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Epidemiology of childhood enterovirus infections in Hangzhou, China, 2019–2023

Jian Sun[1], Yajun Guo[2*], Lin Li[3], Yaling Li[4], Hangyu Zhou[5,6] and We Li[2]

Abstract
Human enteroviruses are highly prevalent world-wide. Up to more than 100 subtypes of enteroviruses can cause several diseases, including encephalitis, meningitis, myocarditis, hand-foot-mouth disease, conjunctivitis, respiratory diseases, and gastrointestinal diseases, thus posing a great threat to human health. This study aimed to investigate the epidemiological characteristics of enterovirus in children in Hangzhou, China before and after the COVID-19 outbreak. Systematic monitoring of enterovirus infections was performed by collecting samples from the children admitted to the inpatient wards and outpatient departments in the Children's Hospital, Zhejiang University School of Medicine, between January 2019 and May 2023. A commercial real-time RT PCR kit was utilized to detect enteroviruses. Among the 34,152 samples collected, 1162 samples, accounting for 3.4% of the samples, were tested positive for enteroviruses. The annual positive rates of the enteroviruses were 5.46%, 1.15%, 4.43%, 1.62%, and 1.96% in 2019, 2020, 2021, 2022, and May 2023, respectively. The positivity rate of the enteroviruses was highest among children aged 3–5 years and 5–7 years. Moreover, the monthly positivity rate of enterovirus infection ranged from 0.32% to 10.38%, with a peak in June and July. Serotypes, especially EV71 and CA16, causing severe symptoms such as HFMD, were decreasing, while the proportion of unidentified serotypes was on the rise. The incidence of enteroviruses in Hangzhou was higher in children aged 1–3 years and 7–18 years.

Keywords Enterovirus, Epidemiology, Children, COVID-19

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Introduction

Enteroviruses (EVs) are among the most common viruses infecting humans worldwide. More than 100 types are currently known to infect humans, most of which are frequently detected globally [1–3]. EVs are single-stranded, positive-sense RNA viruses of the *Picornaviridae* family, whose clinical manifestations include cutaneous, visceral, and neurological symptoms [4, 5]. More importantly, EVs are known to cause several diseases, including encephalitis, meningitis, myocarditis, hand-foot-mouth disease (HFMD), conjunctivitis, respiratory diseases, and gastrointestinal diseases; however, most of these infections remain asymptomatic [6–12]. EVs can be further classified into four species, including *Enterovirus A* (20 serotypes such as EV71 and coxsackievirus A), *Enterovirus B* (59 serotypes), *Enterovirus C* (21 serotypes), and

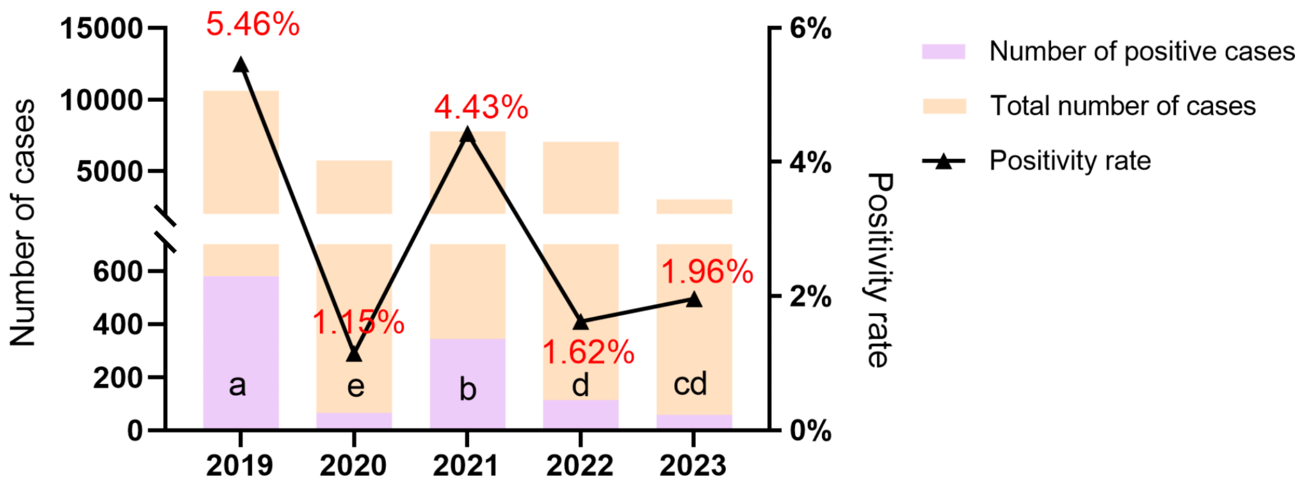

Fig. 1 The positivity rate of enteroviruses (EVs) from 2019 to May 2023 in Hangzhou, China

Enterovirus D (4 serotypes) [4, 13]. Among these, enterovirus 71 (EV71), coxsackievirus A16 (CA16), coxsackievirus A6 (CA6), and coxsackievirus A10 (CA10) are the major subtypes of EVs causing hand-foot-mouth disease (HFMD) [14–20], accompanied with serious complications [21]. EV71 spread in the Netherlands as early as 1963, and its first successful isolation was in 1969 in California, USA [22]. Moreover, EV71 and CA16 are the most common causative pathogens of HFMD in Zhejiang Province, similar to other provinces in China [23–26]. Our previous study evaluated the epidemiology of childhood enterovirus infections in Hangzhou from January 1, 2010, to December 31, 2012. Thus, to better understand the epidemiology of EV infections pre-COVID-19 period to the post-COVID-19 period in Hangzhou, East China, we conducted systematic surveillance of EV infections in 34,152 children from Hangzhou, China, in the present study.

