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Case-fatality of hand, foot and mouth disease associated with EV71: a systematic review and meta-analysis

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SUMMARY

Hand, foot and mouth disease (HFMD) associated with enterovirus 71 (EV71) is a growing public health concern. This study aimed to estimate the case-fatality of HFMD associated with EV71 on the basis of a meta-analysis. We searched PubMed, Cochrane, Web of Science, Elsevier, CNKI, Wanfang, and VIP databases. Two authors independently selected relevant studies. The pooled estimate of case-fatality was calculated using a random-effects model. Potential sources of heterogeneity were explored using subgroup analysis, sensitivity analysis and meta-regression. We identified 14 eligible studies with a total population of 112 546. The random-effects pooled case-fatality was 1·7% (95% confidence interval 1·2–2·4). The funnel plot was asymmetrical. The estimate of case-fatality was highest in mainland China (1·8%). Removal of eight local Chinese studies decreased the original estimate. The pooled case-fatality in the period of 1998–2007 (1·5%) was lower than that in the period 2008–2012 (1·8%). Control measures for HFMD associated with EV71 are essential because of the increased case-fatality over time, especially in East Asia.

Key words: Case-fatality, enterovirus 71, HFMD, meta-analysis.

INTRODUCTION

Hand, foot and mouth disease (HFMD) is a common infectious disease caused by a group of enteroviruses, such as enterovirus 9 (EV9), enterovirus 71 (EV71), coxsackie A2 (CA2), coxsackie A4 (CA4), coxsackie A5 (CA5), coxsackie A6 (CA6), coxsackie A10 (CA10), and coxsackie A16 (CA16) [1–4]. However, EV71 is one of the most clinically important viruses of the *Enterovirus* genus owing to its potential to cause death and disability as a result of severe central nervous system disorders, including meningitis, encephalitis, and acute flaccid paralysis similar to that found in paralytic poliomyelitis [5]. Manifestations of EV71 infection can range from fever, oral ulcerations, and benign skin lesions to severe neurological complications and respiratory and cardiac conditions [6]. EV71 can infect people of all ages, but it more commonly and severely infects children aged $\leqslant 5$ years because older individuals are more likely to be exposed and develop natural immunity [7].

EV71 infection is a growing public health concern, especially in Asia. EV71 was first isolated from the

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stool of an infant suffering from encephalitis in California in 1969 [8]. However, it was not until 1997 that the incidence of EV71 infection increased significantly in South East Asia. Since 2000, EV71 had been implicated in 13 outbreaks in several regions throughout the world [5], such as Japan [9, 10], Australia [11], Malaysia [12, 13] and Taiwan [2]. In 2008, China experienced an outbreak of EV71, prompting authorities to go on national alert. HFMD outbreaks primarily due to EV71 infection occurred recently in China in the years 2007 [14], 2008 [15, 16] and 2009 [16], with increasingly higher incidences each subsequent year. At present, national surveillance systems for HFMD/enteroviruses have been established in some Asian countries.

However, considering the number of HFMD cases and the average population in these countries, the actual incidence is quite low. The focus should be on the estimation of the case-fatality of HFMD caused by EV71. Therefore, the purpose of this study was to assess the case-fatality on the basis of a systematic review and meta-analysis.

METHODS

Search strategy and selection criteria

A literature search was performed using the databases from PubMed, Cochrane, Web of Science, Elsevier, CNKI (China Knowledge Resource Integrated Database), Wanfang (Wanfang Database), VIP (Weipu Database), which were searched on 14 March 2014 with no language or age restrictions. Search terms included '(Enterovirus OR EV OR EV71 OR HFMD OR 'Hand, foot and mouth') AND (fatal OR mortality OR death)' in the abstract/title/keywords sections. The bibliography of the original studies, reviews, and relevant conferences were manually searched.

The inclusion criteria were: (1) surveillance for EV71 or HFMD with information on the period and region of investigation; (2) number of reported cases and deaths of laboratory-confirmed HFMD caused by EV71 on the basis of population studies (diagnosis of laboratory-confirmed HFMD cases followed the guidance of the World Health Organization for the clinical management of HFMD [6]); (3) laboratory-confirmed HFMD cases caused by EV71 reported by a province or country. Furthermore, only one recent or detailed study was chosen for duplicate published studies.

