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Original Article

Epidemiological Characteristics of Hand, Foot and Mouth Disease Reinfection in Guangzhou, Southern China from 2012 to 2017

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Abstract

Background: Hand, foot and mouth disease (HFMD) reinfection is common because of the limited cross-protection from infections of different enterovirus. We aimed to investigate the epidemiological characteristics and its influential factors of HFMD reinfection in Guangzhou, China.

Methods: Data on HFMD patients aged ≤5 yr from 2012 to 2017 were extracted from surveillance system. Influential factors of reinfection were assessed using the logistic regression model.

Results: Of 369,054 HFMD patients, 11,321 patients (3.07%) were classified as reinfection. The reinfection rate in male was higher than in female (χ^2=60.11, P<0.001). The reinfection rate in patients ≤1 yr was 3.86%, which showed a downward trend with age (Z=37.37, P_{trend}<0.001). The highest reinfection rate was observed in the scattered children (3.38%), followed by nursery care children and others (χ^2=514.75, P<0.001). Besides, higher risk of reinfection was detected among those who were male, lower age group, rural residence and other enteroviruses infection compared with their respective counterparts. Seasonality was illustrated according to the number of reinfections peaked from April to July. Time intervals curves revealed the number of reinfections gradually increased after 13 months from the initial infection.

Conclusion: Male ≤4 yr, living rural area, especially those lived scattered and infected with other enteroviruses were more likely to be reinfection.

Keywords: Hand foot and mouth disease; Enterovirus; Reinfection; Epidemiology

Introduction

Hand, foot and mouth disease (HFMD) is a common childhood illness caused by various enteroviruses. Generally, the disease is mild and self-limiting, with common symptoms including fever, painful sores in the mouth and a rash with blisters on hands, feet and buttocks. However, severe symptoms such as meningitis, encephalitis and polio-like paralysis may occur (1). The most

common causative pathogens are coxsackievirus A16 (CoxA16) and enterovirus 71 (EV71), with the latter accounting for the majority of severe and fatal HFMD (2). HFMD is widespread in the Asian-Pacific region, such as Japan, Singapore, Vietnam, Taiwan and Hong Kong (3). As one of the most affected countries, China reported 10,717,283 HFMD cases from May 2008 to June 2014, with 3,046 deaths and a fatality rate of 0.03% (4). Morbidity increased from 146.6/100,000 population in 2015 to 149.4/100,000 population in 2018 and peaked in 2016 at 178.2/100,000 population. The incidence of HFMD also varies geographically (5, 6), with most cases occurring in large, densely populated cities. The situation is more serious in Guangzhou, where the incidence was 4 times higher than the national average (7). Guangzhou is one of the top 3 cities in terms of the total number of HFMD cases reported (8). Thus, HFMD represents a growing public health threat to children and social development.

The first inactivated EV71 vaccine for preventing HFMD became commercially available in China in 2015 (9). Although the EV71 vaccine showed a >90% protective effect against EV71-related HFMD (10, 11), reinfection can occur because of the lack of cross-protection against other viral subtypes. From 2008 to 2015, 398,010 out of an estimated 820,000 HFMD patients were diagnosed as reinfection cases in China (12). The prevalence of HFMD reinfection in different regions has been estimated, with the reinfection rate ranging from 0.10% to 12.68% (13). For example, 8,960 patients (2.02%) were confirmed to have reinfection in Anhui and the annual reinfection rate increased each year from 2008 to 2013 (14). Furthermore, a cohort study reported the reinfection rate was 3.15% in Fujian Province; patients were mainly infected twice, with the cumulative number of up to five reinfections (15).

Nevertheless, few large-scale epidemiological studies have been carried out on HFMD reinfection in Guangzhou. Considering that children aged ≤5 yr represent a high-risk population of HFMD (16), we aimed to describe epidemiological characteristics for HFMD reinfection and examine the relationship between reinfection in patients aged ≤5 yr and influential factors.

