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RESEARCH ARTICLE

Population based hospitalization burden of laboratory-confirmed hand, foot and mouth disease caused by multiple enterovirus serotypes in Southern China

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Abstract

Background

Hand, foot and mouth disease (HFMD) is spread widely across Asia, and the hospitalization burden is currently not well understood. Here, we estimated serotype-specific and age-specific hospitalization rates of HFMD in Southern China.

Methods

We enrolled pediatric HFMD patients admitted to 3/3 county-level hospitals, and 3/23 township-level hospitals in Anhua county, Hunan (CN). Samples were collected to identify enterovirus serotypes by RT-PCRs between October 2013 and September 2016. Information on other eligible, but un-enrolled, patients were retrospectively collected from the same six hospitals. Monthly numbers of all-cause hospitalizations were collected from each of the 23 township-level hospitals to extrapolate hospitalizations associated with HFMD among these.

Results

During the three years, an estimated 3,236 pediatric patients were hospitalized with lab-confirmed HFMD, and among these only one case was severe. The mean hospitalization rate was 660 (95% CI: 638–684) per 100,000 person-years for lab-confirmed HFMD, with higher rates among CV-A16 and CV-A6 associated HFMD (213 vs 209 per 100,000 person-years), and lower among EV-A71, CV-A10 and other enterovirus associated HFMD (134, 39 and 66

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per 100,000 person-years respectively, $p<0.001$). Children aged 12–23 months had the highest hospitalization rates (3,594/100,000 person-years), followed by those aged 24–35 months (1,828/100,000 person-years) and 6–11 months (1,572/100,000 person-years). Compared with other serotypes, CV-A6-associated hospitalizations were evident at younger ages.

Conclusions

Our study indicates a substantial hospitalization burden associated with non-severe HFMD in a rural county in southern China. Future mitigation policies should take into account the disease burden identified, and optimize interventions for HFMD.

Introduction

Hand, foot and mouth disease (HFMD) is a common infectious disease that mainly affects children below 5 years of age [1]. HFMD is caused by multiple serotypes of Enterovirus species A, among which enterovirus 71 (EV-A71) and coxsackievirus A16 (CV-A16) are the most frequently detected [2]. EV-A71 is of particular concern as it can cause neurological and systemic complications, and even fatal outcomes [2–4]. Coxsackie virus A6 (CV-A6) and Coxsackie virus A10 (CV-A10) have become more prevalent among HFMD outbreaks, with their re-emergence first identified in HFMD cases in Europe and Singapore between 2008 and 2011 [5–8]. These serotypes are also responsible for a considerable proportion of cases of HFMD in China since 2013 [9, 10]. Severe complications associated with CV-A6 and CV-A10 have also been reported [11, 12].

Currently, no specific antiviral treatments are available for HFMD. Three inactivated monovalent EV-A71 vaccines have been licensed in mainland China, with high efficacy (90.0%-97.4%) against EV-A71-HFMD, but no cross-protection against other enterovirus serotype-associated HFMD [13, 14]. Bivalent and multivalent enterovirus vaccines are under development [15, 16].

Since 1997, HFMD has widely spread across Asia, including Malaysia, Japan, Singapore, Vietnam, Cambodia, and China [1, 2, 4, 17–21]. Understanding the age and serotype-specific burden of HFMD, including the hospitalization burden, is valuable in informing healthcare systems, vaccine strategies and other intervention policies. However, the hospitalization burden of HFMD has not been thoroughly studied in a well-defined catchment population. Indirect estimates of hospitalization rates of HFMD are hampered by limited availability of population-level incidence and unknown hospitalization rates among HFMD cases, however, population-level incidences of HFMD have been estimated in Japan [22], Singapore [23], Malaysia [24], and China [2, 25]. These estimates were based on notifiable surveillance data, which may underestimate the true prevalence. The risk of hospitalization for HFMD varied between 1.3% and 24.3% [26–30], possibly due to patients with distinct severity and different causative serotypes of enterovirus in different studies. The number of people that were included in these studies ranged from 6,027 to 1,081,046 [26–30]. Additionally, there is an increasing threat of enterovirus serotypes of non-EV-A71 among both mild and severe HFMD [7, 11, 25]. Therefore, the specific hospitalization burden of HFMD caused by CV-A6, CV-A10, CV-A16 and other enterovirus serotypes requires further assessment.

We aim to estimate population level hospitalization rates of HFMD by age and enterovirus serotype in a well-defined catchment population in China, between October 2013 and September 2016.

Methods

Study setting

This study was conducted in Anhua County, Yiyang Prefecture, Hunan Province, China [31]. The total population residing in Anhua County was 1,017,463 according to the 2015 census [32]. A total of 165,050 (16%) of the population were children aged <15 years, and of these 61,123 (6%) were aged <5 years [32]. During the study period, there were three county-level hospitals and 23 township-level hospitals in Anhua County where HFMD patients were admitted. According to vaccination records, the EV-A71 vaccine was initiated in Anhua County on July 10, 2016.

Case definitions

A probable case of HFMD was defined as a patient with a rash on their hands, feet, limbs or buttocks, ulcers or vesicles in the mouth, with or without fever. A lab-confirmed case was defined as a probable case with laboratory evidence of enterovirus infection in specimens of stool, rectal swab or throat swab, detected by real-time RT-PCR (reverse transcription-polymerase chain reaction) or nested RT-PCR. Hospitalized patients were defined as patients admitted to hospital for a period of at least 24 hours.

Virological surveillance of HFMD

Virological surveillance of HFMD was conducted among six hospitals, including three county-level hospitals and three township-level hospitals between October 2013 and September 2016. The six hospitals selected admitted 87% of the reported HFMD patients from Anhua County during 2010–2012. The methods of virological surveillance have been described elsewhere [31]. Briefly, pediatric patients (aged 0–14 years) who were hospitalized for HFMD, were enrolled after their parents/legal guardians provided verbal consent. Throat swabs and stool samples (rectal swab instead if stool samples were unavailable), were collected within 24 hours of enrollment. A standardized form was used to collect data, including basic demographic information, date of illness onset, types of samples (stool, throat swab, or rectal swab), any complications, and clinical outcome.

Swabs were immediately placed in viral transport mediums, and all samples were stored at -70˚C until testing. Viral RNA was extracted using QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). RNA from each sample was tested with generic primers and probes targeting pan-enterovirus conserved regions, and serotype specific primers and probes targeting EV-A71, CV-A16, and CV-A6 (S1 Table). Where a sample tested positive in the generic, but negative in the specific RT-PCRs, nested RT-PCRs were used to amplify VP1 regions. If the sample was also negative in amplifying VP1 regions, additional nested RT-PCRs on VP4-VP2 regions were used for further identification of enterovirus serotype (S2 Table).

