Basic reproduction number of coxsackievirus type A6 and A16 and enterovirus 71: estimates from outbreaks of hand, foot and mouth disease in Singapore, a tropical city-state

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SUMMARY

Coxsackievirus A6 (CV-A6), coxsackievirus A16 (CV-A16) and enterovirus 71 (EV-A71) were the major enteroviruses causing nationwide hand, foot and mouth disease (HFMD) epidemics in Singapore in the last decade. We estimated the basic reproduction number (R_0) of these enteroviruses to obtain a better understanding of their transmission dynamics. We merged records of cases from HFMD outbreaks reported between 2007 and 2012 with laboratory results from virological surveillance. R_0 was estimated based on the cumulative number of reported cases in the initial growth phase of each outbreak associated with the particular enterovirus type. A total of 33 HFMD outbreaks were selected based on the inclusion criteria specified for our study, of which five were associated with CV-A6, 13 with CV-A16, and 15 with EV-A71. The median R_0 was estimated to be 5·04 [interquartile range (IQR) 3·57–5·16] for CV-A6, 2·42 (IQR 1·85–3·36) for CV-A16, and 3·50 (IQR 2·36–4·53) for EV-A71. R_0 was not significantly associated with number of infected children ($P = 0.86$), number of exposed children ($P = 0.94$), and duration of the outbreak ($P = 0.05$). These enterovirus-specific R_0 estimates will be helpful in providing insights into the potential growth of future HFMD epidemics and outbreaks for timely implementation of disease control measures, together with disease dynamics such as severity of the cases.

Key words: Enterovirus, mathematical modelling.

INTRODUCTION

Hand, foot and mouth disease (HFMD) is a common childhood viral infection, characterized by a brief febrile illness, vesicular rashes on the hands and feet and mouth ulcers [1]. The disease is caused by numerous members of the *Enterovirus* genus of the family Picornaviridae, but the most common aetiological agents are coxsackievirus A16 (CV-A16) and enterovirus type 71 (EV-A71) [1, 2].

Epidemiological studies of outbreaks in Europe, Southeast Asia and North America showed that coxsackieviruses usually cause self-limiting infections [3–5]. By contrast, the clinical spectrum of EV-A71 infection ranges from asymptomatic infection to severe cases with neurological and cardiopulmonary complications [6–8]. In the past decade, a number of HFMD epidemics associated with EV-A71 have been reported in a number of Asian countries and regions, including China, Hong Kong, Singapore, Taiwan and Vietnam [9–14]. Severe cases of HFMD and deaths have been reported in these epidemics. In Singapore, fatal cases of HFMD associated with EV-A71 were reported in 2000, 2001 and 2008 [14, 15]. Additionally, there have been seven severe cases of HFMD reported

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since 2006, of which six tested positive for EV-A71 with the remaining case positive for enterovirus.

The basic reproductive number (R_0) measures the infectiousness of a pathogen in a given population. R_0 is generally defined as the average number of secondary cases infected by a typical infected person in a population that is entirely susceptible. R_0 has been estimated for many infectious diseases, including severe acute respiratory syndrome (SARS), avian influenza A(H5N1), the recent influenza A(H1N1) pdm09 pandemic and dengue [16–19]. However, there have been a limited number of publications on the estimation of R_0 of enteroviruses related to HFMD outbreaks. There was a study on the estimation of R_0 of EV-A71 and CV-A16 in HFMD outbreaks in Hong Kong between 2004 and 2009 [20].

HFMD is endemic in Singapore, with >50% of cases occurring in children aged <5 years [21]. Although the predominant circulating enteroviruses change periodically, CV-A6, CV-A16 and EV-A71 were the major enterovirus types causing nationwide HFMD epidemics in recent years [14, 21–23]. The aim of our study was to estimate R_0 of CV-A6, CV-A16 and EV-A71 in order to obtain a better understanding of the transmission dynamics of HFMD outbreaks in Singapore.

MATERIALS AND METHODS

In Singapore HFMD has been a legally notifiable disease under the Infectious Diseases Act since 1 October 2000 [24]. Medical practitioners are required to notify all cases of HFMD to the Ministry of Health (MoH). Educational institutions, including childcare centres, are also required to notify the MoH of any HFMD outbreaks in their institutions. An outbreak is defined as ⩾2 cases of HFMD with onset of illness occurring within 10 days in the same institution for investigation and management by the MoH [21]. We reviewed the records of all HFMD cases and outbreaks reported to the MoH between 2007 and 2012.