Methods

Surveillance data

HFMD (mild/severe) is a collection of laboratory-confirmed cases (EV71/CoxA16/other enteroviruses) and clinically diagnosed cases (unknown enteroviruses) considered notifiable diseases in China. Once diagnosed, cases must be reported to the web-based China Information System for Disease Control and Prevention (CISDCP) within 24 h according to the guidelines for the prevention and control of HFMD (2009 edition) (17), published by the Chinese Ministry of Health. Daily count data for HFMD patients aged ≤5 yr covering from 2012–2017 were obtained from CISDCP. Patients' name, sex, age, date of birth, detailed address, group classification, case classification, date of onset, laboratory results, severity, parents' names and contact number were also collected from the system. Non-resident cases were excluded. In addition to passive surveillance of infectious diseases, the local Centers for Disease Control offices also conducted regular active surveillance to reduce misreporting and underreporting rates.

The Ethics Committee of Guangzhou Center for Disease Control and Prevention approved this study (Grant No.: 2017014).

Screening criteria

The following definitions were used in this study.

Single infection: only one infection between 2012 and 2017; multiple infections during this period but within 14 d after the first onset.

Reinfection: at least two infections during this period; the infections with the same patient's name, date of birth and contact information. If only one item is similar, then the infection can be verified by other items such as parents' names or address; the interval between the two infections was ≥15 d (18). The initial infection refers to the first infection of the reinfected patient; the sec-

ondary infection refers to the second infection of the reinfected patient.

Statistical analysis

The reinfection rate was calculated as follows:

$$\text{Reinfection rate} = \frac{\text{number of reinfections}}{\text{number of reported infections}} \times 100\%.$$

The median and interquartile range (IQR) were used for statistical description of measurement data, and count data were presented as rate and proportion. The chi-squared test or Fisher's exact test was used to compare differences between groups. The Cochran–Armitage trend test was applied to analyze the relationship between reinfection rate and age. Independent risk factors of HFMD reinfection were assessed using logistic regression analysis. Statistical analyses were performed using SAS V9.4. A two-tailed *P*-value <0.05 was considered as significant.

Results

General characteristics

In total, 369,054 HFMD patients aged ≤5 yr were included in analyses. Of these, 11,321 patients had repeatedly suffered from HFMD. The reinfection rate was 3.07%, with 10,934 patients infected twice (2.96%), 376 patients infected three times (0.10%), and 11 patients infected four times (0.003%). Nearly 5.91% (1,154/19,526) of patients have showed HFMD reinfection among laboratory-confirmed cases, and other enteroviruses was the highest reinfection prevalent (6.36%). No severe patients were identified among reinfection cases.

The reinfection rate was significantly higher in males (3.24%, Table 1) than in females (2.79%) (*P*<0.001). Reinfection rates were also age-specific. Patients aged <4 yr (96.54%) accounted for the majority of reinfection cases [median age: 2 yr (IQR=1,3)]. The reinfection rate of patients aged 1 year was the highest (3.86%) and declined with increasing age (P_{trend}<0.001). More reinfection cases (3.38%) occurred in scattered children than those in nursery care children (1.75%) and other (0.81%) children (*P*<0.001). The reinfection rate was higher in rural areas than urban and suburban areas (*P*<0.001). No significant difference in the case severity rate between reinfection and single infection patients was found (*P*>0.05).

Table 1: General characteristics of HFMD patients

Characteristics	*Total (N)*	*Reinfection*		*Single infection*		*χ²*	*P*
		n	%	n	%		
All cases	369,054	11,321	3.07	357,733	96.93	-	-
Sex						60.11	<0.001
Male	226,053	7,330	3.24	218,723	96.76		
Female	143,001	3,991	2.79	139,010	97.21		
Age (yr)						1442.33	<0.001
≤1	177,781	6,866	3.86	170,915	96.14	37.37[b]	< 0.001[&]
2	74,491	2,500	3.36	71,991	96.64		
3	65,459	1,563	2.39	63,896	97.6		