Estimation of hospitalization burden

Some patients who were admitted to hospital for HFMD during the study period were not captured by the above virological surveillance. Therefore, we retrospectively collected monthly numbers of eligible, but unenrolled, hospitalized patients with a diagnosis of HFMD, by age group, at each of the six hospitals. We extracted data from the electronic Hospital Information System (HIS) at the three county-level hospitals. We manually reviewed the paper-based medical charts at the three township-level hospitals. We did not visit the other 20 township-level hospitals to collect the number of HFMD-associated hospitalizations manually. Instead, the number of patients who were hospitalized between October 2014 and September 2016, the

cause of their hospitalization and their age was extracted from the Rural Health Information System (RHIS) of Hunan Province (http://220.170.145.170:8888/chss/) for each of the 23 township-level hospitals.

A multiplier model [33] was used to estimate the hospitalization rates of HFMD in this study (Fig 1). We divided the patients and the population denominators into eight age groups, including <6 months, 6–11 months, 12–23 months, 24–35 months, 36–47 months, 48–59 months, 5–9 years, and 10–14 years. For the model, we assumed that the age stratified admission rates were comparable among the township-level hospitals. We extrapolated the number of HFMD-associated hospitalizations per age group among the 23 township-level hospitals based on data from the three township-level hospitals and number of total hospitalizations among all township-level hospitals (Fig 1). The total number of hospitalizations for HFMD in each age group in Anhua county was the sum of the number of hospitalizations captured and those not captured by the virological surveillance among the county-level hospitals, and number HFMD-associated hospitalizations among all township-level hospitals. We estimated the number of hospitalizations attributable to lab-confirmed HFMD, EV-A71, CV-A16, CV-A6, CV-A10 and other enteroviruses (non-EV-A71 & non-CV-A16 & non-CV-A6 & non-CV-A10) associated with HFMD in Anhua County. We assumed that the age-specific distribution of enterovirus serotype in the enrolled group was equivalent to in the non-enrolled group (Fig 1).

The serotype-specific hospitalization rates were estimated by dividing the serotype-specific number of hospitalizations by the size of the resident population. The 2013–2016 age-specific population denominators of Anhua County were collected from the National Bureau of Statistics of China [32].

Statistical analysis

The Poisson method was used to estimate 95% confidence intervals (CI) of hospitalization rates. The χ^2 or Fisher's exact test was used to analyze categorical data. The Mann-Whitney U test was used to analyze ranked data. Student's t-test was used to analyze continuous data. All data cleaning and analyses were conducted using R (version 3.4.2).

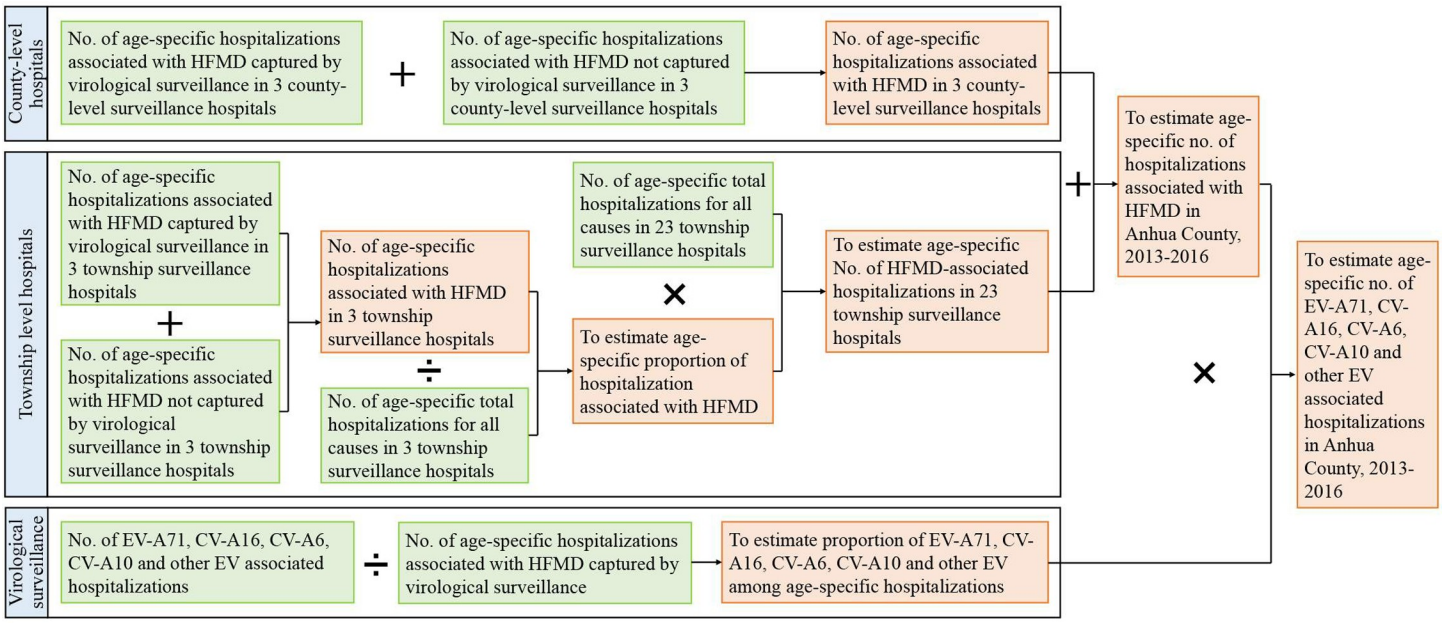

Fig 1. Flowchart of estimating age-specific and serotype-specific hospitalization burden associated with HFMD.

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Ethical approval

This study was approved by the Institutional Review Board (IRB) at the Chinese Center for Disease Control and Prevention (no. 201224) and World Health Organization Regional Office for the Western Pacific (no. 2013.10.CHN.2.ESR). This study was part of a continuing public health outbreak investigation by National Health and Family Planning Commission of China. The IRB approved the use of verbal consent. Verbal informed consent was obtained, and documented in form, from the patients' parents/guardians when sampling and filling out the questionnaire.

