Epidemiological characteristics of hand-foot-and-mouth disease in China, 2008-2012

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Summary

Background Hand–foot–and–mouth disease (HFMD) is a common childhood illness caused by enteroviruses. Increasingly it imposes a substantial disease burden throughout East and Southeast Asia. To better inform vaccine and other interventions, we characterized the epidemiology of HFMD in China based on enhanced surveillance.

Methods We extracted epidemiological, clinical and laboratory data from reported HFMD cases during 2008–2012 and compiled climatic, geographic and demographic information. All analyses were stratified by age, disease severity, laboratory confirmation status and enterovirus subtype.

Findings The surveillance registry captured 7,200,092 probable HFMD cases (annualized incidence, 1·2 per 1,000), of whom 3·7% were laboratory–confirmed and 0·03% died. Incidence and mortality were highest in children aged 12–23 months (in 2012: 38·2 cases per 1,000 and 1·5 death per 100,000). Median durations from onset to diagnosis and death were 1·5 days and 3·5 days respectively. The risk of cardiopulmonary or neurological complications was 1·1% and the severe-case fatality risk was 3·0%, with >90% of deaths associated with enterovirus 71. HFMD peaked annually in June in the North, whereas Southern China experienced semi-annual outbreaks in May and September/October. Geographic differences in seasonal patterns were weakly associated with climate and demographic factors (variance

explained 8-23% and 3–19%, respectively).

Interpretation This is the largest population-based study to date of the epidemiology of HFMD. Future mitigation policies should take full account of the heterogeneities of disease burden identified. Additional epidemiologic and serologic studies are warranted to elucidate local HFMD dynamics and immunity patterns and optimize interventions.

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Key words Hand, Foot and Mouth Disease; Enterovirus 71; Coxsackie virus A16; Epidemiology; Disease burden; Seasonality

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Introduction

Hand-foot-and-mouth disease (HFMD) is a common infectious condition caused by a variety of enteroviruses, with enterovirus 71 (EV-A71) and Coxsackie virus A16 (CA-V16) being the most commonly reported.[1] Children under five years are particularly prone to HFMD, and most patients show self-limiting illness typically including fever, skin eruptions on hands and feet, and vesicles in the mouth. However, a small proportion has been known to rapidly develop neurological and systemic complications that can be fatal, particularly in cases associated with EV-A71.[2]

EV-A71 was first identified in 1969 in California, USA[3] and has become more predominant across the Asia–Pacific region in the past 15 years.[4–11]

China established a national enhanced surveillance system for HFMD in May 2008 partly in response to several large HFMD outbreaks during 2007 and early 2008.[9]

Here we describe the enhanced HFMD surveillance system and characterize the epidemiology of the disease in China, focusing on age, seasonal, and geographic patterns from 2008 to 2012.

Methods

Enhanced national surveillance program

Beginning on January 1, 2008, all probable and laboratory–confirmed HFMD cases were reported on a voluntary basis to the Chinese Centre for Disease Control and

Prevention (China CDC) in Beijing. On May 2, 2008, HFMD was made statutorily notifiable.

Case definitions

A probable HFMD case was defined as a patient with papular or vesicular rash on hands, feet, mouth, or buttocks, with or without fever. A confirmed case was defined as a probable case with laboratory evidence of enterovirus infection (including EV-A71, coxsackievirus A16 (CA-V16), or other non-EV-A71 and non-CA-V16 enteroviruses) detected by reverse-transcriptase polymerase chain reaction (RT-PCR), real-time RT-PCR, or virus isolation.

HFMD patients, whether probable or confirmed, were classified as severe if they experienced any neurological complications (aseptic meningitis, encephalitis, encephalomyelitis, acute flaccid paralysis, or autonomic nervous system dysregulation) and/or cardiopulmonary complications (pulmonary oedema, pulmonary haemorrhage, or cardiorespiratory failure); otherwise, they were categorized as mild cases.

Collection and testing of specimens

Given limited laboratory capacity during the first year of enhanced surveillance (from May 2008 through June 2009), clinical samples were collected from the first five mild, probable cases who visited hospital outpatient departments each week in each of 31

Chinese provinces (Appendix Figure 1). We also attempted to collect specimens from all severe cases. With enhanced capacity after June 2009, samples were collected from the first five mild, probable cases who visited hospital outpatient departments each month in each of the 3,074 counties or districts and from all severe cases (Appendix Figure 1).[12] Depending on the symptoms and clinical status of each case, the appropriate clinical specimens, including throat swab, rectal swab, fecal sample, vesicular fluid, and/or cerebrospinal fluid, were collected. Sampling was systematic and did not follow community outbreaks.

Specimens were placed in sterile viral transport medium and sent to provincial– or prefecture–level CDCs (n=425) for PCR or virus isolation, according to standardized protocols disseminated by the national CDC.[13] Viral ribonucleic acid (RNA) was extracted using available commercial kits (most frequently, QIAamp Viral RNA Mini Kit, Qiagen, Valencia, CA, USA, and Geneaid Viral RNA Mini Kit, Geneaid Biotech, Taiwan China) as per the manufacturer's protocols. RNA from each sample was tested for specific primers and probes that targeted pan-enterovirus, EV-A71, and CA-V16. Testing was carried out in biosafety level 2 facilities. Test results were classified into four categories: enterovirus negative, EV-A71 positive, CA-V16 positive, or positive for another enterovirus without further serotype identification.

A small number of samples underwent virus isolation at provincial–level CDCs. According to national guidelines,[12] at least ten enterovirus strains should be identified

in each province every month. Specimens were inoculated into human rhabdomyosarcoma (susceptible to EV-A71 and CA-V16) and Hep-2 (susceptible to other enteroviruses) cells. If cultures showed cytopathic effect (CPE), serotype identification was obtained by sequencing analysis[14]. A blind passage was done with all cultures showing no CPE after 7 days.

Clinical and epidemiologic data

Probable and confirmed case were reported on-line to the national CDC within 24 hours of diagnosis, using a standardized form including basic demographic information (sex, date of birth, and address), case classification (probable or confirmed), severity (mild or severe), death status, date of symptoms onset, date of diagnosis, and date of death (if applicable), and virus type (EV-A71, CA-V16, other enterovirus) for confirmed cases.

Climate, geographic, and demographic data

To analyze the potential drivers of HFMD seasonality, we collected demographic, economic, geographic, and climatic information for all provinces from 2008 to 2012, including: 1) age–specific population denominators, population density, Gross Domestic Product, and Gross Regional Product;[15] 2) latitude and longitude of province capitals; 3) air, rail, road, and boat passenger data;[16] 4) meteorological data

including daily temperature, rainfall, relative humidity, air pressure, average vapor pressure, and hours of sunshine (Appendix Text 1 for details).[17] Climate variables were aggregated by season (spring: April–June; autumn: September–November), to reflect the seasonal occurrence of HFMD peaks in surveillance data. The 31 provinces were further grouped into six climatic regions (Appendix Figure 2A).

Data analysis

Disease burden and severity of disease

We included all cases with illness onset from January 1, 2008 through December 31, 2012 in the analysis. We estimated age–specific rates of incidence, severe illness, and mortality by combining probable and confirmed cases. 95% confidence intervals (CIs) for rates were estimated with Poisson methods. For rates with denominator >100,000 and numerator ≥20, we calculated 95% CIs assuming a normal distribution. We assessed the geographic distribution of cases across all 31 provinces, grouped into seven regions as shown in Appendix Figure 2B. We estimated the age specific case–severity risk (no. of severe cases/no. of probable and confirmed cases), case–fatality risk (no. of deaths/no. of probable and confirmed cases), and case–fatality risk of severe HFMD cases (no. of deaths/no. of severe cases), overall and stratified by serotype. In serotype-specific analyses, we estimated the number of serotype-specific HFMD cases by applying the distribution of serotypes among

specimens positive for enteroviruses and further serotyped to the universe of probable and confirmed cases.

We calculated the distributions of times from illness onset to diagnosis, illness onset to death, and diagnosis to death by virus serotype. To identify predictors for severe or fatal outcomes, we applied logistic regression and backward selection of demographic and geographic variables, time from onset to diagnosis, and virus type (p-value for exclusion≥0·10), separately for laboratory–confirmed cases and probable cases. All analyses were carried out using SAS version 9.1 (SAS Institute, Cary, USA).

Geographic patterns and seasonality

To quantify HFMD seasonal patterns by province, we created heatmaps of the proportion of HMFD cases identified in each week of the year.[18] We also fitted seasonal multiple linear regression models to weekly HFMD time series, including time trends and harmonic terms representing annual and semi-annual periodic cycles (Appendix Text 2).[18,19] We extracted the amplitude and peak timing of the annual and semi-annual cycles based on model coefficients.[18] The amplitude measures the difference between the maximum and minimum of a seasonal curve.[19]

We used multivariate stepwise linear regression to study the association between HFMD seasonal characteristics and putative seasonal drivers, including geography, human mobility patterns, demographic, economic, and climate variables.[18]

Epidemiologically relevant HFMD regions were identified by hierarchical clustering

using Ward's minimum variance method, and predictors of these regions were identified through discriminant analysis.[18] Time series were standardized for differences in sampling intensity over time and geography by dividing weekly case counts by the annual number of cases.

Results

Incidence description

7,200,092 probable HFMD cases were reported to the China CDC surveillance system during 2008–2012, of which 3·7% were laboratory–confirmed and 0·03% died. The incidence of reported HFMD cases showed a sharp increase since the initiation of surveillance in 2008 (Table 1), due to improvements in reporting and surveillance. Reported disease rates reached a steady state at over 1·2 cases per 1,000 person–years during 2010-2012.

HFMD incidence varied greatly with age, with highest rates in children aged six months to two years (Table 1). The median age of reported cases was 27 months (interquartile range = 18–43 months). Most cases occurred in children below the age of five, and incidence was very low in young infants aged <6 months, older children, and adults. The incidence of HFMD was 1·6 times higher in boys under 5 years than in girls of the same age ($p<0·001$).

