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Letter

Seroprevalence of neutralizing antibodies against HFMD associated enteroviruses among healthy individuals in Shanghai, China, 2022

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Dear Editor,

Hand, foot and mouth disease (HFMD) is one of the most common infectious diseases, particularly in the Asia-Pacific region. In the past two decades, HFMD rises to prominence for its heavy burden, with over one million cases reported annually. Before 2013, enterovirus A71 (EV-A71) and Coxsackievirus A16 (CVA16) were the main pathogens leading to HFMD in the mainland of China (Yang et al., 2017). In recent years, non-EV-A71-non-CVA16 other enteroviruses, such as Coxsackievirus A6 (CVA6), Coxsackievirus A10 (CVA10) and Coxsackievirus A4 (CVA4), were frequently reported and replaced EV-A71 and CVA16 becoming the major causative agents of HFMD (Zhou et al., 2021; Wang et al., 2022).

Currently, there is no specific antiviral drug available for the clinical treatment of HFMD. Neutralizing antibodies (NtAbs) are one of the most important indicators of human immunity to assess the immunogenicity and efficacy of vaccine. NtAbs also play an essential role in defending viral infection and preventing further progression of disease. Systematic surveillance on seroprevalence and geometric mean titer (GMT) values of NtAbs against enteroviruses not only shed light on herd immunity for vaccine development but also provided a supplement way to understand the prevalence of HFMD associated enteroviruses, particularly occult infections in the population.

Each year, two epidemic peaks of HFMD incidence occur in Shanghai. The first peak occurs in late spring and early summer, while the smaller peak occurs in autumn and winter. In the spring of 2022, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) rapidly spread in Shanghai, resulting in a widespread outbreak of novel coronavirus disease 2019 (COVID-19). Subsequently, non-pharmaceutical interventions were implemented in April and gradually lifted in May, which attached the first incidence peak of HFMD to a great extent. Based on public data from the Shanghai Municipal Health Commission, the reported cases of HFMD in 2022 decreased to 82.40% compared with the previous year.

A total of 307 serum samples were collected from healthy individuals between September 27th and December 7th in 2022, prior to the implementation of Category B infectious disease management for SARS-CoV-2 infection. The seroprevalence and GMT values of five HFMD-associated enteroviruses (EV-A71, CVA16, CVA6, CVA10, and CVA4) were assayed. As shown in Supplementary Table S1, the 307 healthy individuals included 160 males and 147 females. Participants were aged from 3 days to 69 years (mean: 28.49 ± 21.33 years). Eight age groups were categorized, including ≤ 1 year (4.23%, 13/307), 1–2 years (5.21%, 16/307), 3–5 years (12.05%, 37/307), 6–10 years (13.68%, 42/307), 11–18 years (15.31%, 47/307), 19–40 years (21.50%, 66/307), 41–59 years (18.24%, 56/307) and ≥ 60 years (9.77%, 30/307). The samples were collected across three local districts in Shanghai, including Jing'an (15.31%, 47/307), Changning (68.40%, 210/307) and Yangpu (16.29%, 50/307) districts.

Clinical isolates of EV-A71C4a, CVA16 B1b, CVA6 D3a, CVA10C and CVA4 C2 evolutionary branches (Supplementary Table S2; Fig. S1) were selected for the quantification of NtAbs using a micro-neutralization test. As shown in Table 1, the overall seroprevalence of the five enteroviruses ranged from 40.07% to 67.43%. However, the GMTs were varied, ranging from 9.80 to 72.14. Among the five enteroviruses, CVA16 NtAbs exhibited the lowest overall seroprevalence and GMT value, with a seropositive rate of 40.07% (123/307, 95% CI: 34.74%–45.64%,

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Table 1
Seroprevalence and GMT values of NtAbs against five EVs in healthy individuals in Shanghai, China.