Results

Virological surveillance

During the three-year virological surveillance, 3,326 patients were hospitalized with a diagnosis of HFMD in the six surveillance hospitals, and 2,836 (85%) patients were enrolled into the study. 490 (15%) patients were not enrolled because they refused to provide clinical specimens. Baseline characteristics were similar between the enrolled and un-enrolled patients, including gender, age, and length of hospital stay [31]. One 27-month old child with infection serotype EV-A71 had symptoms of neurological involvement (frequent jittering and myoclonic jerks after 4 days of fever), but all other patients had uncomplicated illnesses. Enterovirus was detected among 2,517/2,836 (89%) patients via the real-time RT-PCR targeting pan-enterovirus. Nineteen serotypes of enterovirus were successfully identified in 2,513/2,517 (99.8%) patients. Of these, the most commonly detected were CV-A16 (33%, 819), CV-A6 (31%, 785), EV-A71 (20%, 514), and CV-A10 (6%, 149). The genotype of CV-A16 (B1), CV-A6 (D) and EV-A71 (C4a) were identified based on the VP1 sequences. CV-A6 infections were more frequently identified in patients younger than 2 years, while CV-A16 and EV-A71 accounted for a higher proportion of HFMD among children aged $\geq$3 years than CV-A6 ($p<0.001$) (S3 Table). Fever symptom (axillary temperature $\geq$ 37.3˚C) was more common among CV-A10 (82.1%) and CV-A6 (73.6%) infections than EV-A71 (61.9%) and CV-A16 (54.6%). The distributions of rash on body including hand, foot, mouth and buttocks were similar between the serotypes. A seasonal peak in total hospitalizations associated with HFMD was observed between April and June across all years (S1 Fig). EV-A71, CV-A16, CV-A10 and other enterovirus showed similar seasonal patterns compared to total HFMD. However, the peak with CV-A6 infection occurred during the troughs of total HFMD hospitalizations during the first year (October 2013—September 2014) and the third year (October 2013—September 2014) (S1 Fig).

Serotype-specific hospitalization rates of HFMD

Between October 2013 and September 2016, an estimated 3,642 pediatric patients were hospitalized for HFMD in Anhua County. A total of 3,273/3,642 (90%) children were admitted in the three county-level hospitals and 369/3,642 (10%) children were admitted in the 23 township-level hospitals (S4 Table). Among these, 3,236/3,642 (89%) patients were estimated to be positive for enterovirus. The mean hospitalization rates of probable HFMD were estimated as 743 (95% CI: 719–768) per 100,000 person-years, and the mean hospitalization rates of lab-confirmed HFMD were 660 (95% CI: 638–684) hospitalizations per 100,000 person-years during the surveillance period (Fig 2A). Both annual hospitalization rates for probable and lab-confirmed HFMD were highest overall during the 2013–2014 season (1,099 and 1,011 per 100,000 person-years, respectively), and lowest overall during the 2014–2015 season (458 and 422 per 100,000 person-years, respectively) (Fig 2B).

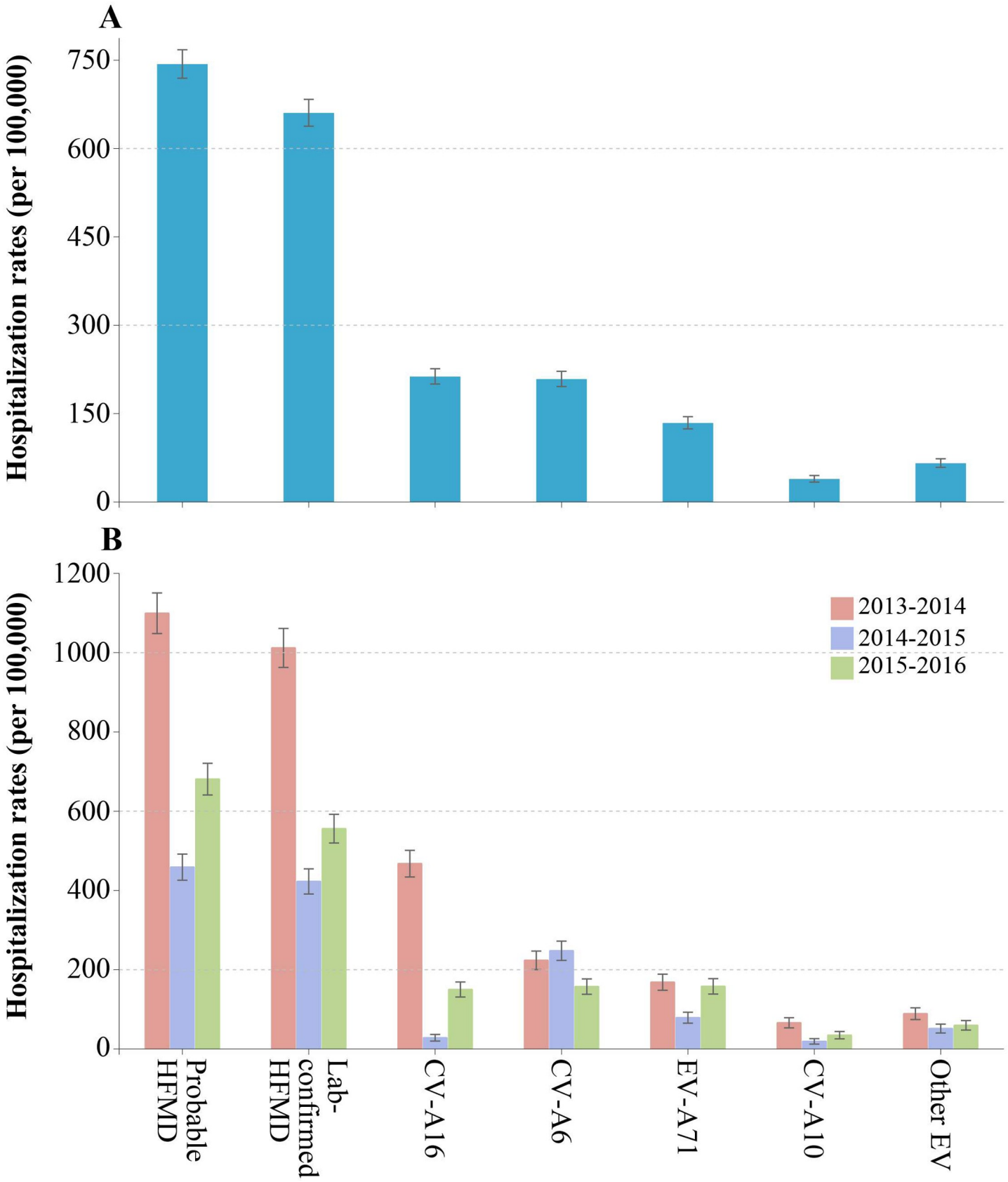
A
Hospitalization rates (per 100,000)
750
600
450
300
150
0
B
Hospitalization rates (per 100,000)
1200
1000
800
600
400
200
0
2013-2014
2014-2015
2015-2016
Probable HFMD
Lab-confirmed HFMD
CV-A16
CV-A6
EV-A71
CV-A10
Other EV

Fig 2. Hospitalization rates of HFMD in Anhua County, China, October 2013—September 2016. (A) Average hospitalization rates of HFMD, overall and stratified by serotype. (B) Annual hospitalization rates of HFMD, overall and stratified by serotype.