EV-A71 predominated among laboratory-confirmed cases, accounting for 45%, 80%,

and 93% of mild, severe, and fatal cases respectively (Figure 1). There was little variation in EV-A71 predominance by age (Figure 2A). EV-A71, CA-V16, and other enteroviruses co-circulated throughout the observation period in all regions (Appendix Figure 3).

Clinical course

The median time from illness onset to diagnosis, illness onset to death, and diagnosis to death were 1·5 days (interquartile range: 0·5–2·5 days), 3·5 days (interquartile range: 2·5–4·5 days), and 0·5 day (interquartile range: 0·5–1·5 days) respectively. The probability density distributions of the onset-to-diagnosis, onset-to-death, and diagnosis-to-death intervals were similar between probable and laboratory–confirmed cases of EV-A71 or other enteroviruses (Figure 3). However, CA-V-16 density distributions showed attenuated signal-to-noise ratio given the small number of deaths (n=25).

Severity of disease

Overall the case–fatality, case–severity, and severe case–fatality risks were 0·03% (range across years = 0·03–0·05%), 1·1% (range = 0·2–1·6%), and 3·0% (range = 2·6–10·4%) respectively. Illness severity and death were inversely related to age (Table 2 and Figure 2B–2D). Consistently across all age groups, EV-A71 infections

were much more severe than CA-V16 infections (Figure 2B–2D). We performed sensitivity analyses on severity estimates by age and viral etiology limited to 2010-2012 (Appendix Figure 4) and only 2012 (Appendix Figure 5), and found results were similar. Multivariate backward logistic regression of laboratory confirmed cases identified several mortality risk factors beyond young age and infection with EV-A71, including rural residence and long onset-to-diagnosis interval. Further, males and rural residents experienced higher risk of severe disease. Including probable cases in the analysis yielded similar risk predictors (Table 2).

Seasonal characteristics

National and province-specific patterns

At the national scale, HFMD showed semi-annual peaks of activity, including a major peak in spring and early summer followed by a smaller peak in autumn, consistent between probable and confirmed time series (Figure 4).

While Southern China experienced two outbreaks of HFMD peaking in May and October of each year, Northern China experienced a single annual peak in June (Figure 5-6). The annual amplitude of HFMD epidemics increased with increasing latitude and semi-annual periodicity was strongest in the South (Figure 5).

Seasonal characteristics by serotype

All enteroviruses exhibited latitudinal gradients in the amplitude of annual and

semi-annual epidemics (Appendix Figures 6–8). Annual peak timing of CA-V16 activity occurred earlier than for other enteroviruses (p<0·001).

Predictors of HFMD seasonality

Putative predictors of HFMD seasonal characteristics were identified through multivariate stepwise regression (Appendix Table 1). We found that the annual amplitude of epidemics was associated with spring rainfall and spring sunshine in overall and serotype–stratified analyses (p<0·05, partial R2: 5%–23%; p<0·05, 8%–23%, respectively). HFMD peak timing was associated with maximum spring air pressure in overall and serotype–stratified analyses (p<0·05, partial R2: 4%–12%). Predictors of semi-annual periodicity of HFMD epidemics were more varied. Population and transportation factors were moderately associated with HFMD seasonal characteristics, explaining 1–19% of the variance in epidemic timing and periodicity. Overall, multivariate models explained a moderate-to-high fraction of the variance in HFMD seasonal characteristics through multiple factors (40–92%).

Epidemiological regions

We identified two main geographic regions that share similar HFMD epidemiological characteristics: a northern region (latitude range 35·5°–46·2°N, plus Tibet, n=14) and a southern region (19·5°–34·8°N, n=17) (Figure 7). Serotype–specific analysis revealed a broadly similar split between Northern and Southern China, with a third epidemiological region including Western provinces identified for EV-A71 and

CA-V16 (Appendix Figures 9–11). Discriminant analysis confirmed that climate factors were the dominant predictors of HFMD epidemiological regions (Figure 7C, Appendix Figures 9–11).

Discussion

Our study relies on a large sample of more than 7·2 million HFMD cases reported to the national enhanced surveillance system during 2008–2012 in China and gives the first comprehensive account of the national burden and epidemiology of the disease (panel). The estimated HFMD incidence is 1·2 per 1,000 person–years in China and the disease is responsible for 350–900 reported deaths annually, predominantly among young children. As in other countries, we observed a predominance of EV-A71 serotype among severe cases, with a particularly young median age at infection of 2·3 years. Our large study covers 31 climatologically diverse provinces and suggests that while HFMD cases tend to occur in warmer months of the year throughout the country, peak timing and periodicity of epidemics varies with latitude. All serotypes causing HFMD appear to follow broadly similar geographic gradients, which are moderately associated with climate differences.

The observed age profile of infection is in agreement with reports from other countries,[4,20–22] and the particularly young mean age at infection suggests that enteroviruses causing HFMD are highly transmissible. Relative sparing of infants

younger than six months was most likely due to protection by maternal antibodies.[23,24] Infection with the causative viruses appears to confer lifelong immunity at least to disease and possibly infection given the virtual absence of adult cases.

Our large-scale analysis of HFMD seasonal patterns identified two main epidemiological regions corresponding to Northern China, where HFMD peaks in summer, and Southern China, which experiences two HFMD peaks in spring and autumn. A highly transmissible, strongly immunizing disease requires constant replenishment of susceptibles to sustain semi-annual, even annual, epidemics. Experience from Asia–Pacific countries has shown biennial[25] or triennial[20,21] outbreaks against a background total fertility rate of 1·3–3 births per woman,[26] compared to China's 1·6 births per woman under the longstanding one–child policy.[27] In contrast, annual epidemics were reported in Japan and Malaysia,[6,25] consistent with observed patterns in Northern China, while semi-annual epidemics were reported in Hong Kong SAR, South Taiwan of China, and Vietnam,[8,10,21], in line with our Southern China data. Further, our data indicate that EV-A71 epidemics occur annually in China, in contrast to Japan and Malaysia where this serotype circulates every three or four years.[6,25] HFMD periodicity may be complicated by interference between the causative enterovirus serotypes, perhaps associated with cross–serotype immunity. Overall, the drivers of HFMD periodicity are not fully understood and worthy of further study.

Although HFMD seasonal patterns were associated with precipitations, sunshine, temperature, and air pressure, no single climatic variable explained the complexity of HFMD seasonality across China. Our results confirm those of previous time series analyses suggesting a relationship between HFMD and climatic factors in Singapore, Hong-Kong, Southern China, and Japan.[29–32] Moreover, the occurrence of poliomyelitis, a disease caused by another enterovirus, may be associated with humidity.[33] Humidity was a weak predictor of HFMD seasonal patterns in our large Chinese study, which encompasses a greater diversity of climate zones and populations than previously studied. The HFMD epidemiological patterns in Hainan and Tibet were apparently different from those in the other provinces (Fig. 4). This divergence could be due to the extreme, unique climate of these two outliers: Hainan is the only tropical region of China; while the climate in Tibet is very particular and complex because of the great altitude. We cannot rule out artefactual surveillance biases, in part due to the lack of financial and human resources (in Tibet in particular). Clearly, more work is needed to clarify the local drivers of HFMD seasonality in South East Asia.

Systematic, population–representative seroepidemiology studies across the age spectrum from mother–infant pairs through the first ten years of life would greatly refine our understanding of HFMD dynamics in different regions. Previous cross–sectional serosurveys have provided age-specific estimates of the cumulative

incidence of infection at certain ages.[34–37] Prospective longitudinal serological studies could provide more detailed information on age–specific incidence of infection and disease, risk factors for infection, and the protection against new infections conferred by previous infections with the same or different viruses. Longitudinal serosurveys were instrumental to elucidate the acquisition of infection and immunity in other complex disease systems involving competition between viruses, which in turn informed vaccination strategies.[38]

EV-A71 infection in early infancy conferred the gravest risks for clinical severity and fatality.[21,39] Several candidate vaccines against EV-A71 are currently undergoing regulatory vetting in China.[40] Whether and how these should be deployed require further assessment of cost–effectiveness, feasibility and contextual considerations in different regions. Transmission models are needed to fully assess the direct and indirect benefits of vaccination, and the risk of serotype replacement given use of a monovalent vaccine.

Our severity data highlight two independent risk factors: 1) each extra day since symptom onset was associated with a 1% higher risk of mortality, and 2) those living in a rural area had a one–quarter excess risk of death from infection. In addition to rural–urban disparity in health care access and advanced life-sustaining technology,[41] widespread and inappropriate use of glucocorticoids and pyrazolones in mild HFMD stages[42,43] could have precipitated critical illness and death.

Several limitations bear mention. First, as for any common, self-limiting illness, surveillance only captures the tip of the clinical iceberg while most cases go undetected because their condition is asymptomatic, or the patient does not seek formal care, or he/she is not diagnosed and reported. Second, access to and provision of health care as well as technical capacity varied between and sometimes within provinces and there was no formal quality assurance or systematic audit for HFMD surveillance. In particular, we cannot rule out that surveillance bias explained the marked differences in HFMD patterns observed in Hainan and Tibet, relative to other provinces (Fig. 4). Third, data collected during the first year of rollout are likely less reliable than in more recent years. Fourth, we did not have data on mixed enterovirus infections[44] and were unable to serotype enteroviruses beyond CV-A16 and EV-A71. In particular, we were unable to monitor serotype CV-A6, which has become more predominant in Southern China.[45] Fifth, some of severe neurological illnesses caused by EV-A71 may have been missed by using a strict HFMD case definition. Sixth, our study was based on a descriptive analysis of surveillance data. We unfortunately cannot yet determine/estimate a threshold for yearly epidemic –this is beyond the scope of our study.

In conclusion, we found substantial HFMD illness and mortality associated with co-circulating EV-A71, CA-V16, and other enterovirus in China, disproportionately

affecting young children aged <5 years. Younger age, infection with EV-A71, and living in rural area are risk factors for severe disease. Our study also uncovered intriguing differences in HFMD seasonality across China, which may be associated with climate. Overall, our study provides robust, population-based, national data to prioritize EV-A71 vaccination and optimize the timing of routine vaccination regionally. Further, our results will serve as a pre-vaccination baseline against which future interventions can be compared. More broadly, further studies are warranted to elucidate the dynamics and immunity patterns of HFMD, a rising public health problem in Asia.