Data extraction and quality assessment

Two researchers (Y.Z. and H.J.) independently evaluated the studies and extracted data. The extracted data included the period and region of investigation, number of cases and deaths by HFMD caused by EV71, causes of death, the proportion of laboratory-confirmed HFMD associated with EV71 cases aged <5 years and <3 years (reported cases were used), the proportion of scattered children (i.e. children living at home who were educated by parents or grandparents, and did not attend nurseries, kindergartens or other educational institutions [17]; reported cases were used), male to female ratio (reported cases were used), virus subtypes of deaths.

Quality was independently assessed by two investigators for each study (Y.Z. and H.J.) according to the STROBE statement [18]. A STROBE-based checklist including six criteria to estimate a summary risk of bias was used to assess the risk of bias in these studies [19]. A consensus was determined with the help of a third author (B.W.), if necessary.

Statistical analysis

Case-fatality per year (%) of the studies was calculated on the basis of a natural logarithm transformation. Subsequently, forest plot was used to indicate the point estimation of case-fatality and 95% confidence interval (CI). We estimated heterogeneity between studies using Cochran's Q and the I^2 statistic. A random-effects model was used for summary statistics because of the higher heterogeneity ($I^2 > 75\%$) [20]. Funnel plot, Egger's test and Begg's test were used to assess publication bias.

Subgroup analysis was performed according to period and region of investigation, whether children were scattered or unknown, and risk of bias. Sensitivity analysis was performed to evaluate the impact of the included studies. Univariate analysis was conducted considering several factors, including period and region of investigation, the proportion of children aged <5 years and <3 years, male:female ratio, the proportion of scattered children, and virus subtypes of deaths. Potential sources of heterogeneity were further investigated using meta-regression analysis, among which the included variables were statistically significant ($P < 0{\cdot}05$) by univariate analysis.

A normality test was conducted using R i386 3.1.0 software [21] and other analyses were performed using Stata software (version 11.0) [22].

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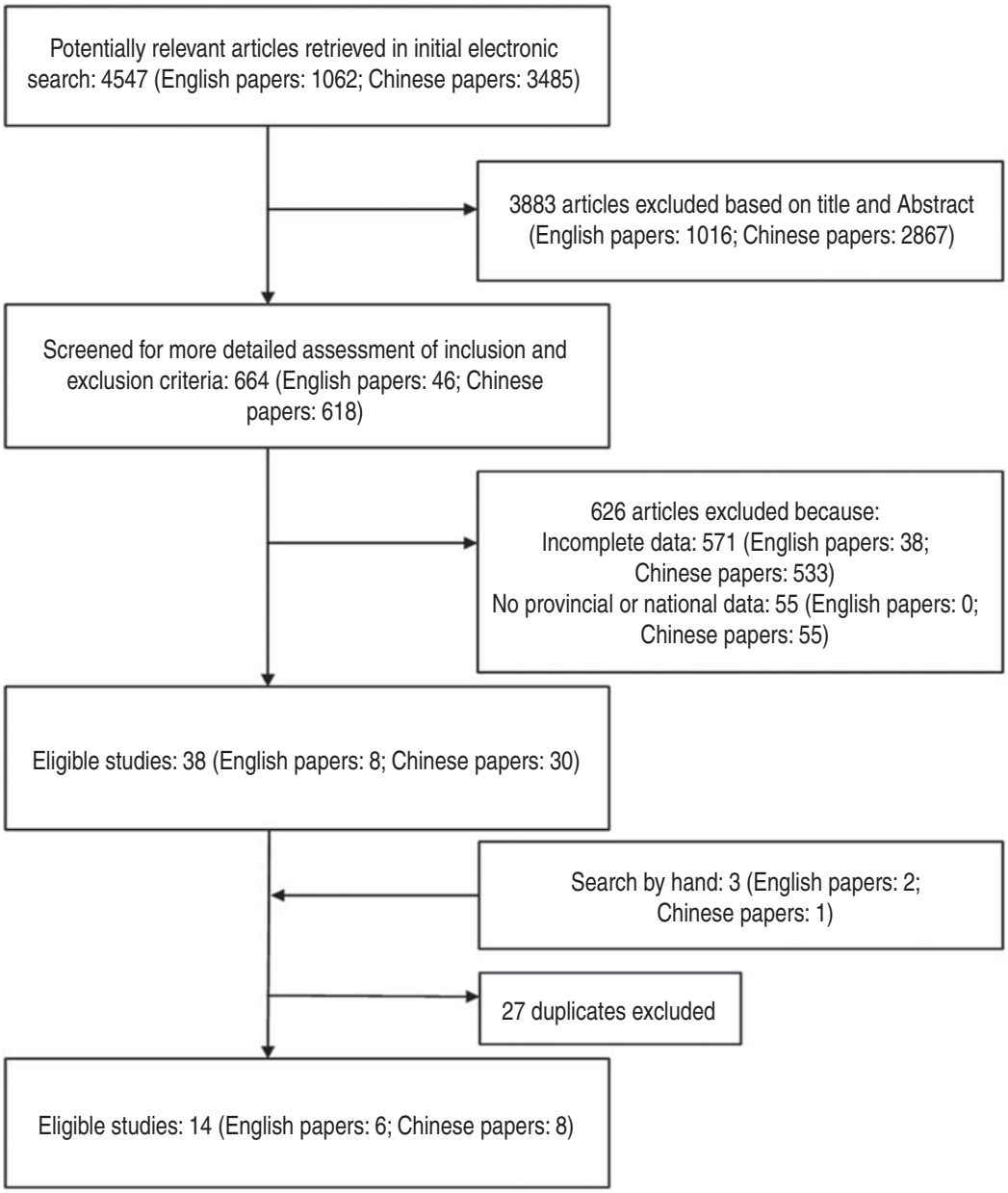