Virological surveillance involves random collection of stool samples, throat and rectal swabs and swabs from vesicular fluid and oral ulcers from outpatients and inpatients of HFMD cases at two public acute-care hospitals, KK Women's and Children's Hospital, and National University Hospital, and outpatients at sentinel general practitioner clinics. About 70% of all hospitalized patients aged ⩽16 years were admitted into these two hospitals during the study period. Samples were also collected from HFMD cases

notified by educational institutions after obtaining parental consent.

Each educational institution was assumed to be a closed system with the source of an outbreak of HFMD originating from a single infected child. As the transmission dynamics in primary schools attended by children aged between 6 and 12 years were different from those of childcare centres and kindergartens attended by children aged between 18 months and 6 years, we confined our study to HFMD outbreaks in childcare centres (18 months to 6 years) and kindergartens (4–6 years). We also assumed that all children were susceptible to infection at the beginning of an outbreak, and infected children would take sick leave after becoming symptomatic.

We merged records of notified cases from HFMD outbreaks and laboratory results from virological surveillance based on unique personal identifiers. If a case from an HFMD outbreak tested positive for a particular enterovirus type, we assumed that all reported cases from that outbreak were associated with the same enterovirus type. We included only outbreaks associated with CV-A6, CV-A16 and EV-A71 in our study. There were no outbreaks with two or all three enterovirus types under study detected. For outbreaks with other enterovirus types detected in addition to CV-A6, CV-A16 or EV-A71, we assumed that the outbreak was primarily associated with the enterovirus type included in our study. The personal identifiers were removed from the final dataset prior to statistical analysis, in order to ensure confidentiality of the notified cases.

We adopted the method used in the study carried out in Hong Kong [20], which estimated R_0 based on the cumulative number of cases at initial growth phase of each HFMD outbreak as determined by its epidemic curve. The initial growth phase refers to the period from the earliest date of onset of symptoms to the date when the number of newly infected cases had peaked or plateaued. The reproductive number was estimated using the following formula:

$$\sum N_{t \cdot i} = 1 + R_0 + R_0^2 + R_0^3 + \cdots + R_0^t$$
$$= \frac{(R_0^{t+1} - 1)}{(R_0 - 1)},$$

where $N_{t \cdot i}$ is the predicted number of incident cases on day $t \cdot i$ and t is the number of days of the initial growth phase of outbreak i divided by the incubation period. The incubation period of HFMD was estimated to be between 3 and 5 days, with the longest duration at 7 days [25, 26]. For the purpose of our analyses, we
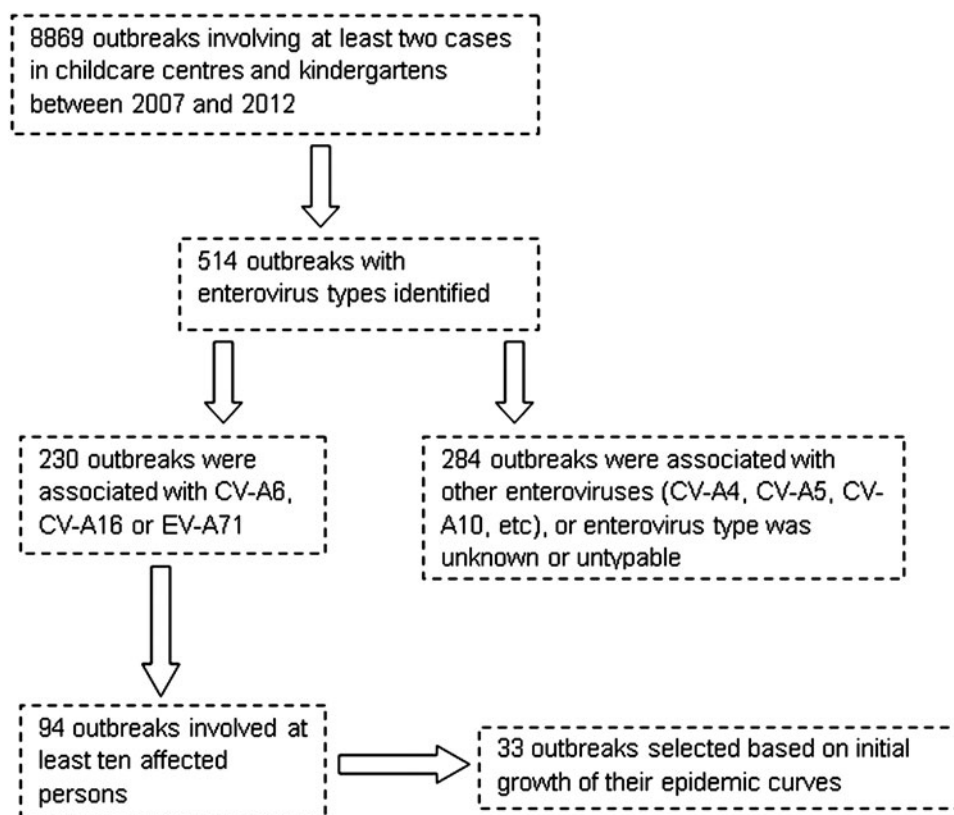
Fig. 1. Flow chart on the selection of hand, foot and mouth disease outbreaks for estimation of R_0 for CV-A6, CV-A16 and EV-A71.