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Table 1. Estimated rates of incidence, severe illness and mortality amongst all HFMD cases by age group

Age group	Incidence rates (95% confidence interval), per 1,000,000					Severe illness rates (95% confidence interval), per 1,000,000					Mortality rates (95% confidence interval), per 1,000,000				
	2008	2009	2010	2011	2012	2008	2009	2010	2011	2012	2008	2009	2010	2011	2012
Total	369.2 (368.2-370.3)	869.3 (867.8-870.9)	1352.9 (1350.9-1354.9)	1221.3 (1219.5-1223.2)	1616.4 (1614.3-1618.6)	0.9 (0.9-1.0)	10.4 (10.2-10.6)	21.3 (21.0-21.5)	14.1 (13.9-14.3)	15.6 (15.4-15.8)	0.1 (0.1-0.1)	0.3 (0.2-0.3)	0.7 (0.6-0.7)	0.4 (0.4-0.4)	0.4 (0.4-0.5)
<6 months	673.7 (655.5-691.8)	1319.9 (1294.9-1345.0)	2259.6 (2227.1-2292.1)	2372.5 (2338.0-2407.1)	4014.7 (3966.9-4062.5)	3.8 (2.6-5.5)	35.9 (31.7-40.0)	60.5 (55.1-65.8)	45.5 (40.7-50.2)	65.0 (58.9-71.1)	0.6 (0.2-1.5)	2.0 (1.2-3.2)	3.3 (2.2-4.8)	2.4 (1.4-3.7)	1.5 (0.7-2.7)
6-11 months	7186.5 (7132.1-7241.0)	18561.8 (18475.6-18647.9)	28042.1 (27937.1-28147.1)	25539.2 (25435.2-25643.2)	28862.1 (28744.5-28979.7)	27.3 (23.9-30.6)	317.1 (305.9-328.4)	589.6 (574.4-604.8)	336.9 (325.0-348.9)	407.4 (393.4-421.4)	3.8 (2.6-5.2)	10.5 (8.5-12.6)	27.1 (23.9-30.4)	11.5 (9.3-13.7)	13.3 (10.8-15.9)
12-23 months	8180.8 (8137.1-8224.4)	19148.5 (19082.5-19214.4)	29480.4 (29399.1-29561.6)	30215.9 (30130.6-30301.2)	38191.1 (38096.4-38285.8)	26.4 (23.9-28.9)	326.8 (318.2-335.4)	626.1 (614.3-637.9)	457.3 (446.8-467.8)	492.4 (481.6-503.1)	3.2 (2.3-4.1)	8.6 (7.2-10.0)	20.2 (18.1-22.4)	12.9 (11.2-14.7)	15.3 (13.4-17.2)
24-59 months	4760.6 (4741.1-4780.1)	11306.9 (11276.9-11336.8)	16848.0 (16811.4-16884.5)	16815.9 (16777.9-16853.8)	23111.3 (23067.3-23155.3)	8.8 (8.0-9.6)	93.2 (90.5-95.9)	204.8 (200.8-208.8)	163.3 (159.5-167.0)	182.1 (178.2-186.0)	0.6 (0.4-0.9)	1.8 (1.4-2.2)	5.2 (4.6-5.9)	3.9 (3.3-4.5)	4.1 (3.5-4.6)

Table 1. (continued)

Age group	Incidence rates (95% confidence interval), per 1,000,000					Severe illness rates (95% confidence interval), per 1,000,000					Mortality rates (95% confidence interval), per 1,000,000				
	2008	2009	2010	2011	2012	2008	2009	2010	2011	2012	2008	2009	2010	2011	2012
5-9 years	580.7 (575.3-586.2)	1045.1 (1037.8-1052.4)	1712.0 (1702.8-1721.2)	1565.3 (1556.2-1574.4)	2706.5 (2694.5-2718.5)	0.7 (0.5-0.9)	5.2 (4.7-5.7)	10.4 (9.7-11.1)	8.8 (8.1-9.5)	10.2 (9.5-10.9)	0.0 (0.0-0.1)	0.1 (0.0-0.1)	0.1 (0.1-0.3)	0.1 (0.0-0.2)	0.1 (0.1-0.2)
10-14 years	68.4 (66.7-70.1)	132.3 (129.9-134.7)	216.3 (213.0-219.5)	186.7 (183.5-189.9)	277.2 (273.4-281.0)	0.1 (0.0-0.1)	0.3 (0.2-0.5)	1.1 (0.9-1.4)	0.9 (0.7-1.2)	0.8 (0.6-1.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.1)
≥15 years	2.3 (2.2-2.4)	4.1 (4.0-4.2)	6.5 (6.4-6.7)	5.9 (5.8-6.0)	7.7 (7.6-7.9)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)

Table 2. ORs and 95%CIs of severe illness and death among probable cases and laboratory-confirmed HFMD cases. Results from multivariate logistic regression with backward selection of demographic and geographic variables, time from onset to diagnosis, and virus type (p-value for exclusion≥0·10).

	Lab-confirmed cases N=267942		Probable cases N=6932150	
Characteristics	No. of cases	Adjusted OR (95% CI)	No. of cases	Adjusted OR (95% CI)
Death				
Enterovirus serotype				
CV-A16	76817	Ref.	NA	
Other enterovirus	56398	4.36 (2.72-6.98)		
EV-A71	134727	34.24 (22.47-52.18)		
Age group				
≥5 years	22666	Ref.	638925	Ref.
24-59 months	131560	3.44 (2.24-5.28)	3274253	4.00 (2.12-7.55)
6-23 months	111238	8.75 (5.73-13.35)	2941707	8.59 (4.59-16.09)
<6 months	2478	20.46 (12.35-33.90)	77265	14.21 (6.59-30.62)
Residence				
Urban	115828	Ref.	3219524	Ref.
Rural	137162	1.19 (1.07-1.33)	3218311	2.45 (2.06-2.92)
Per day increase of onset-to-diagnosis interval	NA	1.01 (1.01-1.02)	NA	1.02 (1.01-1.02)
Sex				
Female	96934	Ref.	4351763	Ref.
Male	171008	1.00 (0.90-1.11)	2580384	1.28 (1.09-1.52)
Severe illness				
Enterovirus serotype				
CV-A16	76817	Ref.	NA	
Other enterovirus	56398	3.91 (3.70-4.14)		
EV-A71	134727	11.17 (10.64-11.74)		
Age group				
≥5 years	22666	Ref.	638925	Ref.
24-59 months	131560	1.90 (1.78-2.01)	3274253	1.99 (1.89-2.10)
6-23 months	111238	3.64 (3.43-3.87)	2941707	3.40 (3.22-3.58)
<6 months	2478	5.68 (5.07-6.36)	77265	4.71 (4.33-5.12)

CI, confidence interval; EV-A71, enterovirus 71; CA-V16, Coxsackievirus A16; Ref., reference; NA, not applicable; OR, odds ratio

Table 2. (continued)

	Lab-confirmed cases N=267942		Probable cases N=6932150	
Characteristics	No. of cases	Adjusted OR (95% CI)	No. of cases	Adjusted OR (95% CI)
Severe illness				
Residence				
Urban	115828	Ref.	3219524	Ref.
Rural	137162	1.08 (1.05-1.10)	3218311	1.67 (1.64-1.71)
Per day increase of onset-to-diagnosis interval	NA	1.00 (1.00-1.00)	NA	1.02 (1.01-1.02)
Sex				
Female	96934		4351763	
Male	171008	1.10 (1.07-1.13)	2580384	1.03 (1.01-1.05)

CI, confidence interval; EV-A71, enterovirus 71; CA-V16, Coxsackievirus A16; Ref., reference; NA, not applicable; OR, odds ratio

Panel

Systematic review

We searched PubMed with the keywords “Hand, Foot and Mouth Disease”, “EV71”, “enterovirus 71” and “coxsackie virus A” for all journal articles written in English between Jan 1, 1995, and June 30, 2013. In the past 15 years, EV-A71–associated HFMD epidemics have been increasingly reported across the Asia–Pacific region, chronologically including Malaysia,[4] Taiwan,[5] Japan,[6] Singapore,[7] Vietnam,[8] mainland China,[9] Hong Kong SAR,[10] and Cambodia.[11] These reports were mostly descriptive, with very few exceptions that estimated epidemiologic parameters, assessed severity or analyzed seasonality. Even fewer had sufficient power to yield definitive conclusions. No previous report had addressed all three sets of interrelated questions. Here we report the largest study to date across a wide geographic expanse through the recently enhanced national surveillance network for HFMD in China.

Interpretation

We described the enhanced HFMD surveillance system, characterized host characteristics, periodicity of epidemics, and geographic patterns from 2008 to 2012. HFMD incidence was 1·2 per 1,000 person–years with 350–900 reported deaths annually during 2009–2012, predominantly among young children. There was strong age dependency, with children aged six months to two years showing the highest rates (Table 1). Boys under five years were 1·6 times more likely than girls of the same age

bracket to register disease. Overall the case–fatality, case–severity, and severe case–fatality risks were 0·03%, 1·1%, and 3·0% respectively. Illness severity and death by enterovirus serotype were inversely related to age (Table 2 and Figure 2, panels B–D). The median time from illness onset to diagnosis, from illness onset to death, and from diagnosis to death were 1·5 days (interquartile range: 0·5–2·5 days), 3·5 days (interquartile range: 2·5–4·5 days), and 0·5 day (interquartile range: 0·5–1·5 days) respectively. HFMD tended to occur during the warmer months of the year throughout the country, although peak timing and the periodicity of epidemics varied substantially with latitude, in particular demonstrating stronger semi-annual cycles in southern China compared to annual outbreaks in the north. Seasonal variation was associated with precipitation, sunshine, temperature, and barometric pressure. However, no single climatic variable was sufficient to explain the complexity of HFMD seasonality across China.

Figure Legends

Figure 1. Proportions of enterovirus serotypes among laboratory-confirmed HFMD cases by clinical severity, 2008-2012, China. A: based on mild cases. B: based on severe cases who survived, C: based on fatal cases.