Methods and methods

Patients

The study was conducted from January, 2019 to May, 2023, and the participants were 34,152 children from the inpatient wards and outpatient departments of the Children's Hospital of Zhejiang University School of Medicine. The inclusion criteria were: (1) all patients who visited the Children's Hospital of Zhejiang University School of Medicine between January, 2019 and May 2023, (2) aged < 18 years with suspected EV infection. The study was approved by the Ethics Committee of the Children's Hospital Affiliated to Zhejiang University School of Medicine, with informed consent from patients.

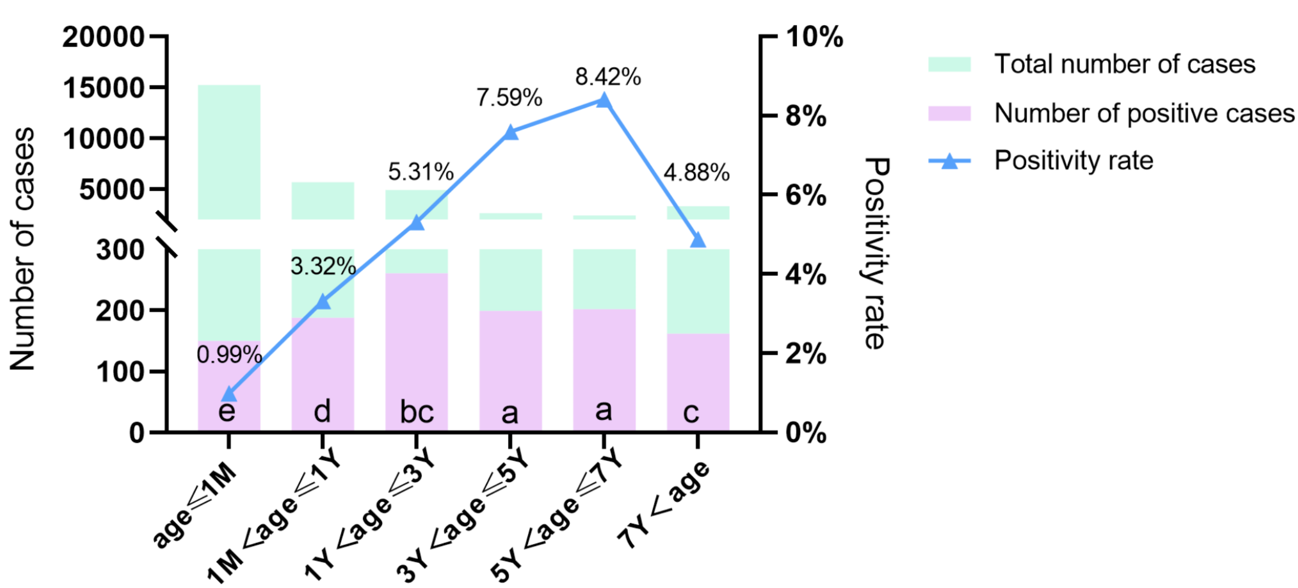

Fig. 2 Age distribution of enterovirus infections among children younger than 18 years