Groups	Seroprevalence									
	EV-A71	GMT value	CVA16	GMT value	CVA6	GMT value	CVA10	GMT value	CVA4	GMT value
Overall	59.61% (54.03%–64.95%)	17.16	40.07% (34.74%–45.64%)	9.80	65.80% (60.33%–70.88%)	36.11	58.96% (53.88%–64.32%)	24.30	67.43% (62.00%–72.43%)	72.14
Age										
≤1	38.46% (17.71%–64.48%)	7.79	30.77% (12.68%–57.63%)	5.51	38.46% (17.71%–64.48%)	8.67	53.85% (29.14%–76.79%)	11.62	76.92% (49.74%–91.82%)	42.91
1–2	50.00% (28.00%–72.00%)	12.61	6.25% (0.32%–28.33%)	4.36	25.00% (10.18%–49.50%)	6.44	25.00% (10.18%–49.50%)	10.83	43.75% (23.10%–66.82%)	10.15
3–5	70.27% (54.22%–82.51%)	32.91	13.51% (5.91%–27.98%)	5.93	29.73% (17.49%–45.78%)	17.74	13.51% (5.91%–27.98%)	6.57	18.92% (9.48%–34.20%)	8.23
6–10	52.38% (37.72%–66.64%)	14.25	11.90% (5.19%–25.00%)	5.80	52.38% (37.72%–66.64%)	35.04	30.95% (19.07%–46.03%)	17.81	52.38% (37.72%–66.64%)	54.71
11–18	29.79% (18.65%–43.98%)	8.55	27.66% (16.94%–41.76%)	8.94	74.47% (60.49%–84.75%)	69.92	53.19% (39.23%–66.67%)	24.90	61.70% (47.43%–74.21%)	79.26
19–40	69.70% (57.78%–79.45%)	24.23	66.67% (54.66%–76.84%)	14.94	86.36% (76.07%–92.66%)	51.87	87.88% (77.86%–93.73%)	44.55	84.85% (74.31%–91.56%)	142.17
41–59	75.00% (62.31%–84.48%)	22.07	67.86% (54.82%–78.60%)	18.91	82.14% (70.16%–90.00%)	64.40	85.71% (74.26%–92.58%)	50.59	83.93% (72.19%–91.31%)	115.93
≥60	66.67% (48.78%–80.77%)	14.42	43.33% (27.38%–60.80%)	10.08	70.00% (52.12%–83.34%)	22.89	66.67% (48.78%–80.77%)	25.69	93.33% (78.68%–98.82%)	440.60
Gender										
Male	52.50% (44.79%–60.09%)	14.80	38.13% (30.96%–45.85%)	9.66	65.63% (57.98%–72.54%)	39.83	58.75% (51.00%–66.09%)	23.78	65.63% (57.98%–72.54%)	61.82
Female	67.35% (59.41%–74.40%)	20.30	42.18% (34.49%–50.26%)	10.06	65.31% (57.31%–72.52%)	33.90	58.50% (50.42%–66.15%)	25.83	68.71% (60.82%–75.65%)	81.02

The 95% confidence interval is in parentheses.

$P < 0.0001$) and the GMT value was 9.80. The seropositive rates of EV-A71, CVA6, CVA10 and CVA4 were 59.61% (183/307, 95%CI: 54.03%–64.95%), 65.80% (202/307, 95%CI: 60.33%–70.88), 58.96 (181/307, 95%CI: 53.38%–64.32%) and 67.43% (207/307, 95%CI: 62.00%–72.43%) respectively, which showed no significant difference.

Similar trends were observed in the seroprevalence of the four serotypes of coxsackieviruses in different age groups. The seroprevalence initially decreased with age and followed by a subsequent increase. The seropositive rate as well as GMT values bottomed out in the 1–2 year-old age group (CVA6 and CVA16) and 3–5 year-old age group (CVA10 and CVA4), and peaked at 19–40 year-old age group (CVA6 and CVA10) and 41–59 year-old age group (CVA16), respectively (Table 1, Fig. 1A and E–H). One exception was the highest seropositive rate of CVA4 NtAbs, which occurred in ≥ 60 years group (Fig. 1A and H). On the other side, EV-A71 showed an entirely different tendency from coxsackieviruses. Two peaks of seroprevalence and GMTs against EV-A71 NtAbs occurred. The first peak occurred in 3–5 year-old age group and bottomed out at 11–18 year-old age group. Another peak occurred in 41–59 year-old age group and then declined slowly in ≥ 60 years age group (Table 1, Fig. 1A and 1D). No significant differences were found in the seroprevalence or GMT values of NtAbs against the five enteroviruses in different age groups.