					1		
4	35,071	361	1.03	34,710	98.97		
5	16,252	31	0.19	16,221	99.81		
Group classification						514.75	<0.001
Scattered children	298,345	10,086	3.38	288,259	96.62		
Nursery care children	70,218	1,231	1.75	68,987	98.25		
School students/Others	491	4	0.81	487	99.19		
Region						146.029	<0.001
Rural	104974	3555	3.16	146604	96.64		
Suburbs	151704	5100	3.36	146604	96.84		
Urban	104974	2666	2.54	102308	97.46		
Clinical manifestation #							>0.05*
Mild	368,897	11,312	3.07	357,585	96.93		
Severe	28	0	0	28	100		
Case classification §						593.77	<0.001
Unknown	349,528	10,167	2.91	339,361	97.09		
EV71	3,741	168	4.49	3,573	95.51		
CoxA16	3,594	211	5.87	3,383	94.13		
Other enteroviruses	12,191	775	6.36	11,416	93.64		

Clinical manifestation was missing for 129 patients; & Cochran–Armitage trend test; * Fisher's exact test; § Of all patients, only 19,526 were laboratory-confirmed cases

Seasonal distributions and time interval

The highest HFMD reinfection rate was reported in 2013 (4.49%), whereas the lowest rate was reported in 2017 (0.20%, data not shown). As expected, the number of initial infections peaked from April to July each year, while a smaller autumnal peak (around September) was evident in 2012–2015 (Fig. 1). The lowest number of patients was seen in February. The seasonal distributions of initial, secondary and single infections were similar.

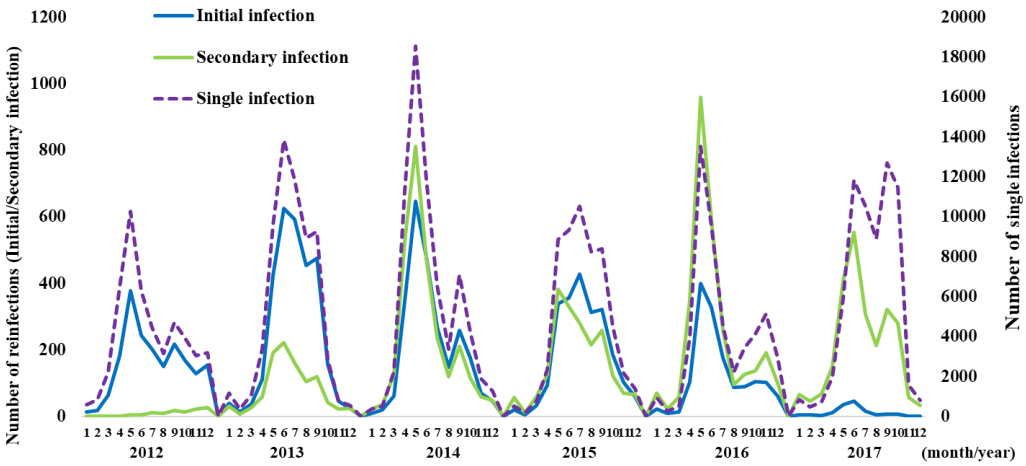

Fig. 1: Seasonal distributions of reinfections and single infections

The median time interval of patients infected twice was 13 months (IQR=10,21). In patients infected thrice, the median time intervals of the antecedent two infections and the latter two infections were 12 months (IQR=8,15) and 13 months (IQR=10,21), respectively. Likewise, in patients infected four times, the median time intervals between adjacent infections was 7 months (IQR=4,19), 7 months (IQR=4,17) and 12 months (IQR=2,16) in order. Reinfections were more likely to occur in the next epidemic season for all age groups. The first peak in the time interval between initial and secondary infections was 13 months, followed by 25 months and 37 months (Fig. 2). For the different age groups (1, 2, 3, 4, and 5 yr), the proportions of cases in which the time interval between the initial and secondary infections was within 2 yr were 78.56%, 84.52%, 91.36%, 100% and 100%, respectively.