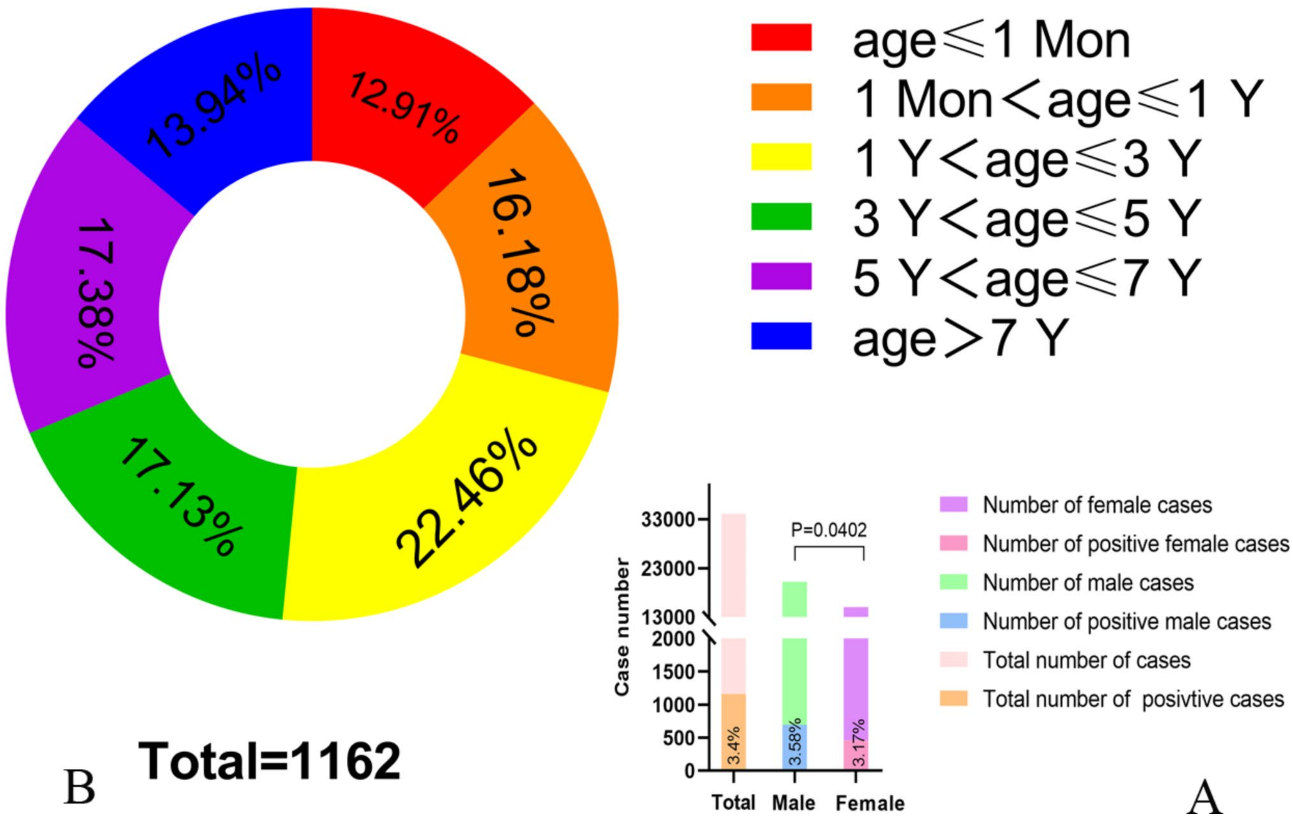

Fig. 3 **A**: Gender composition of the individuals younger than 18 years with positive cases. **B**: Age composition of the individuals younger than 18 years with positive cases

Enterovirus detection

Throat swabs, stool, liquor pericardii, ascites, sputum or cerebrospinal fluid specimens were collected from symptomatic children with suspected enterovirus infection. Personal information on the demographic factors and medical history were obtained from their guardians using a standard questionnaire. RNA was extracted from each specimen using a viral RNA extraction kit (Nucleic acid extraction or purification reagent, Su Tai Medical Device Registration 20180121, mole Bioscience and Liferiver™, China). The detection of EVs (EV A-D) and further classification of EV71 and CVA16 for the EV-positive samples were performed on an ABI7500 system using a commercial one-step real-time RT–PCR assay kit (EV71, CA16 and Other Enteroviral Nucleic Acid Test Kit, mole Bioscience and Liferiver™, China). The real-time RT-PCR

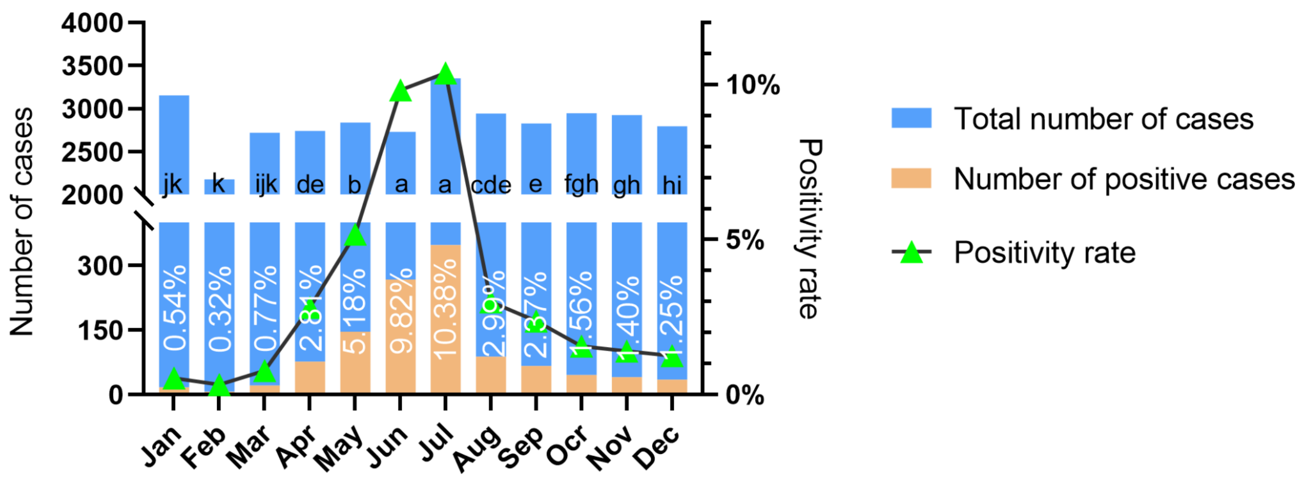

Fig. 4 Monthly distribution of enterovirus infection among children younger than 18 years

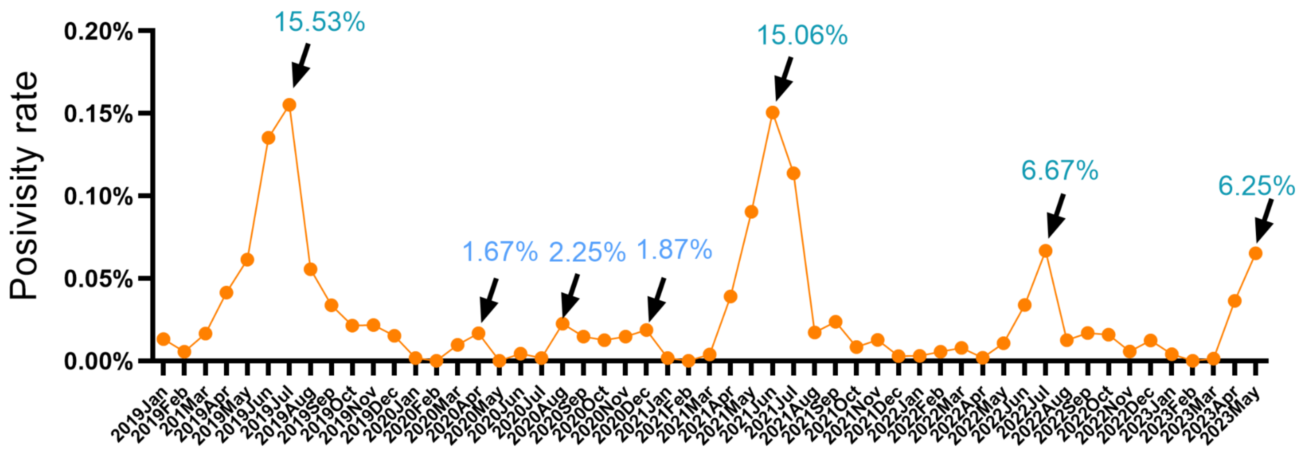

Fig. 5 Positivity rate distribution of enteroviruses (EVs) in each month from 2019 to May 2023

