with NAbs against HAdV-55 in children aged 1 year to 17 years, and no sample with

high NAb titers against HAdV-4, -14, and -11 in children. These results demonstrated that HAdV-4, -55, and -14 were already prevalent in Guangzhou area, mainly in adults, but few in children before 2017. Similarly, in our previous study with sera collected in Dongguan City, a city near Guangzhou, in 2014, the percentage of samples from age groups of 20–29, 30–39, and 40–49 years that were positive

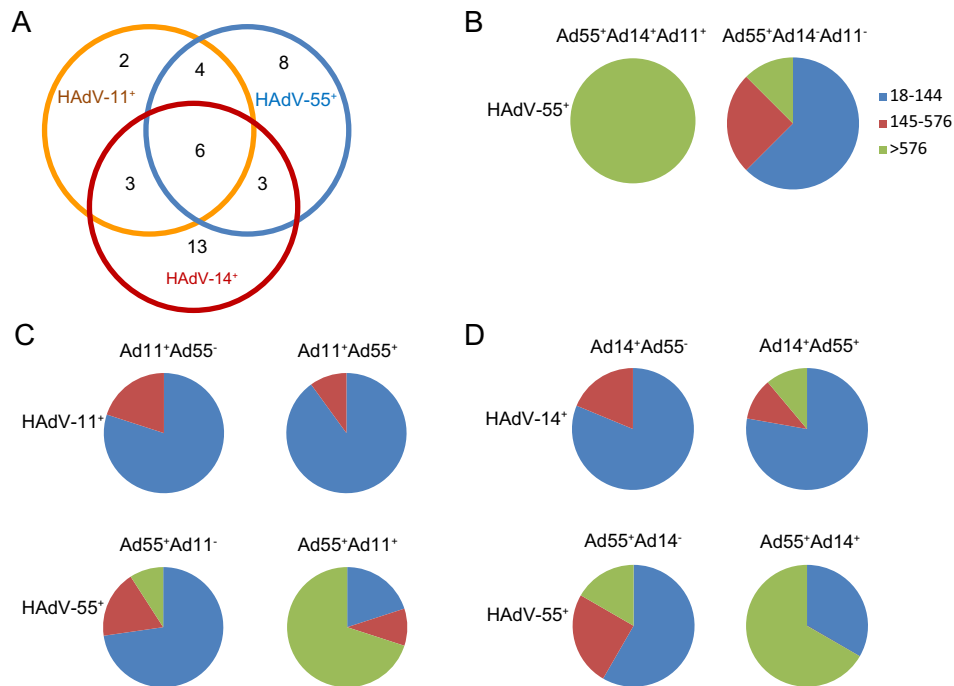


Fig. 4 Profiling of the seropositive rates and NAb titers in single-, double- and tri-positive cases for HAdV-55, HAdV-11 and HAdV-14. **A** The case numbers of single-, double- and tri-positive cases for HAdV-55, HAdV-11 and HAdV-14 NABs. **B** The distributions of HAdV-55-positive donors with different NAb titers in both HAdV-14- and HAdV-11 positive or negative groups. **C** The distributions of HAdV-55-positive cases with different NAb titers in HAdV-11-

positive or negative groups, and that of HAdV-11-positive donors in HAdV-55-positive or negative groups. **D** The distributions of HAdV-55-positive cases with different NAb titers in HAdV-14-positive or negative groups, and that of HAdV-14-positive donors in HAdV-55-positive or negative groups. The difference between the groups was analyzed by χ^2 -test.

for HAdV-55 NABs was 0%, 19.75%, and 40.625%, respectively (Tian *et al.* 2016). This study mainly investigated the pre-existing immunity in young children, so the donors were divided into six groups: 0–5 and 6–11 months old, 1–2, 3–5, 6–17, and 18–49 years old. There were 37–58 donors in each age group. In future work, more detailed study could be done, including more donors in the age groups of 6–17, and over 18 years old which could be divided into more groups, such as 6–8, 9–11, 12–14, 15–17, 18–21, 22–29, 30–39, 40–49, 50–59, 60–69, over 70. The proportion of samples from our donors in Guangzhou that were positive for NABs against HAdV-55 or -14 was low in both children and adults, which suggests that there may be little protection in the Guangzhou population against HAdV-55 or -14 infection. However, the proportion of samples that were positive for NABs against HAdV-4 or -7 was high in adults, but very low in children, which indicated the low level of protective immunity against HAdV-4 and -7 in children. More attention should be paid to these HAdVs circulating in civilian populations. HAdV-4, -7, -55, and -14 vaccinations may be urgently needed for young people under 18 years of age.

HAdV-55 is an inter-typic recombinant of HAdV-11 and HAdV-14, sharing a similar but somewhat different hexon

protein with HAdV-11 and a similar fiber protein with HAdV-14 (Walsh *et al.* 2010). Furthermore, HAdV-11 was indirectly excluded in this study. Furthermore, HAdV-11 was indirectly excluded in this study. Here we showed HAdV-55-seropositive samples tended to be HAdV-11- or HAdV-14-seropositive (Table 3 and Fig. 4). Early studies reported fiber-specific NABs might contribute to the cross-neutralizing against HAdV-14 and HAdV-55 of double-positive sera (Feng *et al.* 2018). The cross-neutralizing against HAdV-55 and HAdV-11 may be attributed to hexon, which elicited predominant NABs in most individuals who were infected by HAdVs. We also found few individuals had high titers of NABs against HAdV-55 but no NAB against HAdV-11 (Fig. 4C). This result may be attributed to the difference among hexon proteins of HAdV-55 and HAdV-11, or NABs targeting other capsid antigens, such as penton base or fiber. However, these speculations should be investigated in future studies. The low seroprevalence and low NAB titers against HAdV-11 in all age groups were consistent with previous studies, which indicated the advantages of being vectors for vaccination, gene therapy, and cancer therapy (Holterman *et al.* 2004). HAdV-11 transduces primary cells including smooth muscle cells, synoviocytes, dendritic cells, and

cardiovascular tissues with higher efficiency than HAdV-5. Furthermore, HAdV-11 vector elicited immune responses both in the presence and absence of anti-HAdV-5 immunity (Lemckert *et al.* 2005; Stone *et al.* 2005).

In summary, we investigated the seroprevalence and distribution of NAbs against HAdV-3, -4, -7, -14, -55, and -11 by age in a civilian population, including children and adults aged between 0 months and 49 years in Guangzhou. These results revealed a low level of herd immunity against HAdV-14, -55, and -11 in all age groups, and a high level of herd immunity against HAdV-3, -4, and -7 in adults. This study also indicates a high level of herd immunity against HAdV-3 in children in the age group of 7–17 years, and the low rate of herd immunity against HAdV-4 and -7 in children of all age groups. These results suggest the importance of monitoring HAdV types, and developing prophylactic vaccines for children and young adults.

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Author contributions TG conceived and designed the experiments. FY, TG, WB, LW, XY, MC, and YP carried out the experiments. TG, FY, LK, and LX analyzed the data. TG and FY wrote the paper. RX and ZR checked and finalized the manuscript. All authors read and approved the final manuscript.

Compliance with Ethics Standard

Conflict of interest The authors declare that they have no conflict of interest.

Animal and human rights statement This study was approved by the Ethics Committee of the Affiliated First Hospital of Guangzhou Medical University. Informed consent was obtained from all the participants.

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