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Based on the assumption of similar distribution of serotypes among the enrolled and un-enrolled groups, 3,236 (89%) patients with lab-confirmed HFMD consisted of 1,043 (32%) with CV-A16, 1,023 (32%) with CV-A6, 657 (20%) with EV-A71, 191 (6%) with CV-A10, and 322 (10%) with other enteroviruses. During the surveillance period the mean hospitalization rates were comparable between CV-A16-associated HFMD (213 per 100,000 person-years) and CV-A6-associated HFMD (209 per 100,000 person-years), and lower for EV-A71-associated HFMD (134 per 100,000 person-years), CV-A10-associated HFMD (39 per 100,000 person-years), and other enteroviruses (66 per 100,000 person-years) infections ($p<0.001$) (Fig 2A).

In the first year, between October 2013 and September 2014, the hospitalization rates for CV-A16 were the highest (467 per 100,000 person-years). While in the second year, CV-A6 had the largest burden of hospitalizations (248 per 100,000 person-years). The hospitalization rates were similar among CV-A16, CV-A6, and EV-A71 infections in the third year ($p = 0.568$) (Fig 2B). The annual hospitalizations varied substantially over years for EV-A71, CV-A16, and CV-A10. However, CV-A6 displayed comparable hospitalization rates over all three years (Fig 2B).

Age-specific hospitalization rates of HFMD

The mean hospitalization rates of HFMD varied with age. The highest rates of lab-confirmed HFMD were among children aged 12–23 months (3,594 per 100,000 person-years), followed by 24–35 months (1,828 per 100,000 person-years) and then 6–11 months (1,572 per 100,000 person-years). Hospitalization rates were lower among infants younger than 6 months (381 per 100,000 person-years) and among children aged 5–14 years (84 per 100,000 person-years) (Fig 3B). Similar age-specific hospitalization rates were observed for probable HFMD (Fig 3A). Among children younger than 5 years the mean rates of probable and lab-confirmed HFMD were 1,829 and 1,638 hospitalizations per 100,000 person-years respectively. Unlike the distribution of yearly rates for children aged $\geq$12 months, the hospitalization rates decreased over three years for children aged 0–11 months (Fig 3C and 3D).

The four most common enterovirus serotypes had highest hospitalization rates among children aged 12–23 months (955 for CV-A16, 1,344 for CV-A6, 640 for EV-A71, and 267 for CV-A10 per 100,000 person-years) (Fig 4A–4D). CV-A6 uniquely had higher rates among children aged 6–11 months than among those aged 24–35 months ($p<0.001$), while CV-A10 had comparable rates between these age groups ($p = 0.849$) (Fig 4A–4D). Statistical analyses suggested that HFMD hospitalization associated with CV-A6 were evident at younger ages, compared to EV-A71 and CV-A16. The distribution pattern of age-specific hospitalization rates for CV-A16, EV-A71, and CV-A10 were consistent across the three years, between October 2013 to September 2016 (S2 Fig). For CV-A6, differences in hospitalization rates in 6–11 months compared to 24–35 months was not evident in the third year, between October 2015 to September 2016 ($p = 0.777$) (S2 Fig).

Discussion

Principal findings

This study provides a comprehensive estimate of hospitalization rates for probable and lab-confirmed HFMD in Anhua County, Hunan Province, China, between October 2013 and September 2016. An average of 743 probable HFMD and 660 lab-confirmed HFMD associated