To further analyze the seroprevalence of these five enteroviruses in healthy individuals, NtAb titers ≥ 16 were defined as protective NtAbs (Zhu et al., 2013) and NtAb titers ≥ 256 were defined as high level NtAbs. The seroprotection rates of coxsackieviruses showed an initial decline followed by a subsequent increase, whereas the protective EV-A71 NtAbs exhibited an age-related increase, accompanied by fluctuations characterized by two distinct peaks (Fig. 1B). However, there was a significant variation in the seroprevalence of high level NtAbs. 6.56% (12/183, 95% CI: 3.79%–11.11%), 8.94% (11/123, 95% CI: 5.07%–15.31%) of seropositive participants carried high level NtAbs against EV-A71 and CVA16 whereas 34.65% (70/202, 95% CI: 28.43%–41.45%), 25.97% (47/181, 95% CI: 20.13%–32.81%) and 62.32% (129/207, 95% CI: 55.55%–68.64%) of the participants carried high level NtAbs against CVA6, CVA10 and CVA4, respectively ($P < 0.05$). Notably, the highest seropositive rate of high level CVA4 NtAbs occurred in ≥ 60 years group (Fig. 1C), ($P < 0.05$). 80.00% (24/30, 95%CI 62.69%–90.50%) of the participants ≥ 60 years old carried high level NtAbs.

No significant gender-specific difference in seroprevalence and GMT values was observed for most of the enteroviruses, except for EV-A71. In details, 52.50% males and 67.35% females showed NtAbs against EV-A71 ($P < 0.05$), while 44.38% males and 59.86% females showed protective NtAbs against EV-A71 ($P < 0.05$) (Table 1). Of all the 307 participants, only 71 (23.13%, 71/307) healthy individuals were negative or positive for only one type of enterovirus, while 35.50% (109/307) and 41.37% (127/307) of the healthy individuals were positive for two or three and four or five types of enteroviruses. Referring to protective and high level NtAbs, 39.41% (121/307) and 33.55% (103/307) of the participants carried two or three and four or five types of protective NtAbs. About 21.17% (65/307) of the participants carried two or three high level NtAbs and none of the healthy individuals carried four or five high level NtAbs (Fig. 1C).

Due to the COVID-19 epidemic, measures such as masking and school closing were taken in the first half of 2022 in Shanghai to reduce the spread of SARS-CoV-2. The anti-COVID-19 nonpharmaceutical interventions also reduced the occurrence of a large number of infectious diseases especially HFMD (Geng and Zhang, 2022; Shen et al., 2022). To gain comprehensive insight into the prevalence of HFMD associated enteroviruses, we conducted the cross-sectional study to investigate the seroprevalence of HFMD associated enteroviruses in healthy individuals in Shanghai after the COVID-19 epidemic.

The seroprevalence of CVA16 NtAbs was surprisingly the lowest among the five enteroviruses, with only 40.07% of healthy individuals in the current study carrying CVA16 NtAbs. Reported seroprevalence of CVA16 NtAbs among participants of all age groups in China ranged from