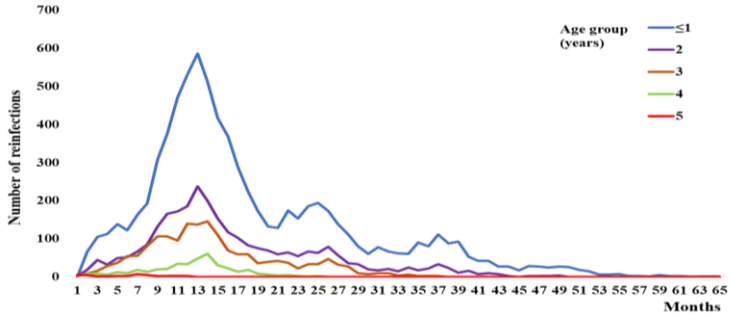

Fig. 2: Time intervals between initial infections and secondary infections in different age groups

Virus subtype of HFMD reinfection

Overall, 1,832 HFMD reinfection were classified as laboratory diagnosed, of which 1,154 patients had initial infection and 678 patients had secondary infection (Table 2). Laboratory results were available for both initial and secondary infections in 301 patients with reinfection. The median age was 2 yr for seven patients infected with EV71 twice, and the median time interval between infection and reinfection was 11 months. In addition, three patients were infected with CoxA16 twice. For patients infected with different enteroviruses between the initial infection and the secondary infection, statistically significant differences in virus types of HFMD reinfection infection were recognized (P<0.001).

Table 2: Comparison of enteroviruses responsible for HFMD reinfection

Initial infection	*Secondary infection*				χ^2	*P*
	Other enteroviruses	CoxA16	EV71	Total		
Other enteroviruses	112	47	49	208	20.90	<0.001
CoxA16	40	3	4	47		
EV71	35	4	7	46		
Total	187	54	60	301		

The proportion of patients diagnosed with EV71-associated HFMD as the secondary infection (24.34%; OR=1.37, 95%CI=1.07–1.75, Table 3) was higher than that in the single infection group (19.45%; OR=1.23, 95%CI=1.04–1.46) and the initial infection group (14.56%). However, there is no statistical significance between secondary infection and single infection.

Table 3: Risk analysis of HFMD causative pathogens in different infection groups

Infection groups	*Laboratory diagnosed*	*Virus subtypes*								
		EV71			CoxA16			Other enteroviruses		
		n (%)	OR (95%CI) §	*P*§	n (%)	OR (95%CI) §	*P*§	n (%)	OR (95%CI) §	*P*§
Reinfection			1.23 (1.04~1.46)	0.012 #		0.88 (0.69~1.13)	0.319 #		0.89 (0.73~1.09)	0.258 #
Initial	1154	168 (14.56)	1.37 (1.07~1.75)	0.016 &	211 (18.28)	0.91 (0.78~1.07)	0.250 &	775 (67.16)	0.94 (0.82~1.06)	0.303 &
Secondary	678	165 (24.34)	1.11 (0.93~1.33)	0.256 *	136 (20.06)	0.97 (0.80~1.17)	0.740 *	377 (55.60)	0.95 (0.81~1.12)	0.553 *
Single infection	18,372	3573 (19.45)	1.00		3,383 (18.41)	1.00		11,416 (62.14)	1.00	

Single infection group vs. Initial infection group; & Secondary infection group vs. Initial infection group; * Secondary infection group vs. Single infection group; § Adjusted for sex and age

Multivariate analysis of influential factors for reinfection

Multivariate analysis indicated that the reinfection risk was higher among males compared with females (OR=1.18, 95%CI=1.14–1.23, Table 4). The risk of reinfection was also 17.95-fold higher in patients aged 2 yr than those aged 5 yr (95%CI=12.57–25.64). Notably, the OR of reinfection in patients aged ≤1 year reached 20.65 (95%CI=14.46–29.48). In addition, the significant association of living area was observed (95%CI=1.14~1.27, 95%CI=1.15-1.27). Interestingly, patients with other enteroviruses had a higher risk of reinfection than those with EV71 infection HFMD (OR=1.21, 95%CI=1.02–1.44).