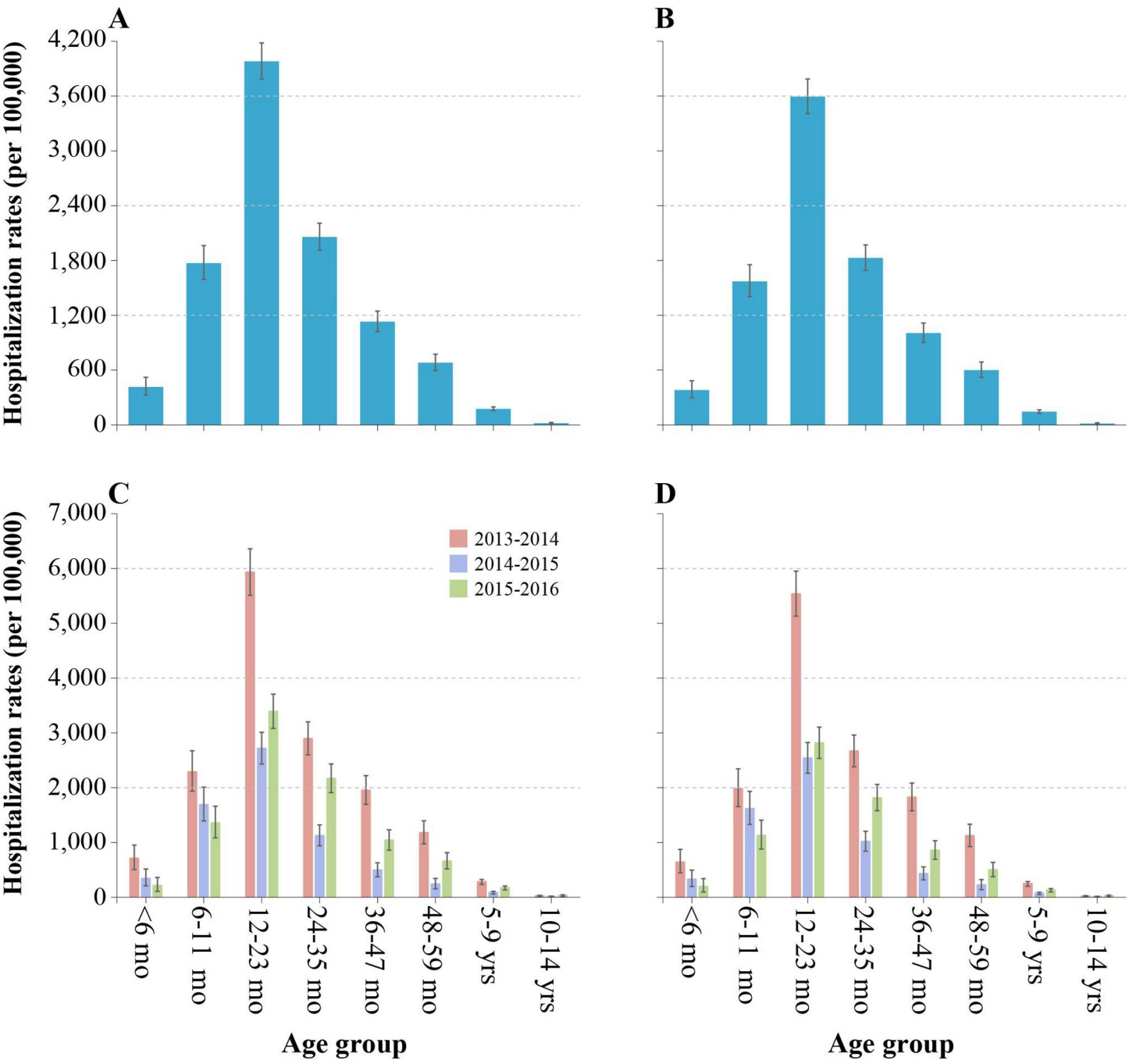

Fig 3. Age-specific hospitalization rates associated with HFMD in Anhua County, China, October 2013—September 2016. (A) Average age-specific hospitalization rates of probable HFMD. (B) Average age-specific hospitalization rates of lab-confirmed HFMD. (C) Annual age-specific hospitalization rates of probable HFMD. (D) Annual age-specific hospitalization rates of lab-confirmed HFMD.

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hospitalizations were estimated per 100,000 person-years in Anhua County, with the highest annual rates during 2013–2014 and the lowest during 2014–2015. CV-A16 and CV-A6 were associated with most (64%) lab-confirmed HFMD hospitalizations, and had higher hospitalization rates than EV-A71, CV-A10 and other enteroviruses during the study period. Hospitalization rates peaked among children aged 12–23 months, and decreased with age among

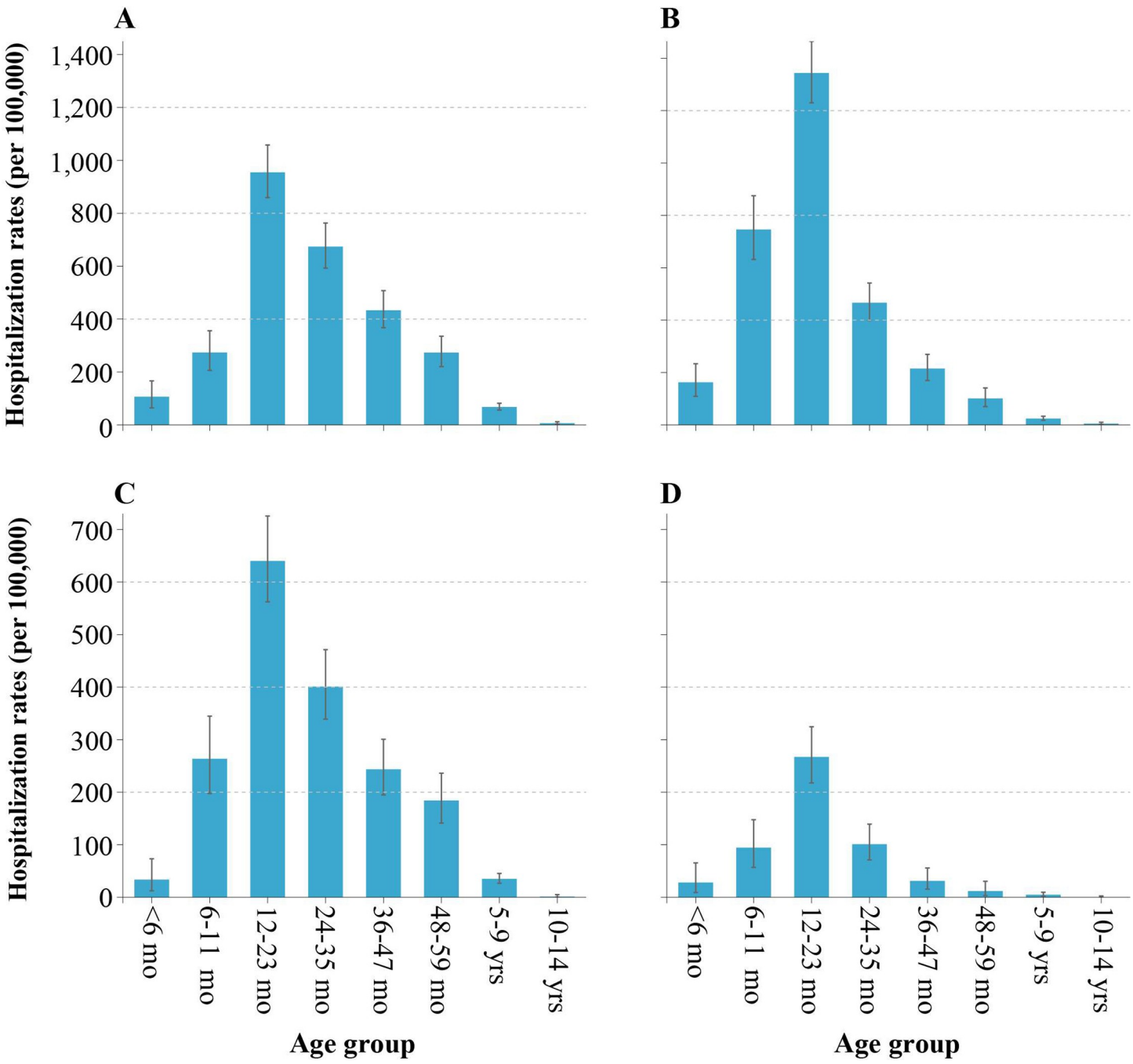

Fig 4. Age-specific and serotype-specific hospitalization rates associated with HFMD in Anhua County, China, October 2013—September 2016. (A) Average age-specific hospitalization rates of CV-A16-associated HFMD. (B) Average age-specific hospitalization rates of CV-A6-associated HFMD. (C) Average age-specific hospitalization rates of EV-A71-associated HFMD. (D) Average age-specific hospitalization rates of CV-A10-associated HFMD.

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EV-A71, CV-A16, CV-A6, CV-A10 and other serotype non-specific enterovirus associated HFMD. Compared to other serotypes, CV-A6 had higher hospitalization rates in children aged 6–11 months than in children aged 24–35 months.