assumed the incubation period to be 5 days, which was the same as that of the study in Hong Kong [20].

Sensitivity analyses were conducted to investigate the impact on the estimation of R_0 based on the selection criteria. We examined whether there was an association between the estimated R_0 of the three enterovirus types and number of children exposed, the number of children infected as well as duration of the selected outbreaks using scatterplots. We checked for statistical difference between the estimated R_0 of the three enterovirus types using the Kruskal–Wallis test. We also investigated the distribution of the estimated R_0 when the incubation period was varied from 3 to 7 days using boxplots. All analyses were carried out using PASW Statistics software, version 18.0 (pasw-statistics.software.informer.com) and statistical software R, version 3.1.0 (https://cran.r-project.org/bin/windows/base/old/3.1.0/).

RESULTS

A total of 8869 HFMD outbreaks involving at least two cases in childcare centres and kindergartens were reported between 2007 and 2012. After merging

notified cases from HFMD outbreaks and laboratory results from virological surveillance, enterovirus types were identified for 5·8% of the outbreaks in the 6-year period (Fig. 1).

The distribution of these 514 HFMD outbreaks with enterovirus types identified is shown in Figure 2. With the exception of 2011, the enterovirus types that were more commonly identified in HFMD outbreaks in each year were the same as those associated with peaks in HFMD incidence at the national level, which were CV-A16 in 2007, EV-A71 in 2008, CV-A16 and CV-A6 in 2009, and CV-A6 in 2010–2012 [27].

Based on the inclusion criteria specified for our study, 33 outbreaks (0·4%) were eventually selected (Fig. 1). Of these outbreaks, 15 (45·5%) were associated with EV-A71, 13 (39·4%) with CV-A16 and the remaining five (15·2%) with CV-A6. All these outbreaks occurred in childcare centres except one in a kindergarten.

The characteristics of the 33 HFMD outbreaks are summarized in Table 1. The median number of children exposed in these outbreaks was 84 [interquartile range (IQR) 70–108]. The median attack rate was 19·8% (IQR 13·7–23·0) and the median duration was
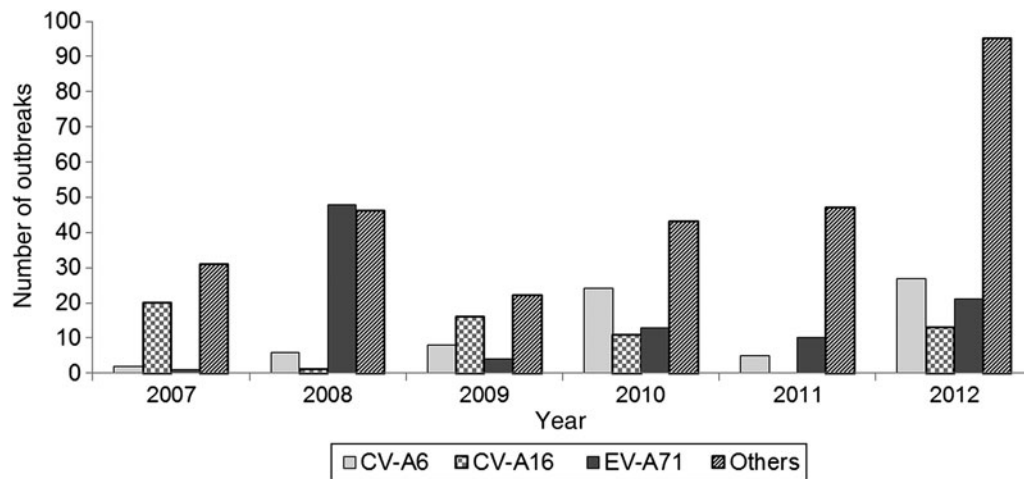
Fig. 2. Annual number of hand, foot and mouth disease outbreaks identified as being associated with CV-A6, CV-A16, EV-A71 and other enterovirus types, 2007–2012.