Fig. 1. Flow chart of the included study. Studies were classified as ineligible and eligible on the basis of inclusion criteria. Citation details of the eligible studies are provided in Table 1.

RESULTS

A total of 4547 titles and abstracts were screened and 664 full articles were retrieved. Fourteen eligible studies ($n = 112\,546$) published from 2007 to 2014 were included in the analysis (Fig. 1). These studies reported case-fatality of hand, foot and mouth disease associated with EV71 in East Asia or South East Asia. Of the 14 studies, three studies were in Singapore, Southern Vietnam and South Korea, while the remaining studies were conducted in China: ten in mainland China and one in Hong Kong (Table 1). Of the 14 included studies, five had a moderate risk of bias, five has a high risk of bias, and four had a very high risk of bias using previously described criteria. The main risk of bias in these studies was confounding bias (Table 2).

Pooled random-effects estimate and publication bias

The estimates of case-fatality varied between 0·4% and 7·7% in different regions (Fig. 2). The heterogeneity observed was substantial ($P < 0{\cdot}001$). The pooled

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Table 1. *Characteristics of the included studies on the case-fatality of hand, foot and mouth disease associated with EV71*

Reference	Region	Year	Deaths/*N*	Case-fatality (%)	Proportion (%)				
					<5 years	<3 years	Scattered children	Male/female	Subtype
Tu (2007) [23]	Southern Vietnam	2005	3/173	1·73	n.a.	78·61	n.a.	n.a.	C5
Ang (2009) [24]	Singapore	2001	1/81	1·23	n.a.	n.a.	n.a.	n.a.	n.a.
Ma (2010) [25]	Hong Kong, China	1998–2007	2/185	1·08	80·40	n.a.	n.a.	1·60:1	C4
Ma (2010) [25]	Hong Kong, China	2008	1/89	1·12	72·40	n.a.	n.a.	1·10:1	C4
Ryu (2010) [26]	South Korea	2009	2/168	1·19	n.a.	n.a.	n.a.	1·70:1	C4a
Mo (2011) [27]	Guangxi, China	2010	133/1728	7·70	94·19	82·04	73·05	1·63:1	n.a.
Sun (2011) [28]	Guangdong, China	2010	54/2967	1·82	n.a.	81·04	73·96	n.a.	n.a.
Wang (2011) [16]	Mainland China	2008	82/4668	1·76	n.a.	59·20	n.a.	1·74:1	n.a.
Wang (2011) [16]	Mainland China	2009	205/11 511	1·78	n.a.	62·10	n.a.	1·69:1	n.a.
Ji (2012) [29]	Jiangsu, China	2010	17/4164	0·41	93·37	73·60	61·64	1·64:1	n.a.
Liu (2012) [30]	Hebei, China	2010	58/2172	2·67	88·68	64·47	78·67	1·67:1	n.a.
Cao (2013) [31]	Mainland, China	2011	299/33 503	0·89	91·17	66·42	73·01	1·69:1	n.a.
Cao (2013) [31]	Mainland, China	2012	408/49 875	0·82	91·17	66·42	73·01	1·69:1	n.a.
Li (2013) [32]	Guizhou, China	2010	14/291	4·81	89·00	59·00	n.a.	1·70:1	n.a.
Mou (2014) [33]	Shenzhen, China	2010	6/112	5·36	88·80	61·60	n.a.	1·67:1	C4a
Yao (2014) [34]	Guizhou, China	Jan.–Jun. 2013	6/685	0·88	87·71	n.a.	n.a.	1·77:1	n.a.
Yu (2014) [35]	Gansu, China	2010	3/174	1·72	87·59	62·29	56·55	1·69:1	C4a