Figure 2. Age distribution and clinical severity of probable and laboratory-confirmed (EV-A71, CA-V16 or other enteroviruses) HFMD cases, 2008-2012, China. A: Age distribution of probable and lab-confirmed cases. B: Risk of fatality among cases by age group and viral etiology. C: Risk of severe illness among cases by age group and viral etiology. D: Risk of fatality among severe cases by age group and viral etiology. EV-A71, enterovirus 71; CA-V16, Coxsackievirus A16; mth, months; y, years. Severity estimates in B-D were calculated by extrapolating the serotype distribution among test-positive cases to untested and test-negative cases. That is, the number of mild cases with serotype X (= EV-A71, CA-V16 or other enteroviruses) was estimated to be (no. of mild cases test-positive for serotype X)/(no. of test-positive mild cases) x (no. of mild cases); severe cases were similarly analyzed. Results were similar if only the 2010-2012 or 2012 data were used (Appendix Figures 4&5).

Figure 3. Estimates of onset-to-diagnosis, onset-to-death, and diagnosis-to-death

distributions of probable and laboratory-confirmed (EV-A71, CA-V16 or other enteroviruses) HFMD cases, 2008-2012, China. A: Onset-to-diagnosis distribution by viral etiology (n=7,200,092). B: Onset-to-death distribution by viral etiology (n=2457). C: Diagnosis-to-death distribution by viral etiology (n=2457). Note that some intervals are negative because diagnosis occurred after death.

Figure 4. Heatmap of HFMD surveillance data from 2008 to 2012 by Chinese province. The provinces were ordered by latitude from Northermost (top) to Southernmost (bottom). A: Time series of weekly probable and lab-confirmed HFMD cases, standardized by the number of annual cases. B: Seasonal distribution of HFMD cases, plotted as the median value of proportion of cases in each week of the year from 2008 to 2012. C: Number of HFMD cases by week of illness onset. The insert is a superposition of the number of cases without probable HFMD cases by week of illness onset.

Figure 5. Latitudinal gradients in periodicity and peak timing of HFMD. A: Amplitude of the annual periodicity. B: Annual peak timing. C: Contribution of the semi-annual periodicity, measured by the ratio of the amplitude of the semi-annual periodicity to the sum of the amplitudes of annual and semi-annual periodicities (higher ratio indicates a stronger semi-annual periodicity). Symbol size is proportional to the number of cases in each province. Black solid line represent linear regression fit

(regression weighted by mean annual number of HFMD cases). P-values are given on the graphs. Colors represent different climatic zones (black: cold-temperate, blue mid-temperate, green warm-temperate, orange subtropical, red tropical).

Figure 6. Amplitude and timing of primary HFMD epidemics in China. A: Amplitude of the annual cycle from yellow (low) to red (high), as indicated in the legend. B: Importance of the semi-annual periodicity, measured by the ratio of the amplitude of the semi-annual cycle to the sum of the amplitudes of annual and semi-annual cycles. Pale green indicates strongly annual influenza epidemics, while dark green indicates dominant semi-annual activity. C: Timing of primary annual HFMD peak, in weeks from Jan 1st. Timing is color coded from pale blue to dark blue.

Figure 7. HFMD epidemiological regions and predictors. A: Identified epidemiological regions based on hierarchical clustering, using the Euclidian distance between weekly standardized HFMD time series. Provinces are color-coded by climatic region (black: cold-temperate, blue: mid-temperate, green: warm temperate, orange: subtropical, red: tropical). B: Map of the three epidemiological regions identified in panel A. C: Climate predictors of the two main clusters identified in panel A, based on stepwise discriminant analysis.

Supporting Information

Appendix Text 1 Additional information regarding climate variables.

Appendix Text 2 Additional information regarding the estimation of seasonal parameters.

Appendix Table 1 Association between HFMD, EV-A71, CA-V16 and other enteroviruses seasonal patterns and geographic, population, economic, transport and climatic factors.

Appendix Figure 1 Flowchart of data and sample collection for HFMD cases, 2008-2012, China.

Appendix Figure 2 Map of the Chinese mainland provinces.

Appendix Figure 3 Proportions of enterovirus serotypes among laboratory-confirmed HFMD cases by week of illness onset and by geographic region, 2008-2012, China.

Appendix Figure 4 Age distribution and clinical severity of probable and laboratory-confirmed HFMD cases, 2010-2012, China

Appendix Figure 5 Age distribution and clinical severity of probable and laboratory-confirmed HFMD cases, 2012, China

Appendix Figure 6 Latitudinal and longitudinal gradients in seasonality of EV-A71, CA-V16 and other enterovirus.

Appendix Figure 7 Periodicity of EV-A71, CA-V16 and other enterovirus epidemics in China.

Appendix Figure 8 Importance of semi-annual seasonality of EV-A71, CA-V16 and other enterovirus.

Appendix Figure 9 EV-A71 epidemiological regions and predictors.

Appendix Figure 10 CA-V16 epidemiological regions and predictors.

Appendix Figure 11 Other enteroviruses epidemiological regions and predictors.

Competing Interests

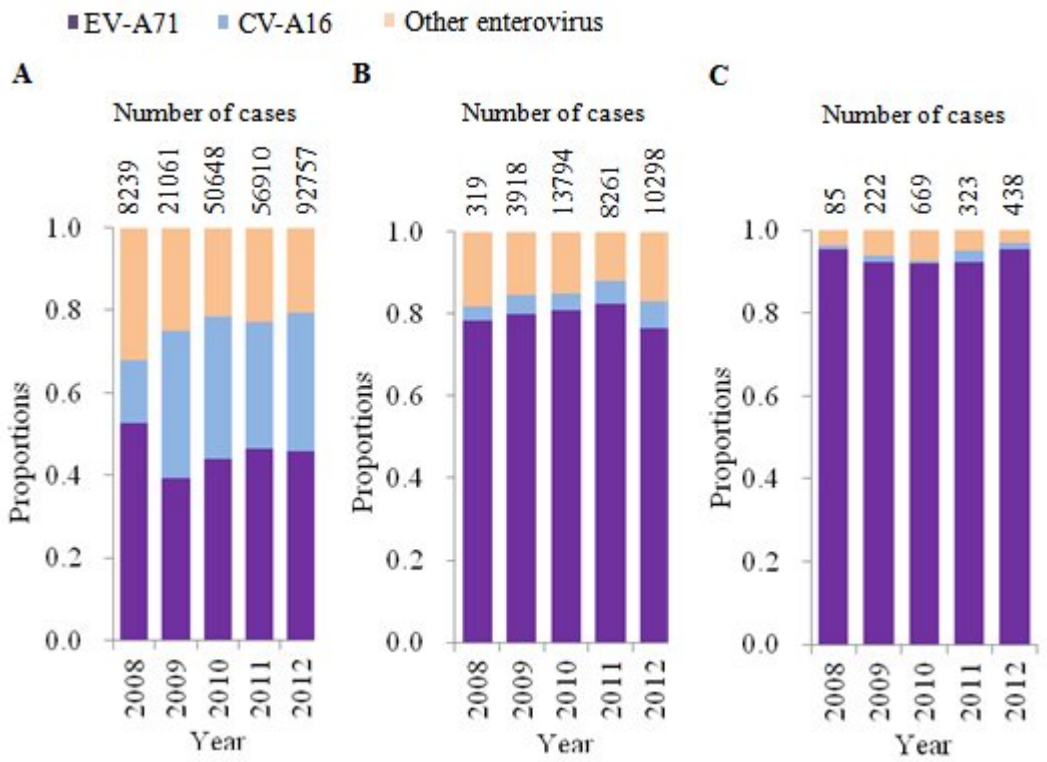
The authors have no competing interests to declare.

Acknowledgments

We thank the hospitals, local health departments and Centers for Disease Control and Prevention in China for assistance in coordinating data collection. We also thank Lin Wang for the data cleaning. We are also grateful to the National Health and Family Planning Commission for supporting this study. The views expressed are those of the authors and do not necessarily represent the policy of the China CDC.

Abbreviations

95% CI, 95% confidence interval; CA-V16, coxsackievirus A16; CDC, Centre for Disease Control and Prevention; CPE, cytopathic effect; EV-A71, Enterovirus 71; HFMD, Hand-Foot-And-Mouth Disease; RD, rhabdomyosarcoma; RNA, ribonucleic acid; RT-PCR, reverse-transcriptase polymerase chain reaction