was conducted under the following conditions: 15 min at 50 °C and 5 min at 95 °C, followed by 40 cycles of 15 s at 94 °C and 45 s at 55 °C. Samples with cycle threshold (CT) values less than 35.0 were identified as positive.

Statistical analysis

Statistical analysis was performed using the Chi-square (χ2) test and the statistical significance was calculated using SPSS 17.0 version (SPSS Inc., Chicago, IL, USA).

Results

Prevalence of the enteroviruses among the patients

Out of 34,152 samples collected during the study period, 1162 (3.4%) tested positive for EV (Fig. 3A). The positivity rates among male and female patients were 3.58% (699/19535) and 3.17% (463/14617), respectively, which was significantly different ($P=0.0402$). Out of all hospitalized patients, 3.28% (1115/33989) had a positive EV result, while the EV infection was proved in a significantly higher proportion of patients from outpatient care, 28.83% (47/163) ($P<0.0001$, Fig. 7).

The epidemiological characteristics of enterovirus infection

The annual EV positive rates ranged from 1.15% observed in 2020 to 5.46% recorded in 2019. The proportion of positive patients in 2019 was significantly higher compared to all other years, while the positivity rate detected in 2020 was significantly lower than rates recorded in all years. Another statistically important difference was found between the proportion of EV-positive patients in 2021 (4.43%) which was higher in comparison to 2020 (1.15%), 2022 (1.62%), and 2023 (1.96%) (Fig. 1). The representatives of the groups containing the same letters in Fig. 1 exhibit no significant statistical difference, while the representatives of the groups containing different letters demonstrate statistically significant disparities.

The EV positivity rate increased with age from 0.99% in the age group of patients less than one-month-old to 8.42% among children 5–7 years of age. Significantly higher positivity rate was found in age group 5–7 years compared to the age group of 1–3 years (5.31%), more than 7-years (4.88%), one-month-old to 1-year (3.32%), less than one-month-old (0.99%) (Fig. 2).

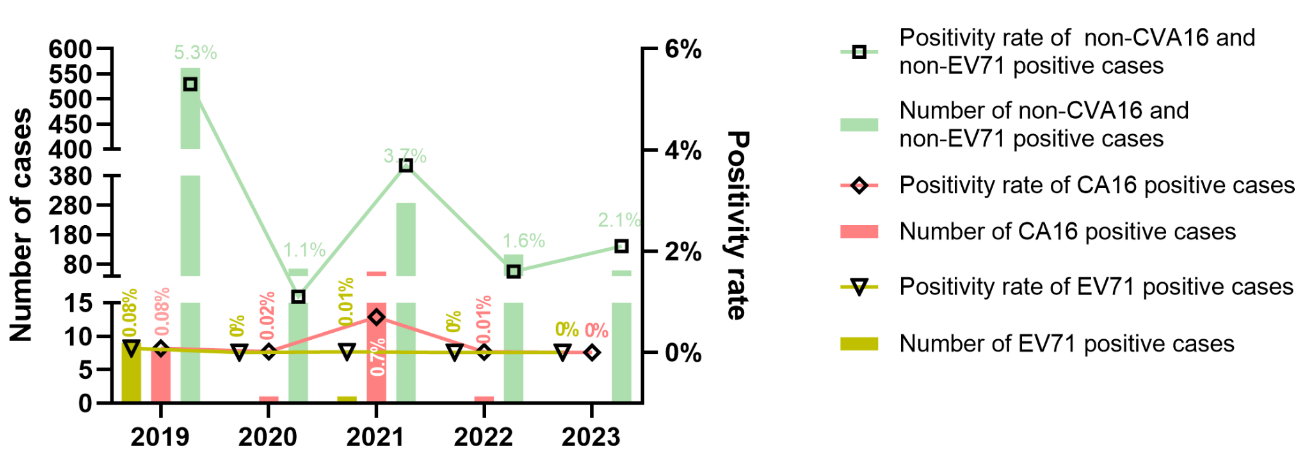

Fig. 6 Positivity rate of different types of enterovirus infection among children younger than 18 years

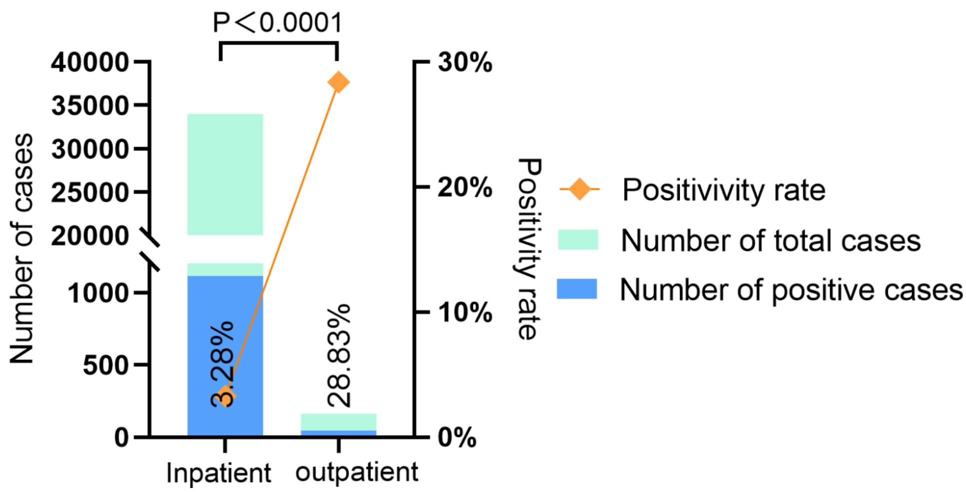

Fig. 7 Positivity rate distribution of enterovirus infection among patients of different health service types younger than 18 years