Table 1. *Characteristics of selected HFMD outbreaks associated with CV-A6, CV-A16 and EV-A71, 2007–2012*

Characteristic	CV-A6	CV-A16	EV-A71	Overall*
Number of outbreaks	5	13	15	33
Median number of children exposed	133 (72–138)	87 (74–99)	77 (63–100)	84 (70–108)
Median number of children infected	17 (15–29)	16 (13–19)	16 (12–17)	16 (13–18)
Median attack rate in each outbreak	21·8% (18·1–23·0)	18·9% (15·5–22·6)	19·6% (12·5–22·5)	19·8% (13·7–23·0)
Median duration of outbreak, days	21 (18–22)	17 (11–19)	15 (12–18)	16 (12–20)

HFMD, Hand, foot and mouth disease.
Values in parentheses are interquartile range.
* Refers to all the three enterovirus types, CV-A6, CV-A16 and EV-A71.

16 days (IQR 12–20). The median attack rate was similar for all three enterovirus types. The duration of outbreaks for CV-A16 and EV-A71 were similar, while that of CV-A6 was higher. However, the difference was not statistically significant ($P = 0·45$).

The median R_0 was 5·04 (IQR 3·57–5·16) for CV-A6, 3·50 (IQR 2·36–4·52) for EV-A71 and 2·42 (IQR 1·85–3·36) for CV-A16 (Table 2).

From the sensitivity analyses, R_0 was not significantly associated with number of children exposed ($P = 0·94$), number of children infected ($P = 0·86$), or duration of the selected outbreaks ($P = 0·05$) (Fig. 3). On the other hand, R_0 of all three enterovirus types increased corresponding to longer incubation period (Fig. 4).

DISCUSSION

In our study, the median R_0 of CV-A6 was highest at 5·04, while that of CV-A16 was lowest at 2·42. The median R_0 of EV-A71 was in between the estimates of CV-A16 and CV-A6 at 3·50. The median R_0 of these

three enterovirus types corresponded to the relative peaks in HFMD incidence at the national level. In the past decade, the HFMD epidemic with the highest incidence of 698·8/100 000 population in 2012 was associated with CV-A6 [23]. The second largest epidemic with incidence of 613·4/100 000 population in 2008 was associated with EV-A71 [21]. By contrast, HFMD epidemics associated with CV-A16 as the dominant circulating enterovirus were of smaller magnitude.

Based on the estimates of the median R_0 from our study, enterovirus infections of CV-A6, CV-A16 and EV-A71 were considered to be less infectious compared to other childhood infectious diseases such as measles ($R_0 \sim 15$) and chickenpox (R_0 between 10 and 12 for varicella) [28].

In a national paediatric seroprevalence survey (NPSS) conducted by the MoH between August 2008 and July 2010, the prevalence of EV-A71-specific neutralizing antibody in children in the 1–6 years age group was 14·3% [95% confidence interval (CI) 11·2–18·0] [21]. In a separate seroprevalence study using a subset of

Table 2. *Quantiles, 95th percentiles and range of estimated R_0 of CV-A6, CV-A16 and EV-A71 in selected HFMD outbreaks, 2007–2012*

Virus type	No. of outbreaks	Minimum	25th percentile	Median	75th percentile	95th percentile	Maximum
CV-A6	5	2·06	3·57	5·04	5·16	10·24	10·24
CV-A16	13	1·64	1·85	2·42	3·36	7·17	7·17
EV-A71	15	1·54	2·36	3·50	4·52	6·02	5·48