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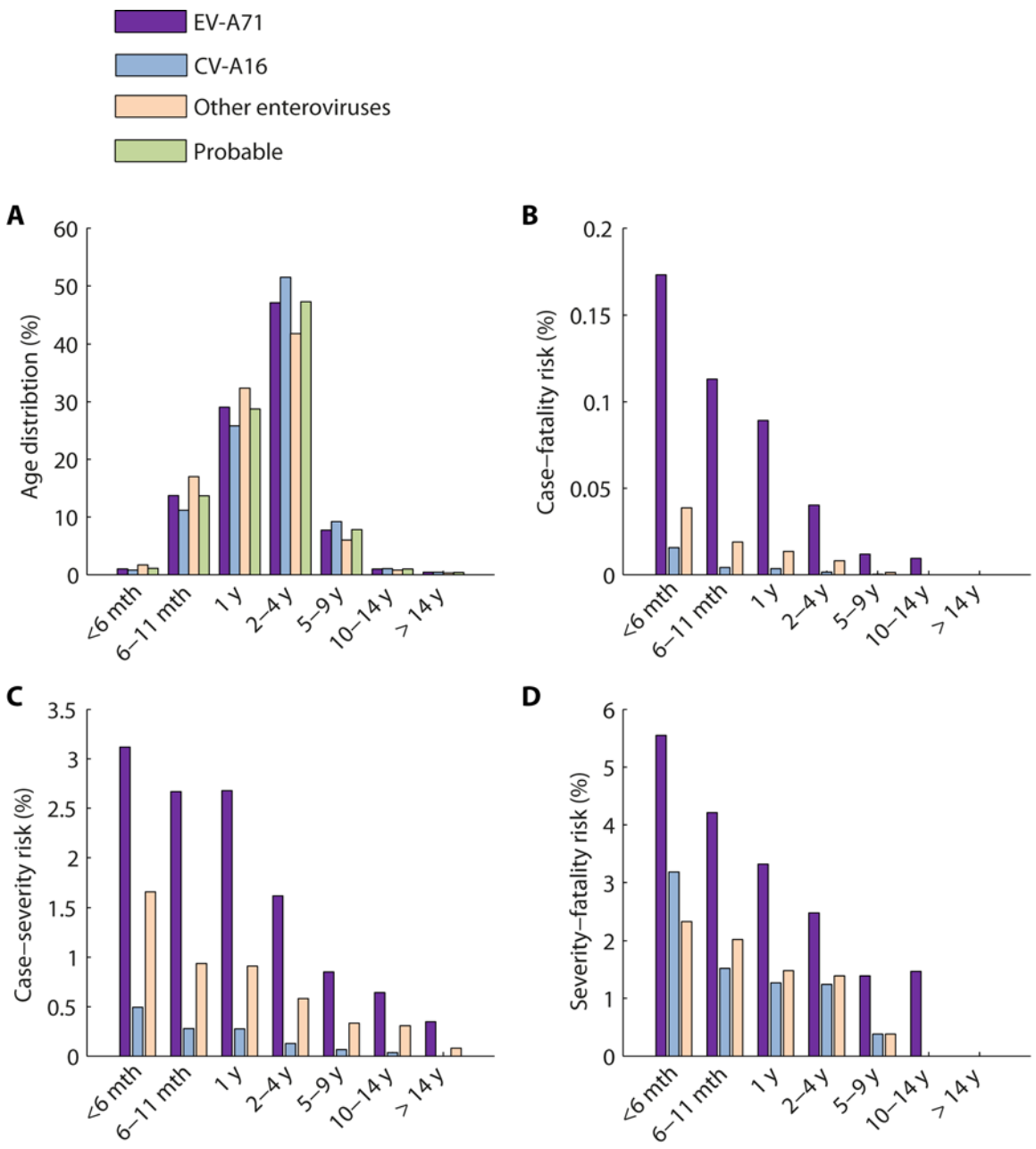


Figure 2. Age distribution and clinical severity of probable and laboratory-confirmed HFMD cases, 2008-2012, China

A: Age distribution of probable and lab-confirmed cases. B: Risk of fatality among cases by age group and viral etiology. C: Risk of severe illness among cases by age group and viral etiology. D: Risk of fatality among severe cases by age group and viral etiology. EV-A71, enterovirus 71; CV-A16, Coxsackievirus A16; mth, months; y, years. Severity estimates in B-D were calculated by extrapolating the serotype distribution among test-positive cases to untested and test-negative cases. That is, the number of mild cases with serotype X (= EV-A71, CV-A16 or other enteroviruses) was estimated to be (no. of mild cases test-positive for serotype X)/(no. of test-positive mild cases) x (no. of mild cases); severe cases were similarly analyzed. Results were similar if only the 2010-2012 or 2012 data were used (Appendix Figures 4&5).

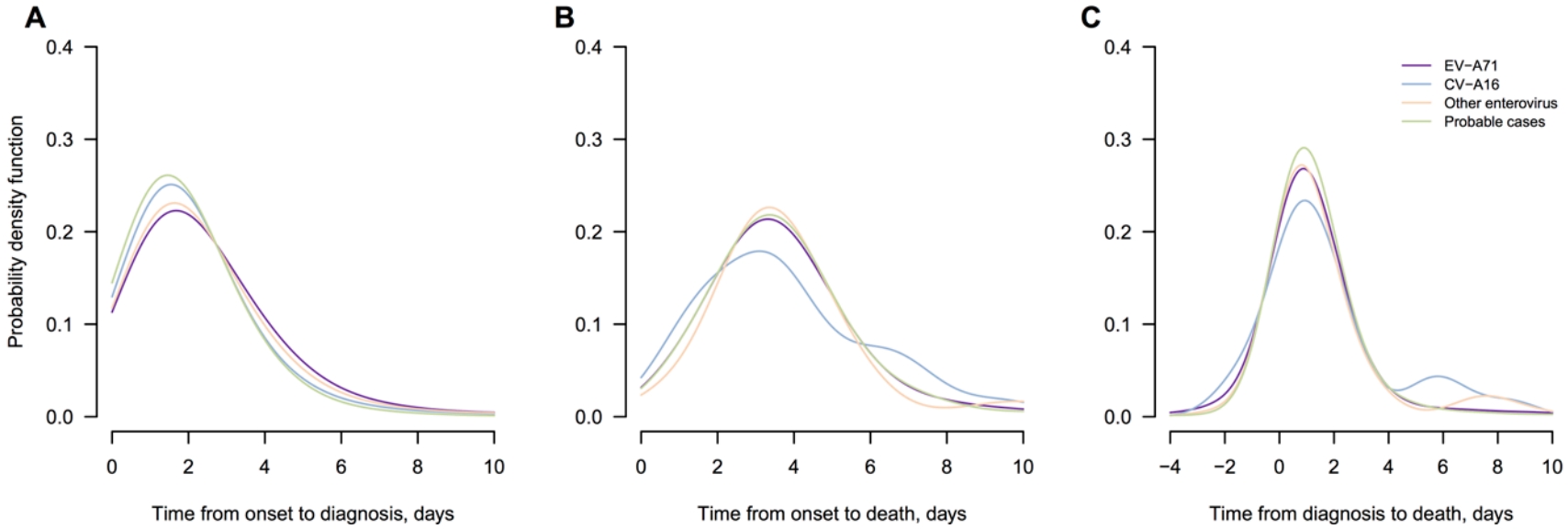
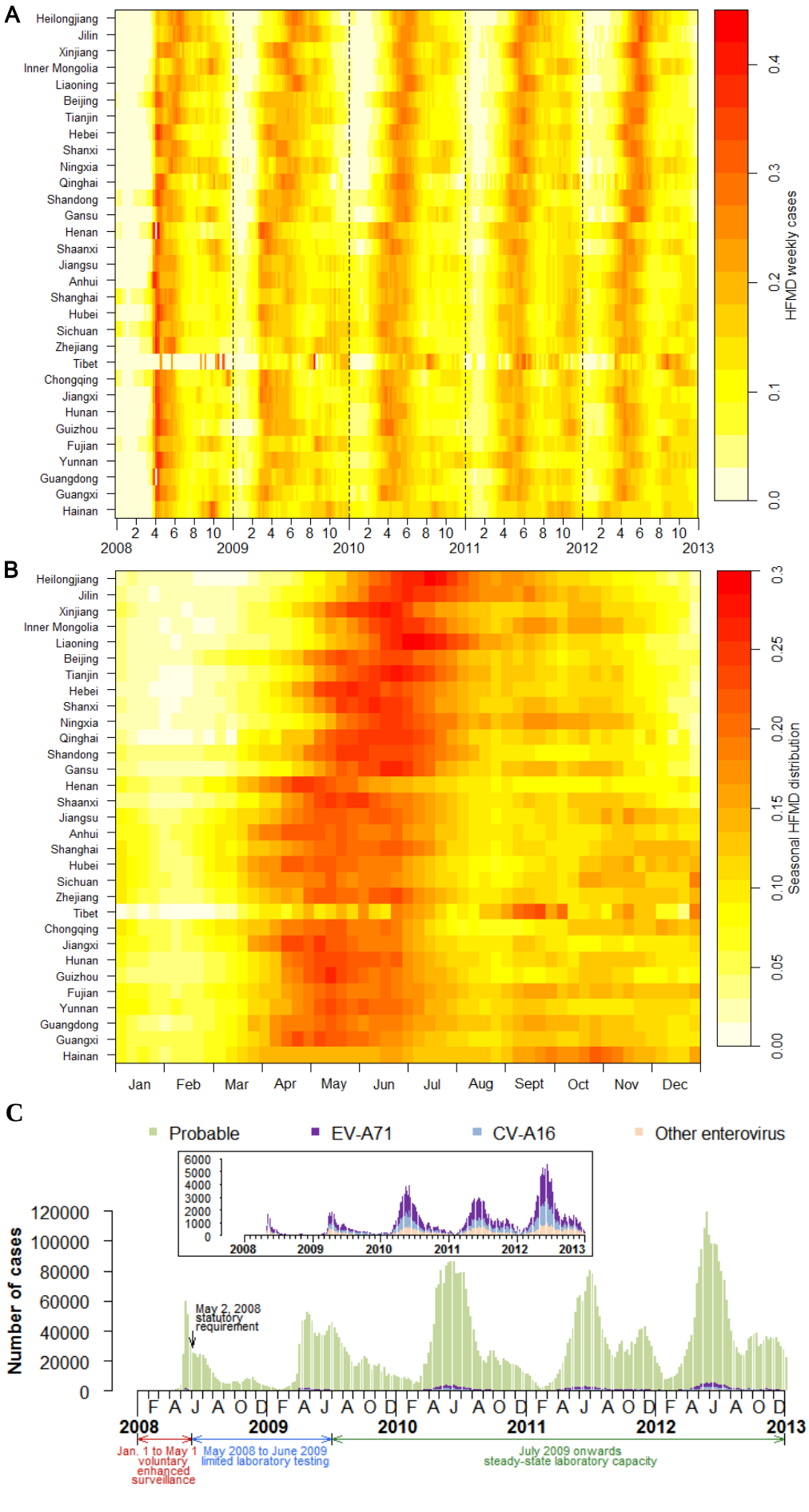


Figure 3. Estimates of onset-to-diagnosis, onset-to-death, and diagnosis-to-death distributions of probable and laboratory-confirmed HFMD cases, 2008-2012, China

A: Onset-to-diagnosis distribution by viral etiology (n=7,200,092). B: Onset-to-death distribution by viral etiology (n=2457). C: Diagnosis-to-death distribution by viral etiology (n=2457). Note that some intervals are negative because diagnosis occurred after death. EV-A71, enterovirus 71; CV-A16, Coxsackievirus A16