The monthly EV detection rates varied throughout the study period, peaking in July 2019 (15.53%), June 2021 (15.06%), and July 2022 (6.67%). In 2023, the highest proportion of positive patients was recorded in May (6.25%) after which the surveillance was ended. The only year without the prominent pick was 2020, during which the highest positivity rates were detected in April (1.67%), August (2.25%), and December (1.87%) (Fig. 5). The age group of 1 to 3 years had the highest proportion (22.46%) among all the positive patients, while those aged less than one-month-old, one-month-old to one year, 3–5 years, 5–7 years, and >7 years accounted for 12.91%, 16.18%, 17.13%, 17.38%, 13.94% of the participants, respectively (Fig. 3, B).

The positive rate of EVs exhibited variability throughout the study period; however, a distinct peak was observed in June and July (Fig. 4), indicating significant seasonality in EV infection. The positive rates during these months were significantly higher ($P<0.0001$) compared to other months, which showed a gradual decline in positive rates (Fig. 4).

The distribution pattern of enteroviruses (EVs) was also analyzed. CA16 displayed a trend characterized by an initial increase followed by a decline, whereas EV71 demonstrated a continuous decrease. In contrast, infections of non-CA16 and non-EV71 subtypes exhibited a rising trend (Fig. 6). The positive rate of CA16 in 2021 was significantly higher ($P<0.0001$) compared to that of EV71. Furthermore, from 2019 to 2023, the positive rates for both non-CA16 and non-EV71 subtypes were significantly elevated ($P<0.0001$) relative to those of CA16 and EV71.The positive rate for the non-CA16 and non-EV71 enterovirus subtype exhibited a fluctuation pattern characterized by a decline, followed by an increase, and then another decline. The significantly highest positive rate, reaching 5.3% (562/10597), was observed in 2019, while the significantly lowest positive rate of 1.13% (65/5736) occurred in 2020. Notably, the positive rate in

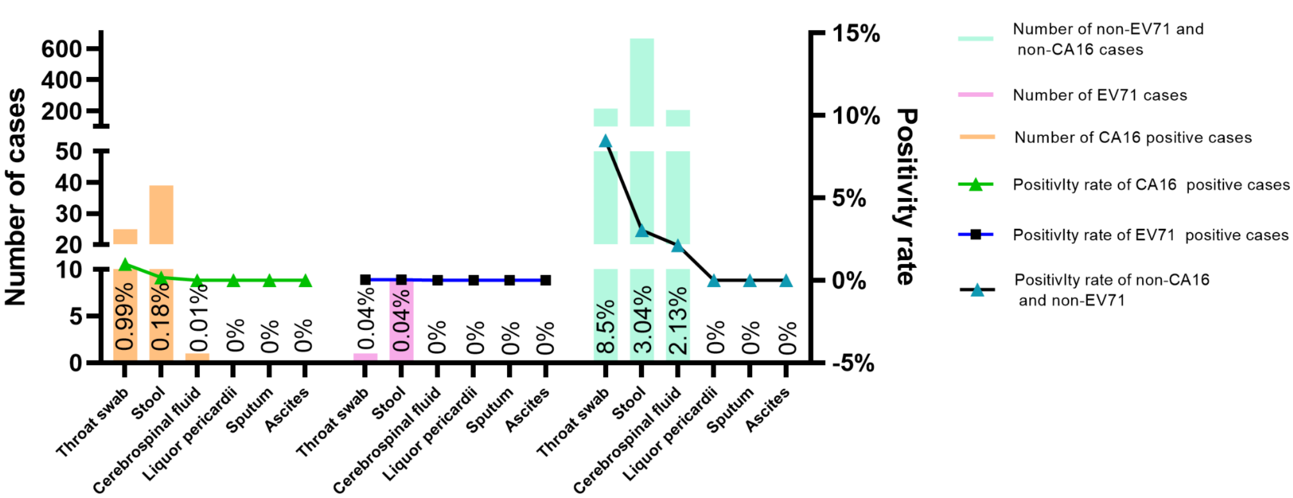

Fig. 8 Positivity rate distribution of enterovirus infection among different sample types from 2019 to May 2023