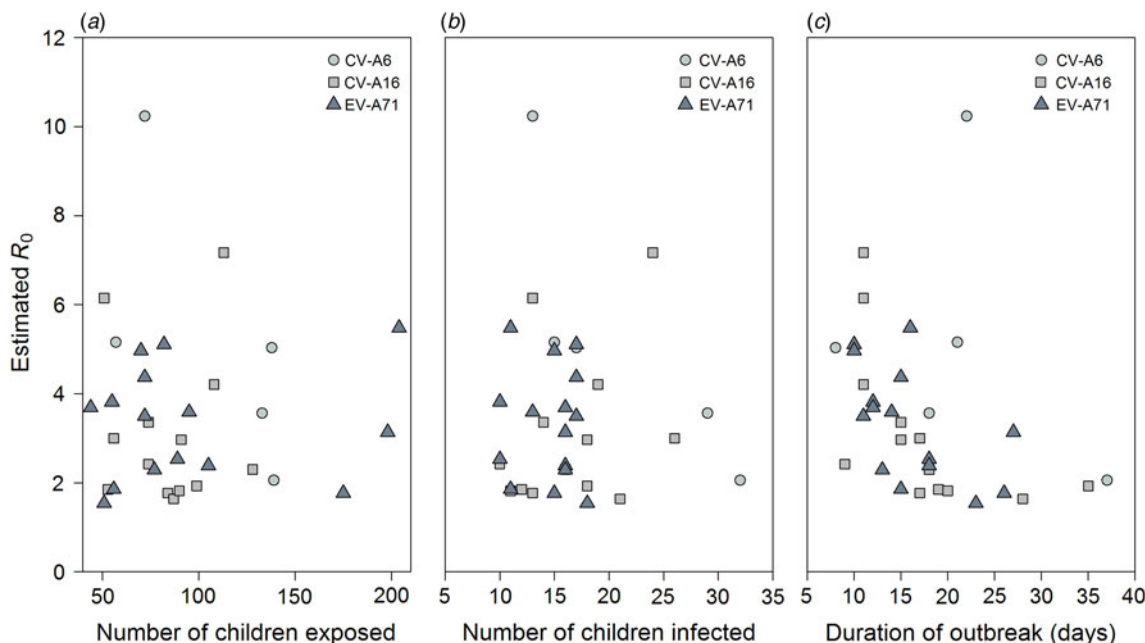HFMD, Hand, foot and mouth disease.

Fig. 3. Scatterplot of estimated R_0 by enterovirus type against (*a*) number of children exposed, (*b*) number of children infected, and (*c*) duration of outbreaks.

residual samples from the aforementioned study, the prevalence of CV-A6- and CV-A16-specific neutralizing antibody in children in the 1–6 years age group was 51·9% (95% CI 42·4–61·3) and 50·0% (95% CI 40·6–59·4), respectively [27]. The effective reproduction number, R, estimates the average number of secondary cases per infectious case in a population made up of both susceptible and non-susceptible hosts. Using results from the NPSS 2008–2010, R, could be computed from the product of R_0 and the proportion of population which is susceptible, which was estimated to be 2·42 for CV-A6, 1·21 for CV-A16 and 3·00 for EV-A71.

Compared to the median R_0 of EV-A71 at 5·48 (IQR 4·20–6·51) in Hong Kong [20], the estimate in our study was lower. The median R_0 of CV-A16 was 2·50 (IQR 1·96–3·67) in Hong Kong [20], which was comparable to that of our study. The median R_0 of EV-A71 was higher than that of CV-A16 in both our study and

the study in Hong Kong. These observations reflect the different epidemiology of these enteroviruses in Singapore and Hong Kong during the study periods. To the best of our knowledge, no studies have been published on the estimation of the R_0 of CV-A6.

A caveat in the interpretation of our findings was the validity of the assumptions that we based our analyses on. We assumed a closed compartment within an institution with only one index case of HFMD at the initial phase of an outbreak, which might not hold. Children could be infected by their family members instead of their fellow schoolmates from childcare centres or kindergartens. The violation of these two assumptions may result in different estimates of R_0 of the three enterovirus types.