Table 4: Multivariate regression analysis of the relationship between influential factors and HFMD reinfection

Influential factors	*β*	*Wald*	*Adjusted OR (95%CI)*	*Adjusted P*
Sex				
Male	0.169	71.440	1.18 (1.14~1.23)	<0.001
Female			1.00	
Age (yr)				
≤1	3.028	277.679	20.65 (14.46~29.48)	<0.001
2	2.888	251.958	17.95 (12.57~25.64)	<0.001
3	2.538	195.053	12.65 (8.86~18.06)	<0.001
4	1.687	81.083	5.40 (3.74~7.80)	<0.001
5			1.00	
Group classification				
Scattered children	0.388	0.588	1.47 (0.55~3.97)	0.443
Nursery care children	0.379	0.559	1.46 (0.54~3.94)	0.455
Others/School students			1.00	
Region				
Rural	0.184	49.683	1.20 (1.14~1.27)	<0.001
Suburbs	0.189	59.593	1.21 (1.15~1.27)	<0.001
Urban			1.00	
Case classification				
Unknown	-0.552	47.668	0.58 (0.49~0.67)	<0.001
Other enteroviruses	0.194	4.900	1.21 (1.02~1.44)	0.027
CoxA16	0.246	5.319	1.28 (1.04~1.58)	0.021
EV71			1.00	

Discussion

We conducted a retrospective study to explore the epidemiological characteristics of HFMD reinfection and its associations with influential factors. The HFMD reinfection rate of patients aged ≤5 yr in Guangzhou from 2012 to 2017 was 3.07%. This finding was consistent with that reported in a study (3.17%) (19), and the rate in Guangzhou was higher than that in Wuhan (1.93%) (18) and lower than that in Wuxi (6.01%) (20). This difference in HFMD reinfection rates may be attributed to variation in geographical regions and time spans. A highly dense population is a risk factor for spread of HFMD (21), similar to dengue fever (22) and influenza (23). The population density in Guangzhou amounted to 1,950 people/square km in 2017, ranking in the top 10 most populated cities in China (24). Consequently, densely populated areas such as Guangzhou should be closely monitored for HFMD infection.

The results obtained from our study and another one were comparable (14), with a higher reinfection rate in males than in females. It is possible that males are more likely to be exposed to pathogens because of their high activity (25). Additionally, it may be related to children's immune constitution (26). However, the susceptibility of males aged ≤1 yr to reinfection remains to be further studied.

A systematic review has demonstrated that the antibody level against EV71 or CoxA16 dramatically increased among children aged 1–4 yr (27). The immune level to enterovirus would accordingly increase with increasing age (28). Our results also clarified that younger children have a greater likelihood of HFMD reinfection. It is necessary to remind younger children to maintain healthy habits, especially good hand washing practices (29).

The present study discovered that scattered children were more prone to HFMD reinfection. A similar finding has also been noticed in Huainan (30). These findings are somewhat contrary to the general belief that nursery care children have more contact with each other and should have a higher risk of reinfection (31). Possible reasons are that most scattered children are young and have poor self-care ability. For instance, because these children are accustomed to sucking their fingers, reinfection may readily occur through the fecal-oral route (32). Moreover, scattered children lack effective early detection methods, such as morning examination. The study also reported relatively higher reinfection risk in rural or suburbs areas compared with urban areas. This finding is somewhat consistent with the general belief that rural or suburbs areas have worse sanitary and should have higher risk.