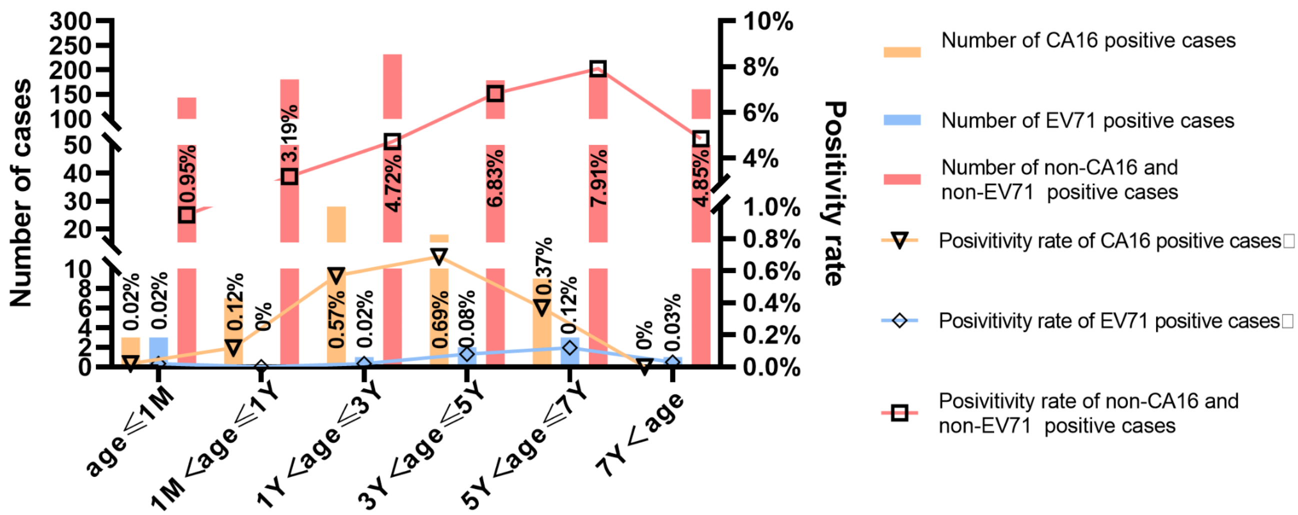

Fig. 9 Age distribution of CA16, EV71 and non-CA16 and non-EV7 infection among children younger than 18 years

2021 (3.71%, 288/7765) was significantly higher than that in 2020 (1.13%) and in 2022 (1.6%, 113/7044), as well as in 2023 (1.96%, 59/3010) ($P<0.0001$). However, there was no significant difference in the positive rates between 2022 and 2023.

Among patients testing positive for CA16, EV71, non-CA16 and non-EV71, the proportions of male cases were as follows: 0.19% (38/19535) for CA16, 0.03% (6/19535) for EV71, 3.35% (655/19535) for non-CA16 and non-EV71, with female proportions at 0.18% (27/14617), 0.03% (4/14617), and 2.96% (432/14617), respectively (Fig. 10). Notably, the positive rates of non-CA16 and non-EV71 cases demonstrated a statistically significant difference between males and females ($P=0.0428$).

Among patients who underwent throat swab testing, the overall positive rate for EVs was 9.5% (241/2531), with 0.99% (25/2531) testing positive for CA16 and 0.04% (1/2531) positive for EV71. The remaining cases, which accounted for 8.5% (215/2531), were identified as non-EV71 and non-CA16 strains. Similarly, in fecal sample analyses, the corresponding proportions were 3.26% (713/21871) for positive EVs, 0.18% (39/21871) for CA16, 0.04% (9/21871) for EV71, and 3.04% (665/21871) for non-EV71 and non-CA16 strains. Notably, cerebrospinal fluid (CSF) samples yielded no positive results for EV71, while CA16 positive samples constituted a minimal 0.01% (1/9740). Additionally, the positivity rates for non-EV71 and non-CA16 enteroviruses were found to be 2.13% (207/9740) (Fig. 8).

The prevalence of CA16 in the 3–5 years age group (0.69%, 18/2621) was significantly higher than in other age groups, with the exception of the 1–3 years age group (0.57%, 28/4912). Furthermore, the positivity rate of CA16 among children aged one month to one year (0.12%, 7/5668) markedly exceeded that of the less than one month age group (0.02%, 3/15227) and those older than seven years (0%, 0/3323). However, it was significantly lower than the positivity rates observed in the

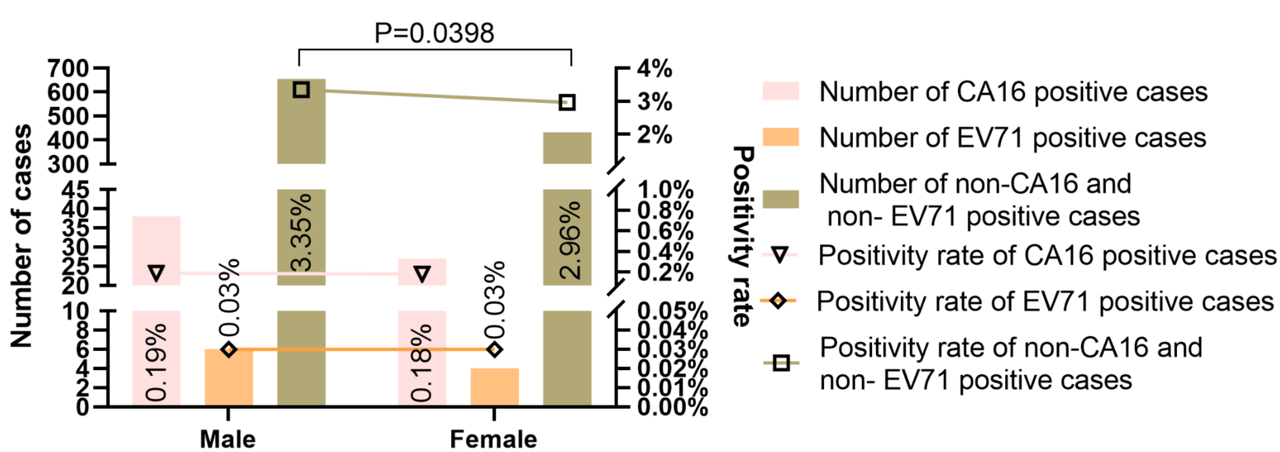

Fig. 10 Gender distribution of CA16, EV71 and non-CA16 and non-EV7 infection among children younger than 18 years from 2019 to May 2023