It has been reported that a large proportion of HFMD cases are asymptomatic, with the asymptomatic rate ranging between 29% and 73% [29–31]. The high proportion of asymptomatic HFMD cases serves as a
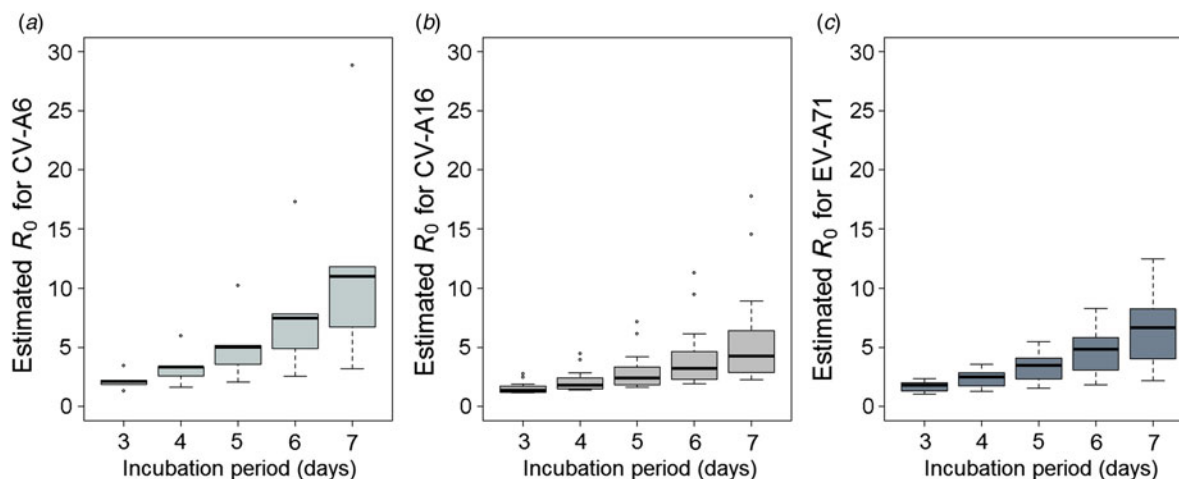
Fig. 4. Boxplots of estimated R_0 by incubation period for (*a*) CV-A6, (*b*) CV-A16 and (*c*) EV-A71.

latent reservoir of enterovirus transmission, and this could affect the estimation of R_0.

Another limitation of our study was the small number of outbreaks included in the analyses, especially for CV-A6. After confining outbreaks to those with at least ten infected children and selection of outbreaks based on the initial growth phase of the epidemic curve, there were only 33 outbreaks left for our analyses. While the peaks in HFMD incidence in 2010–2012 were associated with CV-A6, there were only at most two outbreaks associated with CV-A6 selected for our study for these three years. Increasing the number of outbreaks would provide greater precision of the estimates of R_0.

The effect of control measures on the estimation of R_0 was not considered in our study. Public health measures implemented after the notification of an HFMD outbreak to the MoH would reduce the number of cases and hence R_0 could be lower. In Singapore, the MoH routinely carries out measures year-round to control the spread of HFMD, such as field investigations in educational institutions with active HFMD clusters, and publishes the names of childcare centres and kindergartens with active HFMD clusters of prolonged transmission, and those which have been assessed for breaching specific triggers and are required to close mandatorily for a period of 10 days to break the chain of disease transmission [32]. In addition, the MoH conducts random spot checks on childcare centres, kindergartens and private enrichment centres. When there is an increasing trend in the number of notifications of HFMD, educational institutions are reminded to maintain vigilance including screening children for signs of HFMD

and ensuring high levels of personal and environmental hygiene. We observed that the median number of children infected with HFMD and the median attack rate in the 33 outbreaks included in our study were lower than the triggers required for mandatory closure of affected educational institutions. In addition, the peak of the epidemic curve took place no later than 14 days after the notification of the first case of HFMD for the majority of the 33 outbreaks (97·0%). This was also shorter than the transmission period of >24 days required for mandatory closure of affected educational institutions. Hence, the extent of underestimation of R_0 would most likely be small for our study.

CONCLUSION

The median R_0 of CV-A6, CV-A16 and EV-A71 corresponded to the relative peaks in HFMD incidence associated with these enteroviruses at the national level. These enterovirus-specific R_0 estimates would be helpful in providing insights into the potential growth of future HFMD epidemics and outbreaks for timely implementation of disease control measures, together with disease dynamics such as severity of the cases.

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DECLARATION OF INTEREST

None.

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