A
Heilongjiang
Jilin
Xinjiang
Inner Mongolia
Liaoning
Beijing
Tianjin
Hebei
Shanxi
Ningxia
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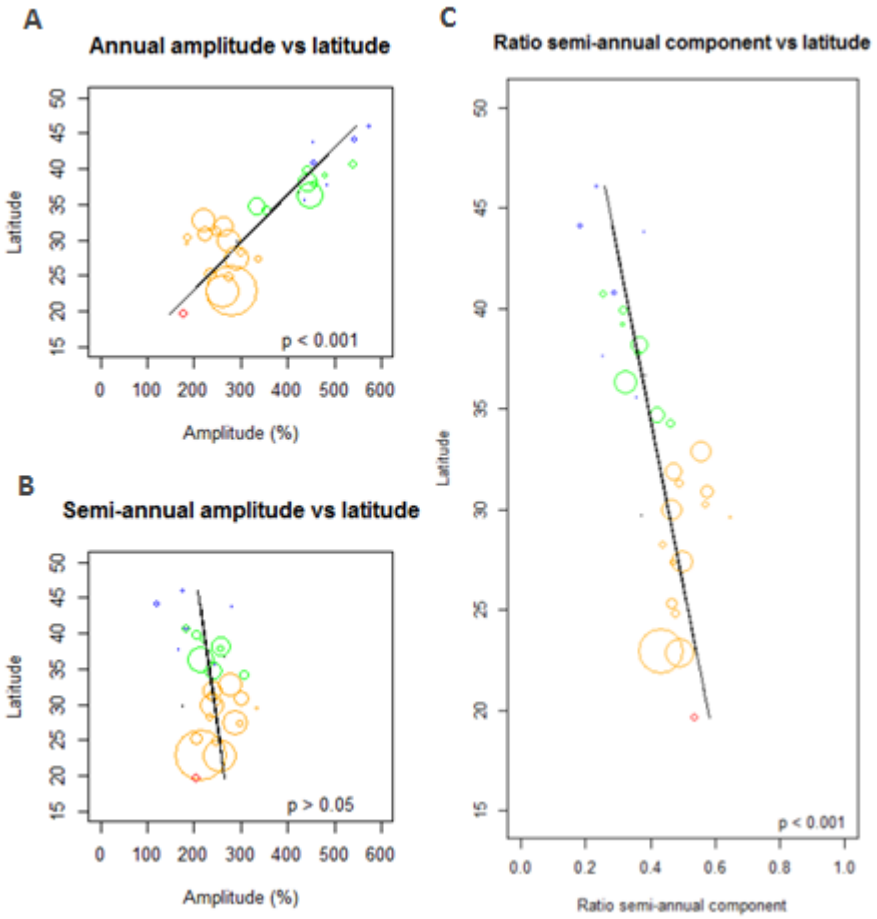
Shandong
Gansu
Henan
Shaanxi
Jiangsu
Anhui
Shanghai
Hubei
Sichuan
Zhejiang
Tibet
Chongqing
Jiangxi
Hunan
Guizhou
Fujian
Yunnan
Guangdong
Guangxi
Hainan
2 4 6 8 10
2008
2009
2010
2011
2012
2013
HFMD weekly cases
0.0
0.1
0.2
0.3
0.4
B
Jan
Feb
Mar
Apr
May
Jun
Jul
Aug
Sept
Oct
Nov
Dec
Seasonal HFMD distribution
0.00
0.05
0.10
0.15
0.20
0.25
0.30
C
Probable
EV-A71
CV-A16
Other enterovirus
Number of cases
120000
100000
80000
60000
40000
20000
0
6000
5000
4000
3000
2000
1000
0
May 2, 2008
statutory
requirement
F A J A O D F A J A O D F A J A O D F A J A O D F A J A O D
2008
2009
2010
2011
2012
2013
Jan. 1 to May 1
voluntary
enhanced
surveillance
May 2008 to June 2009
limited laboratory testing
July 2009 onwards
steady-state laboratory capacity

Figure 4. HFMD surveillance data from 2008 to 2012 by Chinese province and by week

The provinces were ordered by latitude from Northermost (top) to Southernmost (bottom). A: Time series of weekly probable and lab-confirmed HFMD cases, standardized by the number of annual cases. B: Seasonal distribution of HFMD cases, plotted as the median value of proportion of cases in each week of the year from 2008 to 2012. C: Number of HFMD cases by week of illness onset. The insert is a superposition of the number of cases without probable HFMD cases by week of illness onset.



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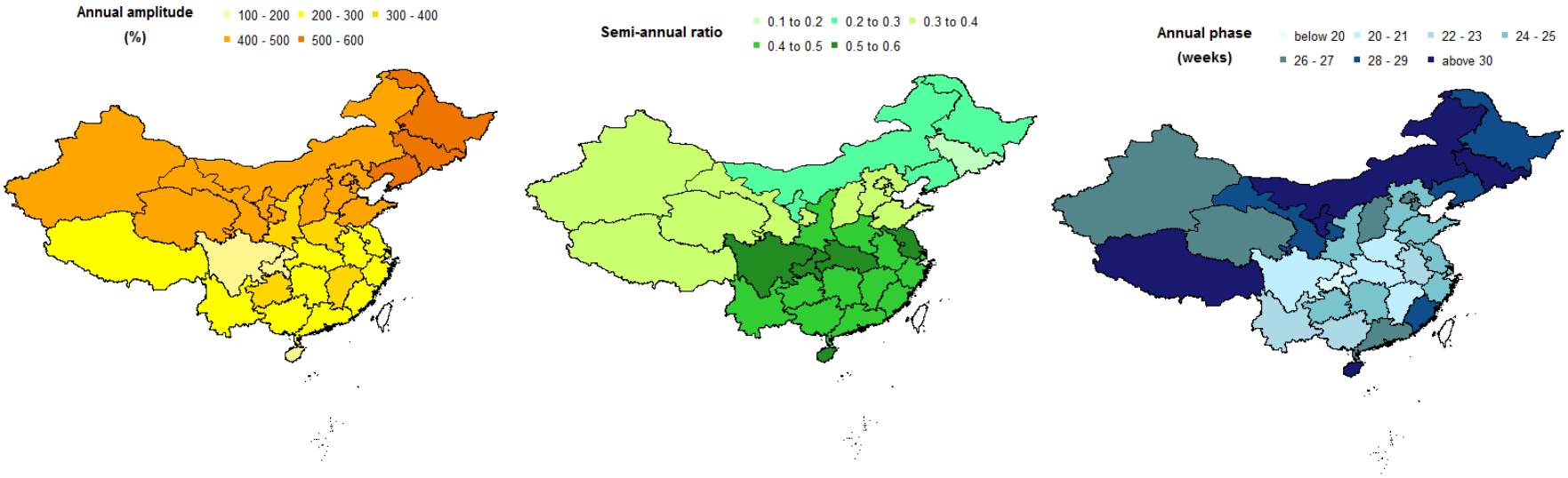

Figure 6. Amplitude and timing of primary HFMD epidemics in China.

A: Amplitude of the annual cycle from yellow (low) to red (high), as indicated in the legend. B: Importance of the semi-annual periodicity, measured by the ratio of the amplitude of the semi-annual cycle to the sum of the amplitudes of annual and semi-annual cycles. Pale green indicates strongly annual influenza epidemics, while dark green indicates dominant semi-annual activity. C: Timing of primary annual HFMD peak, in weeks from Jan 1st. Timing is color coded from pale blue to dark blue.