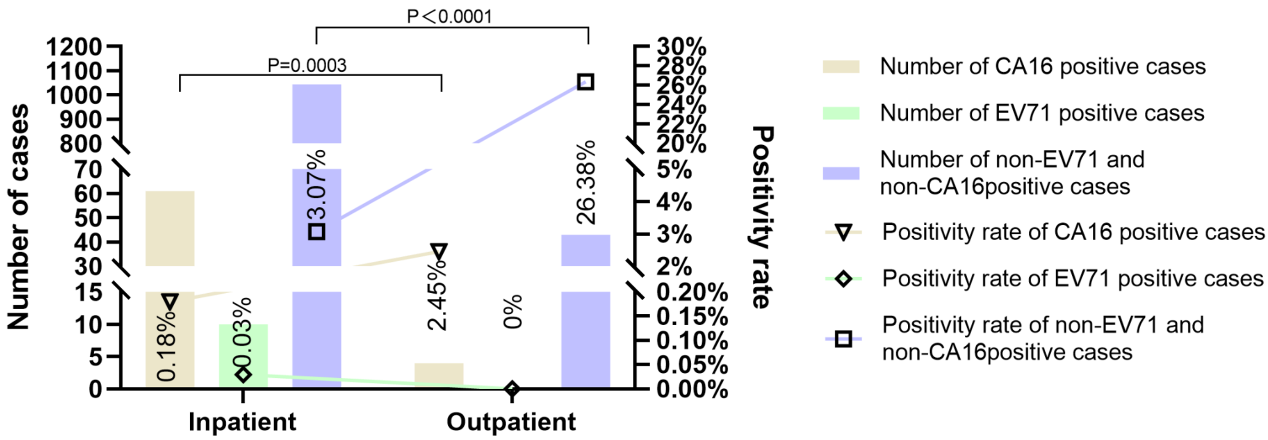

Fig. 11 Positivity rate distribution of enterovirus infection among patients of different health service types younger than 18 years

3–5 years age group (0.69%, 18/2621), the 1–3 years age group (0.57%, 28/4912), and the 5–7 years age group (0.37%, 9/2401).

In the case of EV71, significant differences were noted, particularly when comparing the positivity rate in the 5–7 years age group (0.12%, 3/2401) to both the less than one month age group (0.02%, 3/15227) and the one month to one year age group (0%, 0/5668).

Additionally, the positivity rate for patients testing positive for non-CA16 and non-EV71 demonstrated a notable increase across various age groups: less than one month (0.95%, 144/15227), one month to one year (3.19%, 181/5668), one to three years (4.72%, 232/4912), and those older than seven years (4.85%, 161/3323). The highest positivity rates were observed in the 3–5 years (6.83%, 179/2621) and 5–7 years (7.91%, 190/2401) age groups, which surpassed all other age groups, although no statistically significant difference was found between these two groups.

Moreover, within each age group, the detection rates of non-CA16 and non-EV71 were significantly higher than those of CA16 and EV71 (Fig. 9).

Among inpatients, the positive rates for CA16, EV71, non-CA16 and non-EV71 cases were recorded at 0.18% (61/33989), 0.03% (10/33989), and 3.07% (1044/33989), respectively. In contrast, among outpatients, the corresponding rates were significantly different, with CA16 positivity at 2.45% (4/163), no cases of EV71 detected, and a notably higher rate of non-CA16 positivity at 26.38% (43/163). The rates of non-CA16 and non-EV71 positivity, as well as the CA16 positivity rate among outpatients, were significantly greater than those observed in inpatients (Fig. 11).

Among patients presenting with neuroinvasive symptoms, the proportions of CA16-positive, EV71-positive, non-CA16, and non-EV71 cases were 5.45% (39/715), 0.7% (5/715), and 93.85% (671/715), respectively. Conversely, in patients without symptoms of neurological infection, the respective figures were 5.82% (26/447), 1.12% (5/447), and 93.06% (416/447) (Fig. 12).

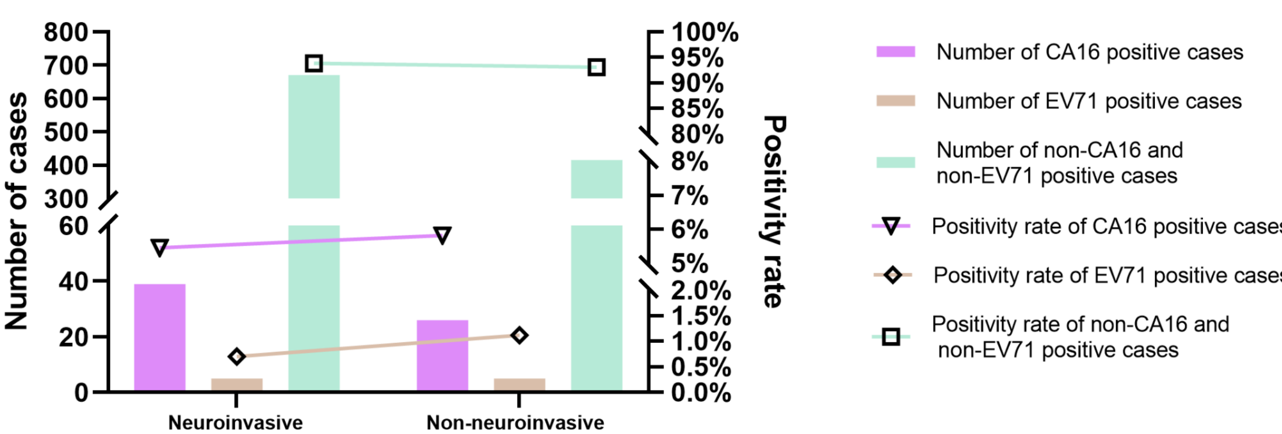

Fig. 12 Positivity rate distribution of CA16, EV71 and non-CA16 and non-EV7 infection among children with or without neuroinvasive symbols from 2019 to May 2023