Remarkably, findings of a seasonal pattern with the peak from Apr to Jul are consistent with other findings (33), which examined the effects of humidity on HFMD reinfection. Relative humidity is associated with elevated risks of HFMD reinfection (33). Adverse effects of relative humidity are generally long term. A humid environment has been reported to promote survival of HFMD-related virus (34). A relatively small number of reinfections were identified in February, which coincided with the school holiday season, reducing contact opportunities among susceptible children. These results agreed with those (35), who suggested the association between holidays and disease epidemics.

More importantly, the median time interval of HFMD reinfection was 13 months. Initially infected patients recovered from the infection at the peak of the epidemic, and then became infected with another virus in the next peak month, resulting in reinfection. Single infection included both patients initially infected before 2012 and patients reinfected after 2017. Therefore, the actual reinfection rate is likely higher than reported, which may also explain the seasonal distribution curve of the lower peak for secondary infection in 2012 and initial infection in 2017.

Laboratory results in patients whose pathogens were detected in two infections support the notion that limited cross-protection against different virus subtypes occurs after natural infection, which is in agreement with observations from the EV71 vaccine study (36) and a modeling study of natural infections (37). One reason underlying EV71 reinfection is that patients have been infected with the variant EV71 genogroup successively. At present, the EV71 genogroup reported in most regions of China is C4a/C4b (38), but there are also reports of EV71 B genogroup causing disease (39). Thus, the current EV71 vaccine may not provide cross-protection between genogroups, allowing for reinfection.

Interestingly, a relatively higher reinfection rate was observed for other enteroviruses compared with EV71. Recently, other enteroviruses have replaced EV71 and CoxA16 as predominant pathogens of HFMD in mainland China, although it is unclear if this is consistent throughout China (6). Additionally, other enteroviruses have been responsible for HFMD outbreaks in Europe (40, 41). These studies provided strong evidence of other enteroviruses as new and important causes of reinfection, thus highlighting the necessity of comprehensive surveillance of HMFD infections caused by other enteroviruses in addition to EV71 and CoxA16 in the future.

In this study, although patients were more susceptible to EV71 during single infection, patients were at greater risk of contracting EV71 during secondary infection than in initial infection if secondary infection occurred. Patients infected with EV71 have increased risk for severe HFMD reinfection (12). This indicates that severe risk of secondary infection may be higher for the reinfected case. However, few severe cases were observed, which requires further verification.

While we characterized HFMD infection over a longer period in this study, some limitations must be discussed. First, influential factors for HFMD include hygiene and social contacts, but we failed to collect this information completely. Second, the results are not representative enough to be extended to other regions.

Conclusion

The HMFD reinfection rate was 3.07% from 2012 to 2017 in Guangzhou. Sex, age and case classification have a significant effect on HFMD reinfection. Targeted health education programs and interventions must be developed to reduce the HFMD reinfection rate in susceptible populations.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors have no conflict of interest to disclose.