Strength and comparison with previous studies

Because HFMD is a self-limiting illness, and the relative scarcity of HFMD associated severe illness (1.1%) [2, 34], the burden of HFMD is substantially underestimated where only notifiable surveillance data is used. In this study, we actively captured HFMD-associated hospitalizations in all healthcare facilities in Anhua county, instead of relying solely on passive surveillance. Additionally, our virological surveillance captured 78% of the total HFMD-associated hospitalizations over three years. This allowed us to attain an accurate representation of the enterovirus serotypes that cause HFMD. Samples were collected in a timely fashion; multiple samples were available and intensive methods were used to identify enterovirus serotypes. This enabled us to make a robust estimation of the population-based hospitalization burden stratified by age group and enterovirus serotype.

In our study, all children with mild illness (except one) were admitted to hospital. The reasons underlying overuse of healthcare resources for mild illnesses include a low hospitalization threshold applied by local physicians to capture all possible sudden deteriorations [3], parents self-requesting hospital admission and the rural healthcare insurance (New Rural Cooperative Medical Scheme) providing higher reimbursement for inpatient than outpatient care [35]. Proportions of mild patients accounted for 80–99% among HFMD-associated hospitalizations in previous reports [26, 29, 30, 34]. The patients without complications were hospitalized in these reports because of high fever, poor feeding, mouth ulcers, vomiting, or dehydration [29]. This indicates that hospitalizations for non-severe HFMD is common in China.

To our knowledge, population-based hospitalization rates for HFMD have not been reported until now. As the threshold for hospitalization of HFMD in our study was relatively low, we made comparisons with the incidence rates estimated using the notifiable surveillance data [2, 22–25]. The hospitalization rate for HFMD estimated in our study (119/100,000 person-years) is comparable to the incidence rates reported in mainland China during 2008–2015 (127/100,000 person-years) [2, 25]. The rates we estimated are higher than the incidence rates in Malaysia during 2011–2014 (20-90/100,000 person-years) [24], and lower than the incidence rates in Singapore during 2001–2007 (126-436/100,000 person-years) [23], and Japan during 2002–2005 (743 vs 2,940–5,740 per 100,000 population) in children aged 0–14 years [22]. The variation in results could be associated with distinct local activity of enterovirus serotypes in different study periods, and differences among the surveillance systems employed. For example, the combination of doctor-driven and teacher-driven surveillance make the data on notified symptomatic presentations of HFMD collected by the Singapore's Ministry of Health unusually complete [34]. The surveillance of HFMD in China and Malaysia rely on all hospitals [2, 24]. The targeted HFMD surveillance in Japan is based on sentinel medical institutions according to the guidelines for surveillance of infectious diseases [22]. Outpatients were not included in our study, therefore the HFMD hospitalization rates we report could be an underestimate of the true incidence rates in Anhua County.

The age pattern of HFMD-associated hospitalizations was consistent with previous reports of HFMD-associated incidence [2, 24, 25]. Relatively low hospitalization rates among children younger than 6 months were likely due to protection by maternal antibodies [36]. Compared to older children who may attend kindergartens or nurseries, children aged 6–23 months have a lower frequency of contact with those of similar ages due to limited mobility. High hospitalization rates among this age group may suggest inherent increased susceptibility and/or possible alternative transmission routes, for example from contact with asymptomatic infected adults or contaminated environments [25]. Further to this, previous studies of serological surveillance revealed low levels of sero-prevalence in children aged 6–23 months [36, 37].

Our study found that CV-A6 presented a higher burden of HFMD-associated hospitalization than EV-A71 during the study period, and had peak hospitalization rates between October 2014 and September 2015. This was consistent with the increasing activity of CV-A6 in China and the regional introduction of CV-A6 in 2012[10, 11, 38–40]. CV-A6-associated hospitalizations were also higher among younger age groups than EV-A71 and CV-A16 [41–43]. This may result from lower maternal immunity against an recently emerging virus serotype. As observed in this study, CV-A6 will likely affect more older children and present a similar age pattern to EV-A71 and CV-A16, since more maternal immunity is attained after introduction and spread of CV-A6, causing the median age of infection to increase. Further studies should be conducted to monitor the potentially changing epidemiology of CV-A6.

Limitations

This study was subject to several limitations. First, due to unavailability of data, the number of HFMD associated hospitalizations in township-level hospitals was estimated as a expected proportion of HFMD-associated hospitalizations compared to all-cause hospitalization. However, the distribution of hospitalization causes is not expected to vary greatly since the demographic, economic and living conditions were very similar among the different townships. Second, virological surveillance was not conducted at the 20 other township-level hospitals, where the number of HFMD hospitalizations was very small (9% of all cause hospitalizations). Third, the HFMD patients hospitalized outside Anhua County were not captured. Due to low proportion of severe disease manifestations (1.1%) among HFMD patients, few cases need referral to prefecture- or provincial-level hospitals. Therefore, hospitalization rates of HFMD estimated in our study should be robust. Finally, as the three-year virological surveillance captured only one child with HFDM-associated neurological complications, the hospitalization burden of severe HFMD could not be estimated in our study. Continuing surveillance over several years should be implemented in different geographical and climactic regions of China, which will help improve our understanding of the HFMD disease burden.

Conclusions

Our study provides a comprehensive analysis of probable HFMD, lab-confirmed HFMD, EV-A71, CV-A16, CV-A6, CV-A10 and other enterovirus associated hospitalization rates in Anhua County, Hunan Province, China between 2013 and 2016. We found a substantial annual hospitalization burden for mild HFMD, caused by multiple enterovirus serotypes in China. During the study period, CV-A16 and CV-A6 predominated hospitalizations compared to EV-A71, CV-A10 and other enteroviruses. Our findings suggest that intensive training on how to quickly detect and treat mild HFMD should be provided to clinicians, especially those working at county-level and township-level hospitals. Furthermore, health education on HFMD should be provided to parents/guardians to address their concerns about HFMD.

Supporting information

S1 Fig. Temporal trends of HFMD-associated hospitalizations by enterovirus serotype in six surveillance hospitals in Anhua County, China, October 2013—September 2016.
(TIF)

S2 Fig. Annual hospitalization rates associated with HFMD stratified by age and enterovirus serotype in Anhua County, China, October 2013—September 2016. (A) Annual age-specific hospitalization rates of CV-A16-associated HFMD. (B) Annual age-specific

hospitalization rates of CV-A6-associated HFMD. (C) Annual age-specific hospitalization rates of EV-A71-associated HFMD. (D) Annual age-specific hospitalization rates of CV-A10-associated HFMD.
(TIF)

S1 Table. Primers and probes used in the real time RT-PCR.
(DOCX)

S2 Table. Primers used in nested RT-PCR.
(DOCX)

S3 Table. The age profile of HFMD-associated hospitalizations stratified by enterovirus serotype in 6 surveillance hospitals in Anhua County, China, October 2013—September 2016.
(DOCX)

S4 Table. Estimated hospitalization rates associated with HFMD by age group in Anhua County, China, October 2013—September 2016.
(DOCX)

S1 Checklist. STROBE checklist.
(DOCX)

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Resources: Hongjie Yu.