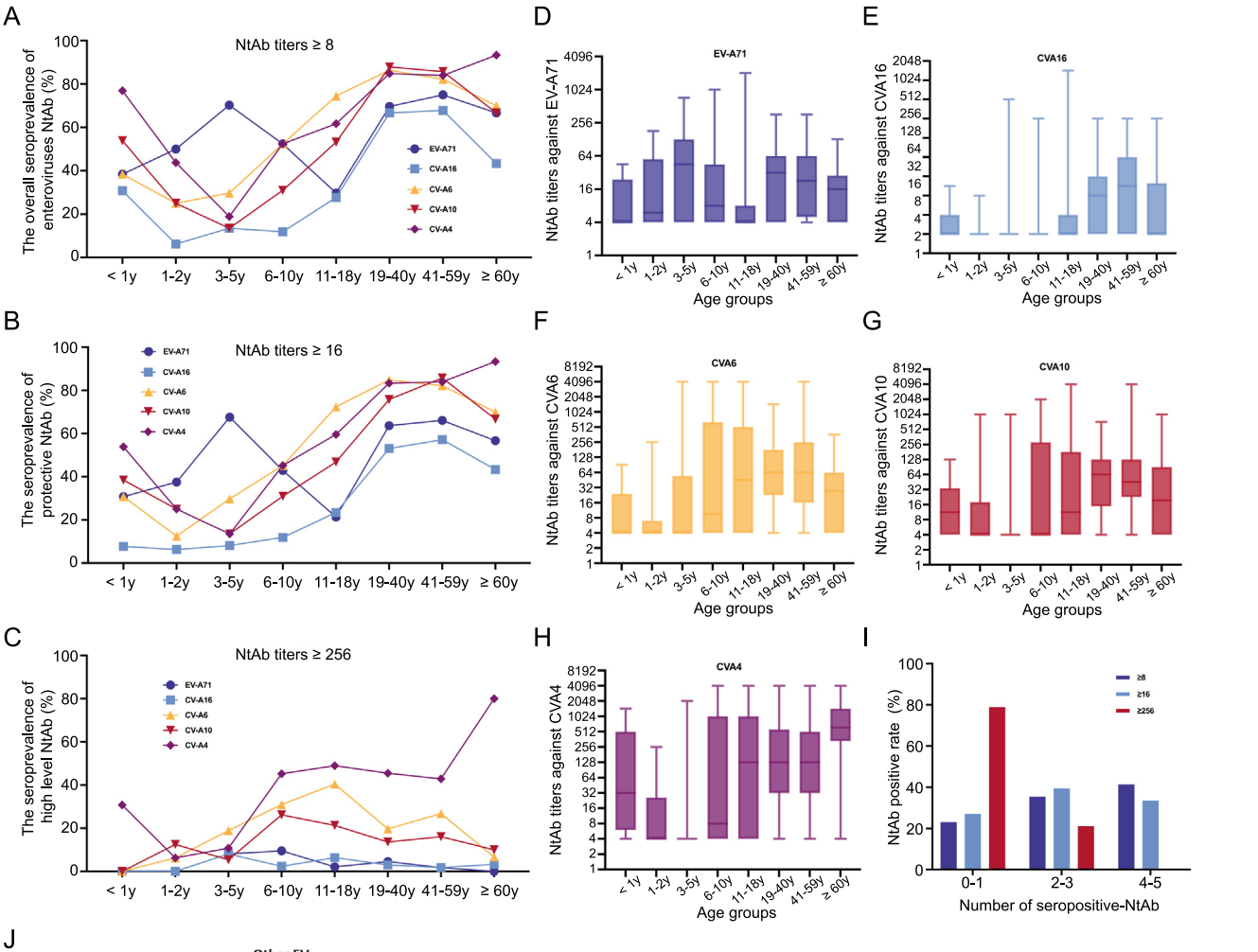

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Fig. 1. Serological and etiological distributions of HFMD associated enteroviruses in Shanghai. **A–C** Seroprevalence of NtAbs against five enteroviruses in healthy individuals among different age groups. The Y-axis represents the seropositive rate of NtAb against the indicated enteroviruses. The X-axis represents different age groups. **A** The overall seroprevalence of enteroviruses NtAbs (NtAb titers ≥ 8). **B** Seroprevalence of protective NtAbs (NtAb titers ≥ 16). **C** Seroprevalence of high level NtAbs (NtAb titers ≥ 256). **D–H** Titer distributions of NtAbs against the five enteroviruses, EV-A71 (**D**), CVA16 (**E**), CVA6 (**F**), CVA10 (**G**) and CVA4 (**H**) in healthy individuals among different age groups. The X-axis represents different age groups. The Y-axis represents NtAb titers against the five enteroviruses. The box plot shows the minimum, first quartile, median, third quartile and maximum titer levels. **I** Co-existence of NtAbs against the five enteroviruses in healthy individuals. The X-axis represents the number of seropositive-NtAbs healthy individuals carried. The Y-axis represents the seropositive rate of NtAbs. **J** Yearly distribution of HFMD associated enteroviruses. The yearly detection rates of CVA16 ranged from 4.41% to 22.20% during 2020–2022. **K** Monthly distribution of enterovirus serotypes in HFMD cases in Shanghai during 2020–2023.