References

1. Guerra AM, Waseem M (2019). Hand Foot and Mouth Disease. In: *StatPearls*. edn. Treasure Island (FL).
2. Li XW, Ni X, Qian SY, et al (2018). Chinese guidelines for the diagnosis and treatment of hand, foot and mouth disease (2018 edition). *World J Pediatr*, 14(5):437-447.
3. Koh WM, Bogich T, Siegel K, et al (2016). The Epidemiology of Hand, Foot and Mouth Disease in Asia: A Systematic Review and Analysis. *Pediatr Infect Dis J*, 35(10):e285-300.
4. Liu SL, Pan H, Liu P, et al (2015). Comparative epidemiology and virology of fatal and nonfatal cases of hand, foot and mouth disease in mainland China from 2008 to 2014. *Rev Med Virol*, 25(2):115-128.
5. Deng T, Huang Y, Yu S, et al (2013). Spatial-temporal clusters and risk factors of hand, foot, and mouth disease at the district level in Guangdong Province, China. *PLoS One*, 8(2):e56943.
6. Wang J, Hu T, Sun D, et al (2017). Epidemiological characteristics of hand, foot, and mouth disease in Shandong, China, 2009-2016. *Sci Rep*, 7(1):8900.
7. Li T, Yang Z, Di B, et al (2014). Hand-foot-and-mouth disease and weather factors in Guangzhou, southern China. *Epidemiol Infect*, 142(8):1741-1750.
8. Xiao X, Gasparrini A, Huang J, et al (2017). The exposure-response relationship between temperature and childhood hand, foot and mouth disease: A multicity study from mainland China. *Environ Int*, 100:102-109.
9. Mao QY, Wang Y, Bian L, et al (2016). EV71 vaccine, a new tool to control outbreaks of hand, foot and mouth disease (HFMD). *Expert Rev Vaccines*, 15(5):599-606.
10. Zhu FC, Meng FY, Li JX, et al (2013). 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*, 381(9882):2024-2032.
11. Li R, Liu L, Mo Z, et al (2014). An inactivated enterovirus 71 vaccine in healthy children. *N Engl J Med*, 370(9):829-837.
12. Huang J, Liao Q, Ooi MH, et al (2018). Epidemiology of Recurrent Hand, Foot and Mouth Disease, China, 2008-2015. *Emerg Infect Dis*, 24(3):432-442.
13. Dang LX, Wang C, Bao WB, et al (2018). A Meta-analysis of recurrent infections prevalence of hand-foot-and-mouth disease among children in China. *Chin J Sch Health*, 39(3):411-414.

14. Chen GP, Wu JB, Wang JJ, et al (2016). Epidemiological characteristics and influential factors of hand, foot and mouth disease (HFMD) reinfection in children in Anhui province. *Epidemiol Infect*, 144(1):153-160.
15. Xie Z, Wang L, Yan Y, et al (2014). [A cohort study on the characteristics of the recurrent epidemics on hand, foot and mouth disease, in Fujian province]. *Zhonghua Liu Xing Bing Xue Za Zhi*, 35(10):1109-1114.
16. Xing W, Liao Q, Viboud C, et al (2014). Hand, foot, and mouth disease in China, 2008-12: an epidemiological study. *Lancet Infect Dis*, 14(4):308-318.
17. The Chinese Ministry of Health (2010). The Guidelines for The Prevention and Control of HFMD (2009 edition). *Chinese Practical Journal of Rural Doctor*, 16: 125-127.
18. Peng Y, Yu B, Kong DG, et al (2018). Reinfection hazard of hand-foot-mouth disease in Wuhan, China, using Cox-proportional hazard model. *Epidemiol Infect*, 146(10):1337-1342.
19. Xiao-Hong J, Qian LI (2017). Characteristic analysis of the repeated infection cases with hand-footand-mouth disease (HFMD) in Chaoyang District of Beijing City between 2008 and 2014. *Modern Preventive Medicine*.
20. Shi C, Liu J, Shi P, et al (2018). Epidemiological characteristics and influential factors of hand, foot, and mouth disease reinfection in Wuxi, China, 2008-2016. *BMC Infect Dis*, 18(1):472.
21. Zhou ZM, Xu Y, Hu CS, et al (2017). Epidemiological Features of Hand, Foot and Mouth Disease during the Period of 2008-14 in Wenzhou, China. *J Trop Pediatr*, 63(3):182-188.
22. Zhu G, Xiao J, Zhang B, et al (2018). The spatiotemporal transmission of dengue and its driving mechanism: A case study on the 2014 dengue outbreak in Guangdong, China. *Sci Total Environ*, 622-623:252-259.
23. Xu B, Tian H, Sabel CE, et al (2019). Impacts of Road Traffic Network and Socioeconomic Factors on the Diffusion of 2009 Pandemic Influenza A (H1N1) in Mainland China. *Int J Environ Res Public Health*, 16(7):1223.
24. Land area and population density in various districts of Guangzhou (2017). http://www.gzstats.gov.cn/gzstats/gzsq/201701/1036f733bf244636b9b62af6f9a504f1.shtml
25. Xie YH, Chongsuvivatwong V, Tan Y, et al (2015). Important roles of public playgrounds in the transmission of hand, foot, and mouth disease. *Epidemiol Infect*, 143(7):1432-1441.
26. Tian H, Zhang Y, Sun Q, et al (2014). Prevalence of multiple enteroviruses associated with hand, foot, and mouth disease in Shijiazhuang City, Hebei province, China: outbreaks of coxsackieviruses a10 and b3. *PLoS One*, 9(1):e84233.
27. Luo L, Xing W, Liao Q, et al (2015). [Research progress on seroepidemiological study of enterovirus 71 and coxsackievirus A16 infection among children]. *Zhonghua Yu Fang Yi Xue Za Zhi*, 49(2):184-188.
28. Zhang D, Chen Y, Chen X, et al (2017). Enterovirus 71 Neutralizing Antibodies Seroepidemiological Research among Children in Guangzhou, China between 2014 and 2015: A Cross-Sectional Study. *Int J Environ Res Public Health*, 14(3):319.
29. Zhang D, Li Z, Zhang W, et al (2016). Hand-Washing: The Main Strategy for Avoiding Hand, Foot and Mouth Disease. *Int J Environ Res Public Health*, 13(6):610.
30. Zhao D, Wang L, Cheng J, et al (2017). Impact of weather factors on hand, foot and mouth disease, and its role in short-term incidence trend forecast in Huainan City, Anhui Province. *Int J Biometeorol*, 61(3):453-461.
31. Zhang Q, Zhou M, Yang Y, et al (2019). Short-term effects of extreme meteorological factors on childhood hand, foot, and mouth disease reinfection in Hefei, China: A distributed lag non-linear analysis. *Sci Total Environ*, 653:839-848.
32. Solomon T, Lewthwaite P, Perera D, et al (2010). Virology, epidemiology, pathogenesis, and control of enterovirus 71. *Lancet Infect Dis*, 10(11):778-790.
33. Yang Y, You E, Wu J, et al (2018). Effects of relative humidity on childhood hand, foot, and mouth disease reinfection in Hefei, China. *Sci Total Environ*, 630:820-826.
34. Cheng Q, Bai L, Zhang Y, et al (2018). Ambient temperature, humidity and hand, foot, and mouth disease: A systematic review and meta-analysis. *Sci Total Environ*, 625:828-836.
35. Eames KT (2014). The influence of school holiday timing on epidemic impact. *Epidemiol Infect*, 142(9):1963-1971.
36. Li JX, Song YF, Wang L, et al (2016). Two-year