Supervision: Hongjie Yu.

Writing – original draft: Shuanbao Yu.

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References

1. Solomon T, Lewthwaite P, Perera D, Cardosa MJ, McMinn P, Ooi MH. Virology, epidemiology, pathogenesis, and control of enterovirus 71. The Lancet Infectious diseases. 2010; 10(11):778–90. https://doi.org/10.1016/S1473-3099(10)70194-8 PMID: 20961813
2. Xing W, Liao Q, Viboud C, Zhang J, Sun J, Wu JT, et al. Hand, foot, and mouth disease in China, 2008–12: an epidemiological study. The Lancet Infectious diseases. 2014; 14(4):308–18. https://doi.org/10.1016/S1473-3099(13)70342-6 PMID: 24485991
3. Ooi MH, Wong SC, Lewthwaite P, Cardosa MJ, Solomon T. Clinical features, diagnosis, and management of enterovirus 71. The Lancet Neurology. 2010; 9(11):1097–105. https://doi.org/10.1016/S1474-4422(10)70209-X PMID: 20965438
4. Ho M, Chen ER, Hsu KH, Twu SJ, Chen KT, Tsai SF, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. The New England journal of medicine. 1999; 341(13):929–35. https://doi.org/10.1056/NEJM199909233411301 PMID: 10498487
5. Blomqvist S, Klemola P, Kaijalainen S, Paananen A, Simonen ML, Vuorinen T, et al. Co-circulation of coxsackieviruses A6 and A10 in hand, foot and mouth disease outbreak in Finland. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology. 2010; 48(1):49–54.
6. Wu Y, Yeo A, Phoon MC, Tan EL, Poh CL, Quak SH, et al. The largest outbreak of hand; foot and mouth disease in Singapore in 2008: the role of enterovirus 71 and coxsackievirus A strains. International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases. 2010; 14(12):e1076–81.
7. Mirand A, Henquell C, Archimbaud C, Ughetto S, Antona D, Bailly JL, et al. Outbreak of hand, foot and mouth disease/herpangina associated with coxsackievirus A6 and A10 infections in 2010, France: a large citywide, prospective observational study. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2012; 18(5):E110–8.
8. Montes M, Artieda J, Pineiro LD, Gastesi M, Diez-Nieves I, Cilla G. Hand, foot, and mouth disease outbreak and coxsackievirus A6, northern Spain, 2011. Emerging infectious diseases. 2013; 19(4).
9. He SZ, Chen MY, Xu XR, Yan Q, Niu JJ, Wu WH, et al. Epidemics and aetiology of hand, foot and mouth disease in Xiamen, China, from 2008 to 2015. Epidemiology and infection. 2017; 145(9):1865–74. https://doi.org/10.1017/S0950268817000309 PMID: 28367766
10. Lu J, Zeng H, Zheng H, Yi L, Guo X, Liu L, et al. Hand, foot and mouth disease in Guangdong, China, in 2013: new trends in the continuing epidemic. Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2014; 20(7):O442–5.
11. Yang F, Yuan J, Wang X, Li J, Du J, Su H, et al. Severe hand, foot, and mouth disease and coxsackievirus A6-Shenzhen, China. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2014; 59(10):1504–5.
12. Chen M, He S, Yan Q, Xu X, Wu W, Ge S, et al. Severe hand, foot and mouth disease associated with Coxsackievirus A10 infections in Xiamen, China in 2015. Journal of clinical virology: the official publication of the Pan American Society for Clinical Virology. 2017; 93:20–4.
13. Zhu FC, Meng FY, Li JX, Li XL, Mao QY, Tao H, et al. Efficacy, safety, and immunology of an inactivated alum-adjuvant enterovirus 71 vaccine in children in China: a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet (London, England). 2013; 381(9882):2024–32.
14. Li R, Liu L, Mo Z, Wang X, Xia J, Liang Z, et al. An inactivated enterovirus 71 vaccine in healthy children. The New England journal of medicine. 2014; 370(9):829–37. https://doi.org/10.1056/NEJMoa1303224 PMID: 24571755
15. Caine EA, Fuchs J, Das SC, Partidos CD, Osorio JE. Efficacy of a Trivalent Hand, Foot, and Mouth Disease Vaccine against Enterovirus 71 and Coxsackieviruses A16 and A6 in Mice. Viruses. 2015; 7(11):5919–32. https://doi.org/10.3390/v7112916 PMID: 26593938
16. Ku Z, Liu Q, Ye X, Cai Y, Wang X, Shi J, et al. A virus-like particle based bivalent vaccine confers dual protection against enterovirus 71 and coxsackievirus A16 infections in mice. Vaccine. 2014; 32(34):4296–303. https://doi.org/10.1016/j.vaccine.2014.06.025 PMID: 24950363
17. Seiff A. Cambodia unravels cause of mystery illness. Lancet (London, England). 2012; 380(9838):206.
18. Chan LG, Parashar UD, Lye MS, Ong FG, Zaki SR, Alexander JP, et al. Deaths of children during an outbreak of hand, foot, and mouth disease in sarawak, malaysia: clinical and pathological characteristics of the disease. For the Outbreak Study Group. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2000; 31(3):678–83.
19. Fujimoto T, Chikahira M, Yoshida S, Ebira H, Hasegawa A, Totsuka A, et al. Outbreak of central nervous system disease associated with hand, foot, and mouth disease in Japan during the summer of 2000: detection and molecular epidemiology of enterovirus 71. Microbiology and immunology. 2002; 46(9):621–7. PMID: 12437029

20. Chan KP, Goh KT, Chong CY, Teo ES, Lau G, Ling AE. Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. Emerging infectious diseases. 2003; 9(1):78–85. https://doi.org/10.3201/eid1301.020112 PMID: 12533285

21. Tu PV, Thao NT, Perera D, Huu TK, Tien NT, Thuong TC, et al. Epidemiologic and virologic investigation of hand, foot, and mouth disease, southern Vietnam, 2005. Emerging infectious diseases. 2007; 13 (11):1733–41. https://doi.org/10.3201/eid1311.070632 PMID: 18217559

22. Kawado M, Hashimoto S, Murakami Y, Izumida M, Ohta A, Tada Y, et al. Annual and weekly incidence rates of influenza and pediatric diseases estimated from infectious disease surveillance data in Japan, 2002–2005. Journal of epidemiology. 2007; 17 Suppl:S32–41.