Discussion

Humans are generally considered to be the only hosts of human EVs, and it has been noted that asymptomatic EV infections are sources of HFMD. The virus can be detected in the pharynx and stool of an infected person several days before the onset of illness, and it is usually most contagious for a week after symptoms appear [27]. EVs are known to elicit a range of clinical manifestations that encompass dermatological, visceral, and neurological disorders [4]. Illnesses such as hand, foot, and mouth disease (HFMD), herpangina, and viral meningitis are prominently associated with these viral infections. This multifaceted symptomatology underscores the significant impact of enteroviruses on public health and highlights the necessity for continued epidemiological research in this area. In our previous study [28], the positivity rate of EVs in Hangzhou was 14.23% in 2012, which was significantly reduced compared with that in 2010 (32.78%). The significant decrease in the positivity rate of EVs may be due to the decrease in HFMD incidences in China in 2011 and 2012 [17, 28–30]. In the present study, the positivity rates of EVs were 5.46%, 1.15%, 4.40%, 1.60%, and 2.07% in 2019, 2020, 2021, 2022, and May 2023, respectively. Moreover, the distribution analysis of EVs revealed that the positivity rate of EV71 exhibited a downward trend. In 2020, the first year after the outbreak of COVID-19, the positivity rates of EVs decreased significantly compared to 2019. Intensive epidemic prevention and control measures, such as school closures for preschool and school-age children, enhanced hand hygiene, improved management of public places, continued mask protection, restricted crowd gatherings, and enhanced environmental disinfection, might have partially limited the transmission of EVs, resulting in reduced positive rates. However, the positive rates of EVS increased again in 2021, which might have been related to the increase in HFMD in Zhejiang Province, China in 2021. Wu et al. [31] found that the combined incidence of CA16, EV71 and other enteroviruses was 4.48 cases per 100,000 people in 2020, compared to 6.32 cases per 100,000 people in 2021. Notably, the incidence of other infectious diseases decreased significantly during the COVID-19 epidemic but gradually increased in 2021 [32].

Some studies reported that preschool children were at a higher risk of infection with EV71 [33, 34] since children aged 2–4 years accounted for 50% of probable and confirmed EV71-associated HFMD cases [35]. In the current study, the positive rate of EVs increased with age from 0.99% in 0–1 months old to 7.60% in 3 to 5-year-olds and 8.42% in 5 to 7-year-olds. This indicated that the EV age distribution shifted towards older individuals. Starting in 2016, China began to promote three monovalent EV71 vaccines [34, 36], and Zhejiang Province also began EV71 vaccination at almost the same time. Thus, the possible reason for the age distribution shift in the positive rates of EVs might be the increased resistance to EV71 infection among the previously high-risk populations after vaccination [34, 37].

In our previous study [28], we found that EV infections occurred year-round but with a well-defined seasonality. According to our present study, May to June was the peak season for EV infections. Humidity and temperature seem to play a potential role in seasonal epidemics of HFMD [38–42]. Urashima et al. [39] reported that EV inactivation is faster during the dry season than in the rainy season, which could explain the seasonality. A similar result was reported by Wang et al. [43]. While any causal relationship between climate and HFMD is unclear, studies speculate that a lower incidence of HFMD might be due to reduced social contact during the winter months in temperate areas [44, 45]. Thus, there is a need to further explore the correlation between the positivity rate of EVs, climate and seasonal social contact.

Historically, EV71, CA16, CA6, and CA10 have all caused HFMD outbreaks worldwide [46]. In Hangzhou, China, the positive rate of EV71 and CA16 declined year by year from 2019 to May 2023 until it reached zero. A probable reason may be the increased use of the EV71 vaccine [27, 31, 34, 46], reduced population susceptibility [46], and reduced HFMD cases. Another possible explanation for this could be the circulation changes of EV subtypes in Hangzhou, with increasing EV71 and decreasing CA16 circulation. In contrast, our data showed a continued rise in the positivity rate of other EVs from 2019 to May 2023. The EV71 vaccine currently in the market in China does not induce cross-immunity to other EVs; thus, it is urgent to develop a multivalent EV vaccine. Furthermore, it is necessary to monitor all EV subtypes to understand their population, age, disease type, region, and season so as to provide a basis for disease prevention and control.

Conclusion

In a comprehensive analysis of enterovirus infections among children in Hangzhou from 2019 to 2023, we observed notable variations in the prevalence of enterovirus types (CA16, EV71, non-CA16, and non-EV71) related to factors such as year, month, gender, age, health service type, and neurological symptoms. On the whole, infections caused by CA16 and EV71 have exhibited a declining trend, whereas cases of non-CA16 and non-EV71 subtypes have increased. These findings underscore the necessity for future prevention and control strategies to prioritize the emerging subtypes and to enhance surveillance and preventive measures within vulnerable populations. Overall, this study offers valuable insights into the epidemiological patterns of enteroviruses in the pediatric population of Zhejiang Province and establishes a foundational framework for subsequent public health initiatives.

Acknowledgements
Not applicable.

Author contributions
SJ, ZHY and LW designed the study. LW and GYJ performed the real-time PCR. LL, LYL analyzed the data, and all authors participated in writing and revising the manuscript. All authors have read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of the Children's Hospital Affiliated to Zhejiang University School of Medicine(2023-IRB-0242-P-01).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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