58.82% to 76.52% (Wang et al., 2016; Zhu et al., 2018; Song, 2020). However, results varied across different regions and periods. Based on meta-analysis, the seroprevalence of CVA16 was 55.1% (95% CI: 44.1%–66.1%) in the Chinese population (Li et al., 2021). In Shanghai, the seroprevalence of CVA16 was 54.23% among healthy individuals ≤18 years old between 2014 and 2016 (Wang et al., 2018), whereas it fell to 18.06% in the same age group in the current study ($P < 0.0001$). EV-A71 and CVA16 have been the major pathogens of HFMD in the mainland of China since 2008. With the outbreak of non-EV-A71-non-CVA16 enteroviruses and the introduction of EV-A71 inactivated vaccine, the detection rate of EV-A71 in Shanghai has rapidly declined since 2018. Unlike EV-A71, CVA16 remained the second most prevalent causative agent associated with HFMD in Shanghai during 2020–2022 (Fig. 1J). However, consistent with the serological results, the monthly detection rate of CVA16 has rapidly declined since September 2022 and remained at a low level (Fig. 1K). Continuous surveillance is needed to better understand the changes in the prevalence of CVA16 in the post-COVID 19-pandemic period.

Although no statistical differences were found, the seroprevalence of EV-A71 and coxsackieviruses NtAbs exhibited distinct distributions among different age groups. As the leading pathogens of HFMD, the seroprevalence of coxsackieviruses protective NtAbs drops to low levels in children under five years old, indicating common susceptibility to coxsackieviruses infection. Due to the lack of the detailed immunization coverage rates of the EV-A71 vaccine, the reason for the different distribution of the NtAbs still remains unclear. Unexpectedly, a high proportion of the elderly population carried high levels of NtAbs against CVA4. CVA4 was first discovered in the United States and has been extensively spread in China's mainland since 2014. In Shanghai, CVA4 was found to be highly prevalent in both HFMD and herpangina cases in 2021. However, there is still a lack of study on CVA4 infection in adults. In the current study, 80% of the elderly participants carried high level CVA4 NtAbs (titers value ≥ 256), which is significantly higher than in other age groups. High levels of NtAbs are usually considered as indicator of recent infection. According to the official National Guidelines for HFMD Control and Prevention (2009 edition), high level NtAbs (titer value ≥ 256) of a single serum sample can be used as the diagnostic criteria for HFMD. Despite the lack of NtAb response kinetics in CVA4 infection, Zhou *etc*. Confirmed that NtAb titers value against CVA16 drop to over 64 until 26 months after infection (Zhou et al., 2022), suggesting that high levels of NtAb titers against coxsackievirus cannot last for long time. The high seroprevalence of high level CVA4 NtAbs in elderly group in the current study strongly indicates the prevalence and spread of CVA4 during a period in the past. Further studies are needed on enterovirus infection in adult groups.

The co-circulation of different enterovirus serotypes is commonly reported, especially during HFMD epidemics (Zhou et al., 2021). However, co-infection was relatively limited according to our surveillance system. In the current study, over 70% of participants carried protective NtAbs against at least two types of enteroviruses and more than 20% of the participants carried high levels of NtAbs against two or three types of enteroviruses. Studies have confirmed that NtAbs against enteroviruses do not provide cross-protection against other serotypes (Huang et al., 2012; Gao et al., 2021). Thus the coexistence of NtAb against multiple enteroviruses is probably the result of previous infections or immunization.

In conclusion, we investigated the seroprevalence and GMT values of five enteroviruses associated with HFMD in healthy individuals, and analyzed the distribution of NtAbs among different age groups. Our result showed a notable reduction in the seroprevalence of CVA16 in 2022. The seroprotection rates of coxsackieviruses were low among children under five years old, indicating a high risk of outbreak and spread among this susceptible population. Serological surveillance also confirmed the presence of a latent outbreak and circulation of CVA4 among the elderly population in the past. This study provides background information on the seroprevalence of HFMD-associated enteroviruses. We believe our data may contribute to reflecting the history of previous infection as well as herd immunity among healthy individuals, and provide a scientific basis for disease control and prevention.

Footnotes

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