23. Ang LW, Koh BK, Chan KP, Chua LT, James L, Goh KT. Epidemiology and control of hand, foot and mouth disease in Singapore, 2001–2007. Annals of the Academy of Medicine, Singapore. 2009; 38 (2):106–12. PMID: 19271036

24. NikNadia N, Sam IC, Rampal S, WanNorAmalina W, NurAtifah G, Verasahib K, et al. Cyclical Patterns of Hand, Foot and Mouth Disease Caused by Enterovirus A71 in Malaysia. PLoS neglected tropical diseases. 2016; 10(3):e0004562. https://doi.org/10.1371/journal.pntd.0004562 PMID: 27010319

25. Yang B, Liu F, Liao Q, Wu P, Chang Z, Huang J, et al. Epidemiology of hand, foot and mouth disease in China, 2008 to 2015 prior to the introduction of EV-A71 vaccine. Euro surveillance: bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin. 2017; 22(50).

26. Ma E, Lam T, Chan KC, Wong C, Chuang SK. Changing epidemiology of hand, foot, and mouth disease in Hong Kong, 2001–2009. Japanese journal of infectious diseases. 2010; 63(6):422–6. PMID: 21099093

27. Liu DP, Wang TA, Huang WT, Chang LY, Wang ET, Cheng SH, et al. Disease burden of enterovirus infection in Taiwan: Implications for vaccination policy. Vaccine. 2016; 34(7):974–80. https://doi.org/10.1016/j.vaccine.2015.12.026 PMID: 26768128

28. Xu W, Liu CF, Yan L, Li JJ, Wang LJ, Qi Y, et al. Distribution of enteroviruses in hospitalized children with hand, foot and mouth disease and relationship between pathogens and nervous system complications. Virology journal. 2012; 9:8. https://doi.org/10.1186/1743-422X-9-8 PMID: 22230340

29. Wang YR, Sun LL, Xiao WL, Chen LY, Wang XF, Pan DM. Epidemiology and clinical characteristics of hand foot, and mouth disease in a Shenzhen sentinel hospital from 2009 to 2011. BMC infectious diseases. 2013; 13:539. https://doi.org/10.1186/1471-2334-13-539 PMID: 24225231

30. Zeng M, Li YF, Wang XH, Lu GP, Shen HG, Yu H, et al. Epidemiology of hand, foot, and mouth disease in children in Shanghai 2007–2010. Epidemiology and infection. 2012; 140(6):1122–30. https://doi.org/10.1017/S0950268811001622 PMID: 21878145

31. Gao L, Zou G, Liao Q, Zhou Y, Liu F, Dai B, et al. Spectrum of Enterovirus Serotypes Causing Uncomplicated Hand, Foot, and Mouth Disease and Enteroviral Diagnostic Yield of Different Clinical Samples. Clinical infectious diseases: an official publication of the Infectious Diseases Society of America. 2018; 67(11):1729–35.

32. National Bureau of Statistics of China. National census in China in 2015. Available from: http://www.stats.gov.cn/tjsj/ndsj/2015/indexeh.htm cited 15 August 2017.

33. Reed C, Angulo FJ, Swerdlow DL, Lipsitch M, Meltzer MI, Jernigan D, et al. Estimates of the prevalence of pandemic (H1N1) 2009, United States, April-July 2009. Emerging infectious diseases. 2009; 15 (12):2004–7. https://doi.org/10.3201/eid1512.091413 PMID: 19961687

34. Koh WM, Badaruddin H, La H, Chen MI, Cook AR. Severity and burden of hand, foot and mouth disease in Asia: a modelling study. BMJ global health. 2018; 3(1):e000442. https://doi.org/10.1136/bmjgh-2017-000442 PMID: 29564154

35. Sun Y, Gregersen H, Yuan W. Chinese health care system and clinical epidemiology. Clinical epidemiology. 2017; 9:167–78. https://doi.org/10.2147/CLEP.S106258 PMID: 28356772

36. Yang B, Wu P, Wu JT, Lau EH, Leung GM, Yu H, et al. Seroprevalence of Enterovirus 71 Antibody Among Children in China: A Systematic Review and Meta-analysis. The Pediatric infectious disease journal. 2015; 34(12):1399–406. https://doi.org/10.1097/INF.0000000000000900 PMID: 26368058

37. Zhu R, Cheng T, Yin Z, Liu D, Xu L, Li Y, et al. Serological survey of neutralizing antibodies to eight major enteroviruses among healthy population. Emerging microbes & infections. 2018; 7(1):2.

38. Hongyan G, Chengjie M, Qiaozhi Y, Wenhao H, Juan L, Lin P, et al. Hand, foot and mouth disease caused by coxsackievirus A6, Beijing, 2013. The Pediatric infectious disease journal. 2014; 33 (12):1302–3. https://doi.org/10.1097/INF.0000000000000467 PMID: 25037037

39. Han JF, Xu S, Zhang Y, Zhu SY, Wu DL, Yang XD, et al. Hand, foot, and mouth disease outbreak caused by coxsackievirus A6, China, 2013. The Journal of infection. 2014; 69(3):303–5. https://doi.org/10.1016/j.jinf.2014.03.015 PMID: 24704297

40. Zhang C, Zhu R, Yang Y, Chi Y, Yin J, Tang X, et al. Phylogenetic analysis of the major causative agents of hand, foot and mouth disease in Suzhou City, Jiangsu province, China, in 2012–2013. Emerging microbes & infections. 2015; 4(2):e12.

41. Mirand A, le Sage FV, Pereira B, Cohen R, Levy C, Archimbaud C, et al. Ambulatory Pediatric Surveillance of Hand, Foot and Mouth Disease as Signal of an Outbreak of Coxsackievirus A6 Infections, France, 2014–2015. Emerging infectious diseases. 2016; 22(11):1884–93. https://doi.org/10.3201/eid2211.160590 PMID: 27767012

42. Xu M, Su L, Cao L, Zhong H, Dong N, Dong Z, et al. Genotypes of the Enterovirus Causing Hand Foot and Mouth Disease in Shanghai, China, 2012–2013. PloS one. 2015; 10(9):e0138514. https://doi.org/10.1371/journal.pone.0138514 PMID: 26398767

43. Huang Y, Zhou Y, Lu H, Yang H, Feng Q, Dai Y, et al. Characterization of severe hand, foot, and mouth disease in Shenzhen, China, 2009–2013. Journal of medical virology. 2015; 87(9):1471–9. https://doi.org/10.1002/jmv.24200 PMID: 25951788