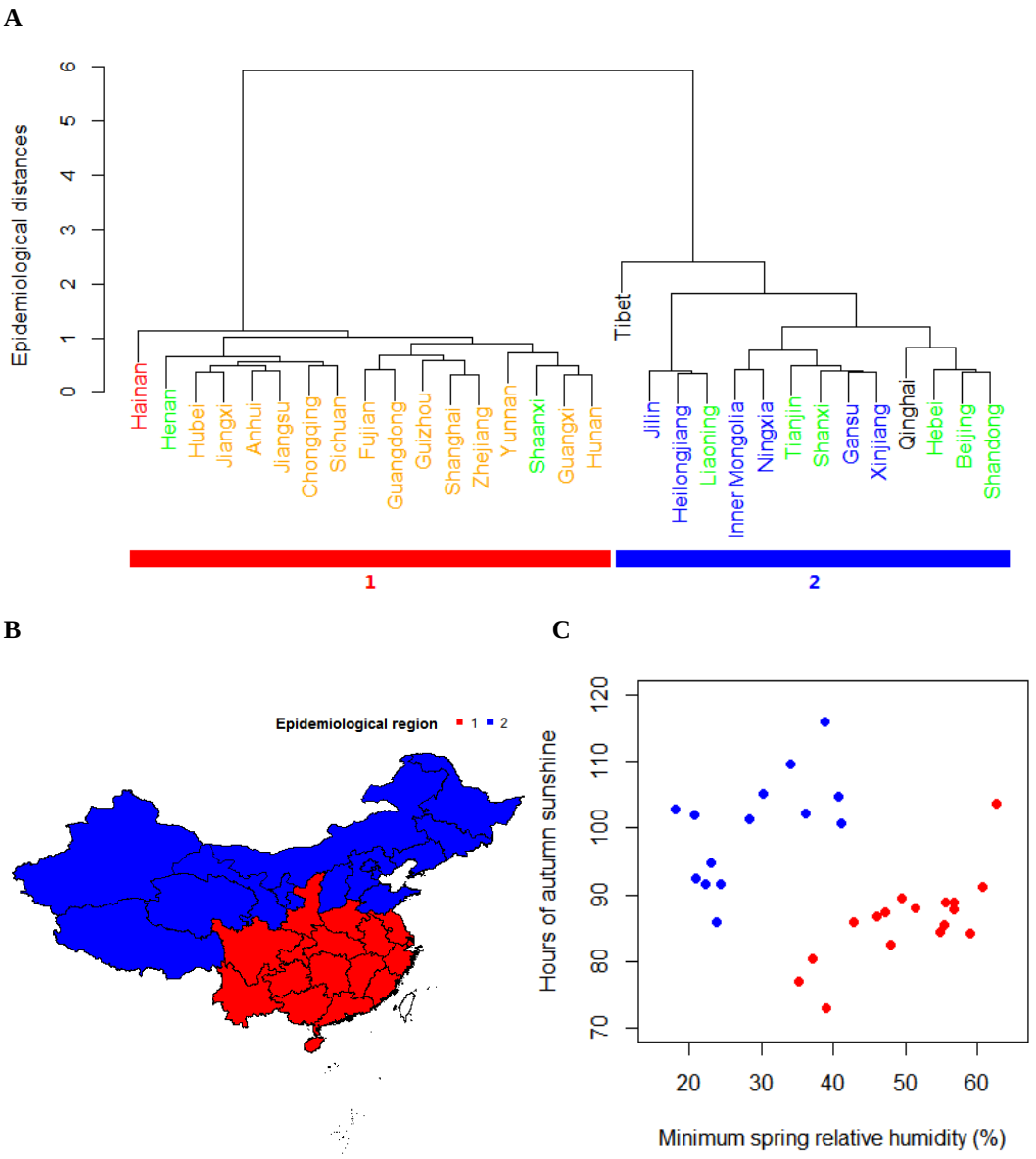

Figure 7. HFMD epidemiological regions and predictors

A: Identified epidemiological regions based on hierarchical clustering, using the Euclidian distance between weekly standardized HFMD time series. Provinces are color-coded by climatic region (black: cold-temperate, blue: mid-temperate, green: warm temperate, orange: subtropical, red: tropical). B: Map of the three epidemiological regions identified in panel A. C: Climate predictors of the two main clusters identified in panel A, based on stepwise discriminant analysis.

Appendix Text 1. Additional information regarding climate variables

Meteorological data collected included daily temperature (min, max, mean), rainfall, relative humidity (min, mean), air pressure (min, max, mean), average vapor pressure, and hours of sunshine recorded at 756 Chinese ground weather stations from 2008–2012.[1]

In order to aggregate daily- station-specific meteorological data at the weekly and province level, we first averaged station–specific daily values at the weekly level (except for rainfall where the weekly sum was taken) and took the average across all stations located within a province. Preliminary analysis of HFMD surveillance data revealed that two HFMD peaks were typically observed in recent years in China in spring and autumn. Therefore, province–specific climatic variables were further summarized for relevant seasons, by averaging the weekly values corresponding to the spring (April–June) and autumn (September–November).

Reference

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Appendix Text 2. Additional information regarding the estimation of seasonal parameters

1. Regression model

We used to following linear regression model to estimate the peak timing and amplitude of the annual and semi-annual periodicities of influenza activity in each province, as described in[1,2]:

hfmdi(t)=a+ bi *cos(2Pi*t/52.17) + ci *sin(2Pi*t/52.17) + di*cos(4Pi*t/52.17) + ei *sin(4Pi*t/52.17) + ε(t)

where hfmdi(t) are the weekly standardized counts of HFMD cases (or EV-A71, or CV-A16, or other enterovirus) isolates in province i, where standardization is obtained by dividing weekly values by the annual number of HFMD cases, t is a running index for week, and a,b, c, d and e are the intercept and seasonal terms to be estimated from the data.

Specifically,

the amplitude of the annual periodicity is estimated as AnnAmpi=sqrt(bi 2+ ci 2),

the annual peak timing is estimated as AnnPeakTimingi=-atan(ci / bi),

the amplitude of the semi-annual periodicity is estimated as

SemiAnnAmpi=sqrt(c2+d2)

To evaluate the relative importance of annual and semi-annual HFMD periodicities by province, we calculated the ratio between the amplitude of the semi-annual periodicity and the sum of the amplitudes of annual and semi-annual periodicities. A ratio close to 1 is indicative of dominant semi-annual periodicity while a ratio close to 0 indicates

dominant annual periodicity.

The ratio is estimated as Ratioi= SemiAnnAmpi / (SemiAnnAmpi + AnnAmpi)

To control for different levels of HFMD activity across provinces, we compare the relative amplitudes of annual and semi-annual periodicity, obtained by dividing AnnAmpi and SemiAnnAmpi by the mean of the hfmdi(t) time series.

2. Sensitivity analyses

We considered alternative seasonal regression models using a Poisson distribution and a log link (given that we were using modified disease counts), with or without an offset for the number of specimens tested. Given that all three approaches gave sensibly similar results, we elected to use the linear model in the main analysis for the sake of simplicity.

Reference

1. Naumova EN, Jagai JS, Matyas B, DeMaria A, Jr., MacNeill IB, Griffiths JK. Seasonality in six enterically transmitted diseases and ambient temperature. *Epidemiol Infect.* 2007; **135**: 281-92.
2. Yu H, Alonso WJ, Feng L, Tan Y, Shu Y, Yang W, Viboud C. Characterization of regional influenza seasonality patterns in China and implications for vaccination strategies: spatio-temporal modelling of surveillance data. *PLoS Med.* 2013: Nov 19. In Press

Appendix Table 1. Association between HFMD, EV71, CA16 and other enteroviruses seasonal patterns and geographic, population, economic, transport and climatic factors

Analyzed factors	Overall cases					EV71 confirmed cases				
	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)
	Annual cycle (p-value, R^2)	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*		*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	
Latitude	<0.001, 0.12						0.001, 0.05			0.004, 0.20
Longitude	0.032, 0.03			0.016, 0.09	0.012, 0.06	0.002, 0.15				
Population size	<0.001, 0.19					0.048, 0.05	<0.001, 0.06			
Area	0.021, 0.04		<0.001, 0.09				0.004, 0.03	0.013, 0.11		
Density	0.032, 0.03									
Passengers										
by railways			0.006, 0.03						0.005, 0.21	0.025, 0.11
by highways		0.018, 0.04	0.006, 0.03				<0.001, 0.07		0.005, 0.21	0.025, 0.11
by waterways			0.006, 0.03						0.005, 0.21	0.025, 0.11
by airways			0.008, 0.03				0.038, 0.01			

Analyses were based on stepwise multivariate regression

Appendix Table 1. (continued)

Analyzed factors	Overall cases					EV71 confirmed cases				
	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)
	Annual cycle (p-value, R^2)	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*		*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	
Total passengers		0.019, 0.04	0.006, 0.03		0.037, 0.04		<0.001, 0.08		0.005, 0.21	0.025, 0.11
GRP per capita	0.029, 0.03		0.001, 0.06						0.038, 0.10	0.027, 0.11
Rainfall[1]			0.003, 0.05					0.031, 0.08		
Sunshine[1]	<0.001, 0.12		<0.001, 0.08					0.018, 0.10		
Air pressure[1]										
max	0.019, 0.04					0.016, 0.08				
mean			0.023, 0.02							
min			0.009, 0.03			0.014, 0.08				
Relative humidity[1]										
mean			0.04, 0.02			<0.001, 0.23				
min						<0.001, 0.26				

[1]Spring weather parameters

Appendix Table 1. (continued)

Analyzed factors	Overall cases					EV71 confirmed cases				
	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)
	Annual cycle (p-value, R^2)	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*		*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	
Temperature[1]										
max	0.036, 0.03					0.019, 0.07				
mean			0.007, 0.03			0.049, 0.05				
min			0.007, 0.03							
Vapor pressure[1]	0.022, 0.04									
Rainfall[2]		0.003, 0.08								
Sunshine[2]		0.006, 0.06		0.017, 0.09			0.045, 0.01		0.009, 0.17	
Air pressure[2]										
max		0.024, 0.04		0.009, 0.11			<0.001, 0.21			
mean		0.032, 0.03					<0.001, 0.08			
min				0.032, 0.07			<0.001, 0.11			

[1]Spring weather parameters

[2]Autumn weather parameters

Appendix Table 1. (continued)

Analyzed factors	Overall cases					EV71 confirmed cases				
	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)
	Annual cycle (p-value, R^2)	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*		*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	
Temperature[2]										
max				0.027, 0.07			<0.001, 0.06			
mean				0.010, 0.11	0.043, 0.04		<0.001, 0.06			
min				0.012, 0.10	0.033, 0.04		<0.001, 0.06		0.044, 0.10	0.048, 0.08
Relative humidity[2]										
mean										
min							<0.001, 0.07			
Vapor pressure[2]		0.044, 0.03			0.030, 0.05					
Total explained variance[3]	0.832	0.817	0.910	0.623	0.755	0.671	0.915	0.646	0.397	0.457

[2]Autumn weather parameters

[3]Explained variance by multivariate best fit models selected by stepwise regression

Appendix Table 1. (continued)

Analyzed factors	CA16 confirmed cases					Other enterovirus confirmed cases				
	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)
	Annual cycle (p-value, R^2)	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*		*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	
Latitude	<0.001, 0.16	<0.001, 0.23	0.011, 0.07							
Longitude	0.003, 0.10			<0.001, 0.41	0.009, 0.16					0.002, 0.21
Population size							0.007, 0.05			
Area			0.028, 0.05	0.007, 0.09		0.040, 0.07		0.034, 0.06		
Density			0.011, 0.07						0.027, 0.06	
Passengers										
by railways		0.003, 0.13							0.003, 0.12	
by highways		0.003, 0.13							0.003, 0.12	
by waterways	0.004, 0.09	0.003, 0.13		0.002, 0.14		0.048, 0.06	0.021, 0.04		0.003, 0.12	
by airways		0.042, 0.05								

Analyses were based on stepwise multivariate regression

Appendix Table 1. (continued)

Analyzed factors	CA16 confirmed cases					Other enterovirus confirmed cases				
	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)
	Annual cycle (p-value, R^2)	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*		*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	
Total passengers	0.018, 0.06	0.003, 0.13							0.003, 0.12	
GRP per capita			0.049, 0.03	0.008, 0.09	0.012, 0.15		0.005, 0.06	0.006, 0.12	0.012, 0.08	
Rainfall[1]			<0.001, 0.23					0.005, 0.12		
Sunshine[1]			<0.001, 0.23					0.006, 0.12		
Air pressure[1]										
max	0.034, 0.05					0.010, 0.12				
mean	0.032, 0.05		0.020, 0.05			0.008, 0.13				
min			0.008, 0.08							
Relative humidity[1]										
mean	0.004, 1.10									
min	0.001, 0.13					0.003, 0.17				