efficacy and immunogenicity of Sinovac Enterovirus 71 vaccine against hand, foot and mouth disease in children. *Expert Rev Vaccines*, 15(1):129-137.

37. Takahashi S, Liao Q, Van Boeckel TP, et al (2016). Hand, Foot, and Mouth Disease in China: Modeling Epidemic Dynamics of Enterovirus Serotypes and Implications for Vaccination. *PLoS Med*, 13(2):e1001958.
38. Zhang Y, Tan X, Cui A, et al (2013). Complete genome analysis of the C4 subgenotype strains of enterovirus 71: predominant recombination C4 viruses persistently circulating in China for 14 years. *PLoS One*, 8(2):e56341.
39. Huang ML, Chiang PS, Chia MY, et al (2013). Cross-reactive neutralizing antibody responses to enterovirus 71 infections in young children: implications for vaccine development. *PLoS Negl Trop Dis*, 7(2):e2067.
40. Blomqvist S, Klemola P, Kaijalainen S, et al (2010). Co-circulation of coxsackieviruses A6 and A10 in hand, foot and mouth disease outbreak in Finland. *J Clin Virol*, 48(1):49-54.
41. Mirand A, Henquell C, Archimbaud C, et al (2012). Outbreak of hand, foot and mouth disease/herpangina associated with coxsackievirus A6 and A10 infections in 2010, France: a large citywide, prospective observational study. *Clin Microbiol Infect*, 18(5):E110-118.