n.a., Data were not searched; EV71, enterovirus 71.

Table 2. *Quality assessment of included studies*

Reference	Methods for selecting study participants	Methods for measuring exposure and outcome variables	Methods to control confounding	Design-specific sources of bias and comparability in groups	Statistical methods	Declaration of conflict	Summary risk of bias
Tu (2007) [23]	Yes	Yes	Partially	Yes	Yes	n.a.	Moderate
Ang (2009) [24]	Yes	Yes	No	Yes	Yes	n.a.	High
Ma (2010) [25]	Yes	Yes	No	Yes	Yes	No	Moderate
Ryu (2010) [26]	Yes	Yes	Partially	Yes	Yes	n.a.	Moderate
Mo (2011) [27]	Partially	NA	No	Partially	Yes	n.a.	Very high
Sun (2011) [28]	Partially	NA	No	Partially	Yes	n.a.	Very high
Wang (2011) [16]	Partially	NA	No	Partially	Yes	n.a.	Very high
Ji (2012) [29]	Partially	NA	Partially	Partially	Yes	n.a.	High
Liu (2012) [30]	Partially	Yes	No	Partially	Yes	n.a.	High
Cao (2013) [31]	Partially	NA	Partially	Partially	Yes	n.a.	High
Li (2013) [32]	Partially	NA	No	No	Yes	n.a.	Very high
Mou (2014) [33]	Partially	Yes	Partially	Partially	Yes	No	Moderate
Yao (2014) [34]	Partially	Yes	No	Partially	Yes	n.a.	High
Yu (2014) [35]	Partially	Yes	Partially	Partially	Yes	n.a.	Moderate

n.a., Result was not searched.

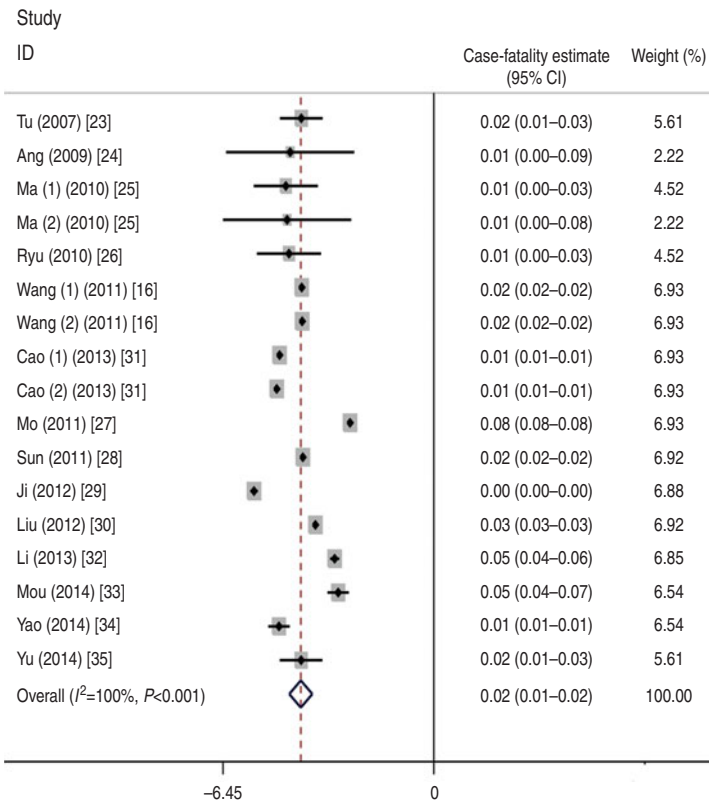

Fig. 2. Forest plots of the estimated case-fatality of hand, foot and mouth disease caused by enterovirus 71. X label values and graphic size were transformed by natural logarithm except the numbers of case-fatality estimate and weight. (1) and (2) indicate the different period of investigation in the same region reported by one author in ascending order. CI, Confidence interval.

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Table 3. *Subgroup and sensitivity analysis of case-fatality of hand, foot mouth disease associated with EV71*

Variable	Effect size*	95% CI	I^2 (%)	Cochran's Q	P value
Subgroup analysis					
All studies	1·7	1·2–2·4	100·0	∞	
Region					<0·001†
Mainland China	1·8	1·2–2·7	100·0	∞	
Hong Kong	1·1	0·5–2·6	0	0	
Other region	1·5	0·9–2·6	0	0·44	
Period of investigation					0·001†
1998–2007	1·5	0·9–2·5	0	0·65	
2008–2013	1·8	1·2–2·6	100·0	∞	
Scattered children					<0·001‡
Yes	1·5	0·9–2·7	100·0	86043·52	
Unknown	2·2	1·9–2·5	96·5	258·37	
Risk of bias					<0·001†
Moderate	1·9	0·9–3·7	80·8	26·04	
High	0·9	0·8–1·1	99·9	5010·51	
Very high	2·9	1·4–6·2	100·0	28330·07	
Sensitivity analysis					
Removal of eight local Chinese studies	1·3	0·9–1·7	100·0	23211·94	0·001§
Removal of two smaller studies (<100 participants)	1·7	1·2–2·5	100·0	∞	<0·001§

CI, Confidence interval; EV71, enterovirus 71.
* Effect size, case-fatality per year (%); ∞, Cochran's Q ($\chi 2$ value) was too high ($\chi 2 = 1{\cdot}0 \times 10^5$).
† P value was calculated on the basis of the univariate analysis.
‡ P value was calculated on the basis of the proportion of scattered children rather than their classification.
§ P value was calculated on the basis of the analysis of heterogeneity.

random-effects model estimate a case-fatality of 1·7% (95% CI 1·2–2·4). Although Egger's test and Begg's test did not suggest the presence of publication bias ($P > 0{\cdot}05$), the funnel plot was asymmetrical.

Considering the high heterogeneity of the studies included, subgroup analysis, sensitivity analysis and meta-regression analysis were used to explore the source of heterogeneity.

Subgroup analysis

Table 3 shows the estimated case-fatality was highest in mainland China, corresponding to 1·8%, followed by 1·5% in other regions and 1·1% in Hong Kong. The pooled case-fatality in the period 1998–2007 (1·5%) was lower than that in 2008–2012 (1·8%), subgroup analysis of the quality assessment demonstrated that articles with high bias had the lowest case-fatality.

Sensitivity analysis

The pooled estimates had similar results as the original analysis after the removal of any one study (data not shown), and the estimated case-fatality changed from 1·5% to 1·9%. Removal of eight local Chinese studies (conducted in mainland China, Hong Kong, Southern Vietnam, South Korea and Singapore) caused the original estimate to decrease from 1·7% to 1·3% (Table 3). Case-fatality of mainland China in 2010 was 2·5% (95% CI 1·3–5·1) using seven local Chinese studies.

Meta-regression analysis

The univariate analysis showed that the period of investigation ($P = 0{\cdot}001$), and geographical region ($P < 0{\cdot}001$) were significantly associated with case-fatality. Including these two factors in multiple variable models were not the covariates that significantly affected case-fatality.

DISCUSSION

Our systematic review and meta-analysis of HFMD associated with EV71 indicated a case-fatality of 1·7% (95% CI 1·2–2·4) according to the pooled random-effects model, and case-fatality in the included studies varied between 0·4% and 7·7%. In the subgroup analysis, the highest case-fatality associated with EV71 occurred in mainland China (1·8%), which

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was 60 times higher than the case-fatality of all HFMD (0·03%) from an epidemiological study in China between 2008 and 2012 [36]. Five of the Chinese studies reported a case-fatality of >1·8%. However, the case-fatality decreased to 1·3% after the removal of eight local Chinese studies (Table 3). In fact, outbreaks of HFMD mainly due to EV71 infection occurred in China in the years 2007 [14], 2008 [15, 16] and 2009 [16]. Therefore, the implementation of a national surveillance system for HFMD in China is essential because of its increasingly high number of cases and deaths. Furthermore, experience from cases that occurred in China should also prompt the authorities overseas to go on national alert.

We explored some potential sources of heterogeneity. First, the period and region of investigation may affect case-fatality, although the P value did not indicate the significant connection in multiple variable meta-regression. The high epidemics in mainland China may be primarily related to the virus variability, overpopulation, etc. Undeniably, case-fatality was affected by the fact that HFMD has been a reportable disease in China since 2 May 2008 [37]. The painful experience with the SARS outbreak in 2003 promoted Chinese professionals to timely and openly release information on HFMD caused by EV71, which may explain the high estimate of case-fatality reported in 2008–2012. In addition, the pooled case-fatality of mainland China in 2010 was highest in 2008–2012, but the representativeness of the data used to calculate case-fatality for mainland China in 2010 should be further evaluated.

Moreover, virus subtypes of deaths ($P = 0{\cdot}06$), the proportion of scattered children ($P = 0{\cdot}09$), and the proportion of children aged <3 years ($P = 0{\cdot}11$) might also affect case-fatality. However, the data on subtypes was incomplete in the included studies and more information on subtypes should be publicly reported. The results of Wu *et al.* [38] indicat that scattered children could not be an independent risk factor for HFMD. In fact, scattered children play at home and in the neighbourhood and might lack the systematic healthcare education given at school. These children could come in contact with a variety of people including patients with HFMD caused by EV71 [39, 40]. Cai *et al* [41] suggested that scattered children are a risk factor for severe HFMD. Additionally, kindergarten children also play a role in the transmission of EV71 [42]. Chang *et al.* [43] found that preschool children playing at home or in kindergarten were the major sources of disease transmission during the widespread EV71 epidemic in 1998 in Taiwan. It should be noted that, owing to the limitation of the study design, the presence of scattered children was closely associated with the age of subjects, which were risk factors of severe HFMD [44, 45]. Therefore, further studies on the relationship between scattered children and case-fatality of HFMD caused by EV71 are essential.

The meta-analysis has some limitations. First, most of the included studies were conducted in China, and lacked the representativeness of cases reported overseas or in Asia. Data were lacking for regions or countries with a low economy and poor sanitary conditions, such as Cambodia and Maynmar. Higher rates of underlying malnutrition, concurrent infectious diseases, and limited access to healthcare may lead to higher case-fatality of HFMD associated with EV71. Regarding this aspect, the point estimate of case-fatality might be underestimated throughout the world or in Asia. Second, ascertainment of HFMD associated with EV71 is likely to remain incomplete. Only some of the collected samples were detected in China, except for severe cases and cases resulting in death, which were diagnosed on an individual basis [37]. However, this method might decrease the total number of HMFD cases associated with EV71 and overestimate case-fatality. On some occasions, the absolute number of HFMD cases associated with EV71 was missing, as reported by Ho *et al.* [2]. In addition, the meta-analysis used observational data and did not provide a weighted case-fatality or an accurate assessment of the relationship between risk factors and case-fatality. The comparison of the case-fatality for HFMD caused by EV71 and the case-fatality for HFMD caused by other enteroviruses is necessary for subjects having the same age and gender. Moreover, the potential differences between sporadic cases and outbreaks of HFMD are also important in estimating the case-fatality of HFMD associated with EV71.

Owing to the growing public health concern regarding HFMD in many countries, an international surveillance system is urgently needed to assess the disease burden of HFMD associated with EV71. For example, The Asia-Pacific Economic Cooperation (APEC) platform might be used to establish the Asian working group [46]. In addition, a direct and effective measure, such as a novel vaccine, is needed to control and prevent the occurrence of HFMD associated with EV71. To date, some research groups have evaluated five EV71 vaccine candidates in clinical trials [47]. Three research groups from

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China have completed phase 3 trials to assess the efficacy of EV71 vaccines [48–50].

In conclusion, to reduce the disease burden of HFMD associated with EV71, an international surveillance network should be established to evaluate the distinct regions affected by the disease; further, vaccines against EV71 should be developed to control HFMD caused by EV71.

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DECLARATION OF INTEREST

None.

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