[1]Spring weather parameters

Appendix Table 1. (continued)

Analyzed factors	CA16 confirmed cases					Other enterovirus confirmed cases				
	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)
	Annual cycle (p-value, R^2)	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*		*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	
Temperature[1]										
max			0.020, 0.05			0.014, 0.10				
mean			0.010, 0.07					0.025, 0.07		
min			0.008, 0.08					0.025, 0.07		
Vapor pressure[1]						0.005, 0.14				
Rainfall[2]				0.005, 0.10			<0.001, 0.11		0.003, 0.12	0.032, 0.09
Sunshine[2]		0.004, 0.12					0.005, 0.06		0.034, 0.05	
Air pressure[2]										
max				0.033, 0.05			0.22, 0.04			
mean							0.018, 0.04		<0.001, 0.27	0.007, 0.15
min				0.046, 0.05					<0.001, 0.17	0.009, 0.14

[1]Spring weather parameters

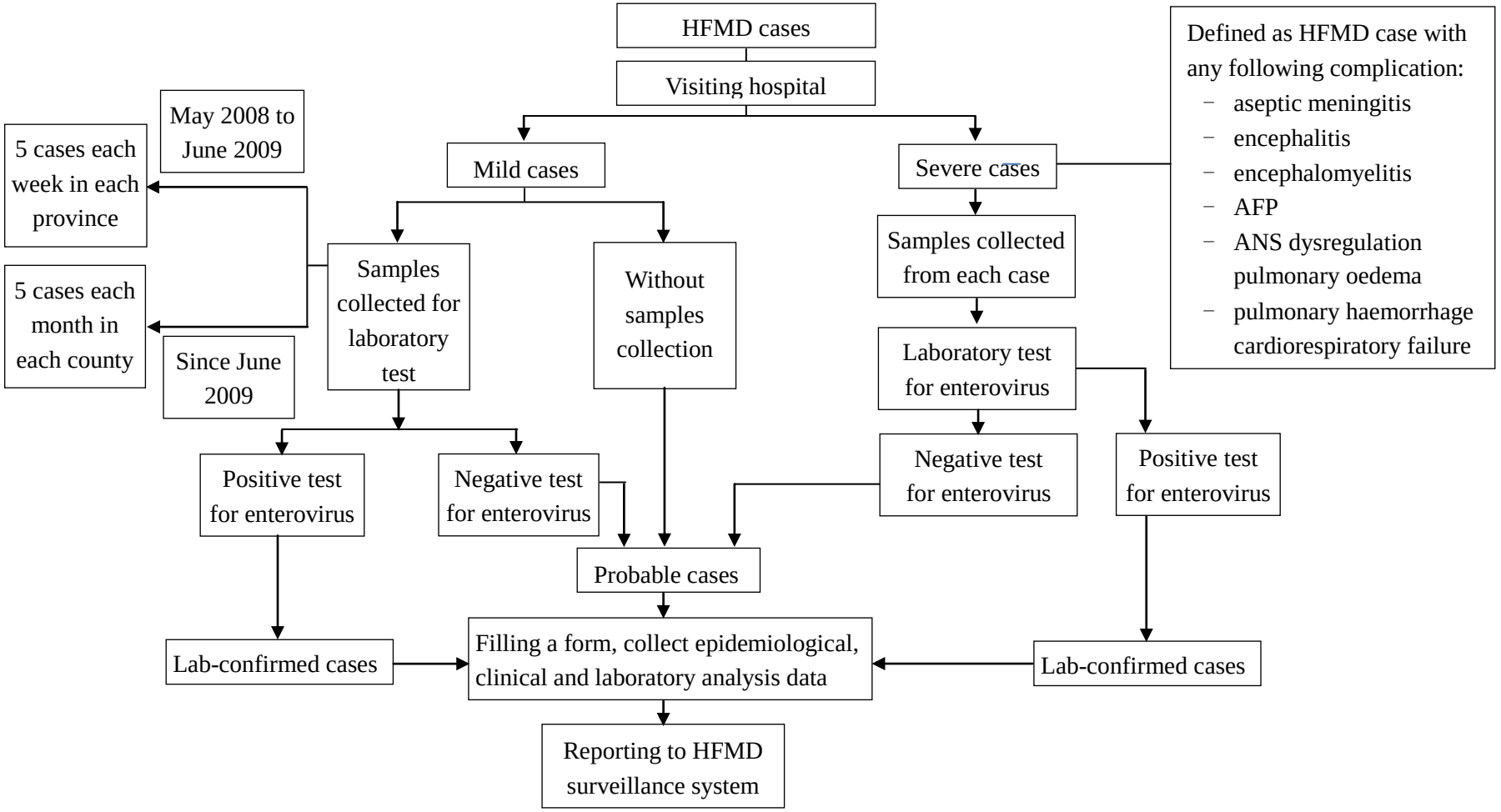
[2]Autumn weather parameters

Appendix Table 1. (continued)

Analyzed factors	CA16 confirmed cases					Other enterovirus confirmed cases				
	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)	Timing (phase)		Amplitude		Importance semi-annual periodicity (p-value, R^2)
	Annual cycle (p-value, R^2)	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*		*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	*Annual cycle (p-value, R^2)*	*Semi-annual cycle (p-value, R^2)*	
Temperature[2]										
max				0.002, 0.13						0.020, 0.11
mean				0.002, 0.13						0.011, 0.13
min				0.007, 0.09					0.007, 0.09	
Relative humidity[2]										
mean				0.015, 0.07					0.014, 0.07	
min				0.017, 0.07						
Vapor pressure[2]		0.001, 0.17								0.002, 0.21
Total explained variance[3]	0.739	0.704	0.799	0.701	0.401	0.591	0.823	0.708	0.719	0.507

[2]Autumn weather parameters

[3]Explained variance by multivariate best fit models selected by stepwise regression

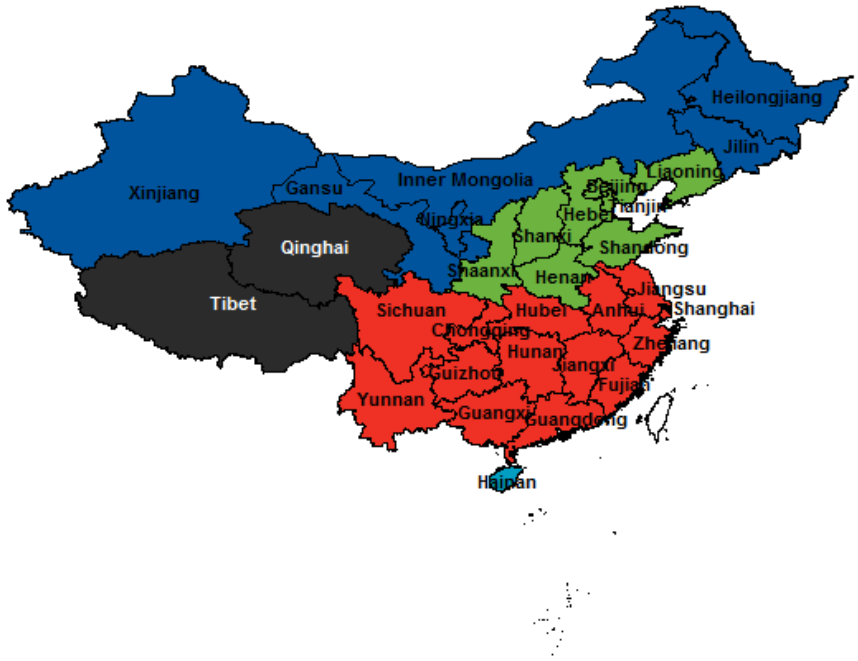


Appendix Figure 1. Flowchart of data and sample collection for HFMD cases, 2008-12, China

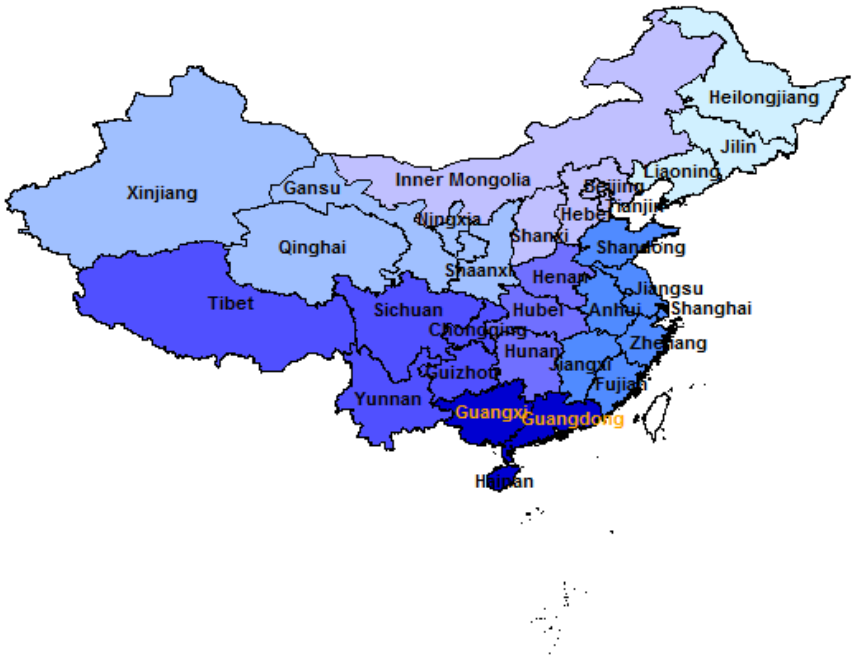
The enhanced surveillance system requires that each probable or confirmed case be reported on-line to the national CDC within 24 hours of

diagnosis. Data are updated as appropriate throughout the course of illness, and particularly at the conclusion of each episode. AFP=acute flaccid paralysis. ANS=autonomic nervous system.

A



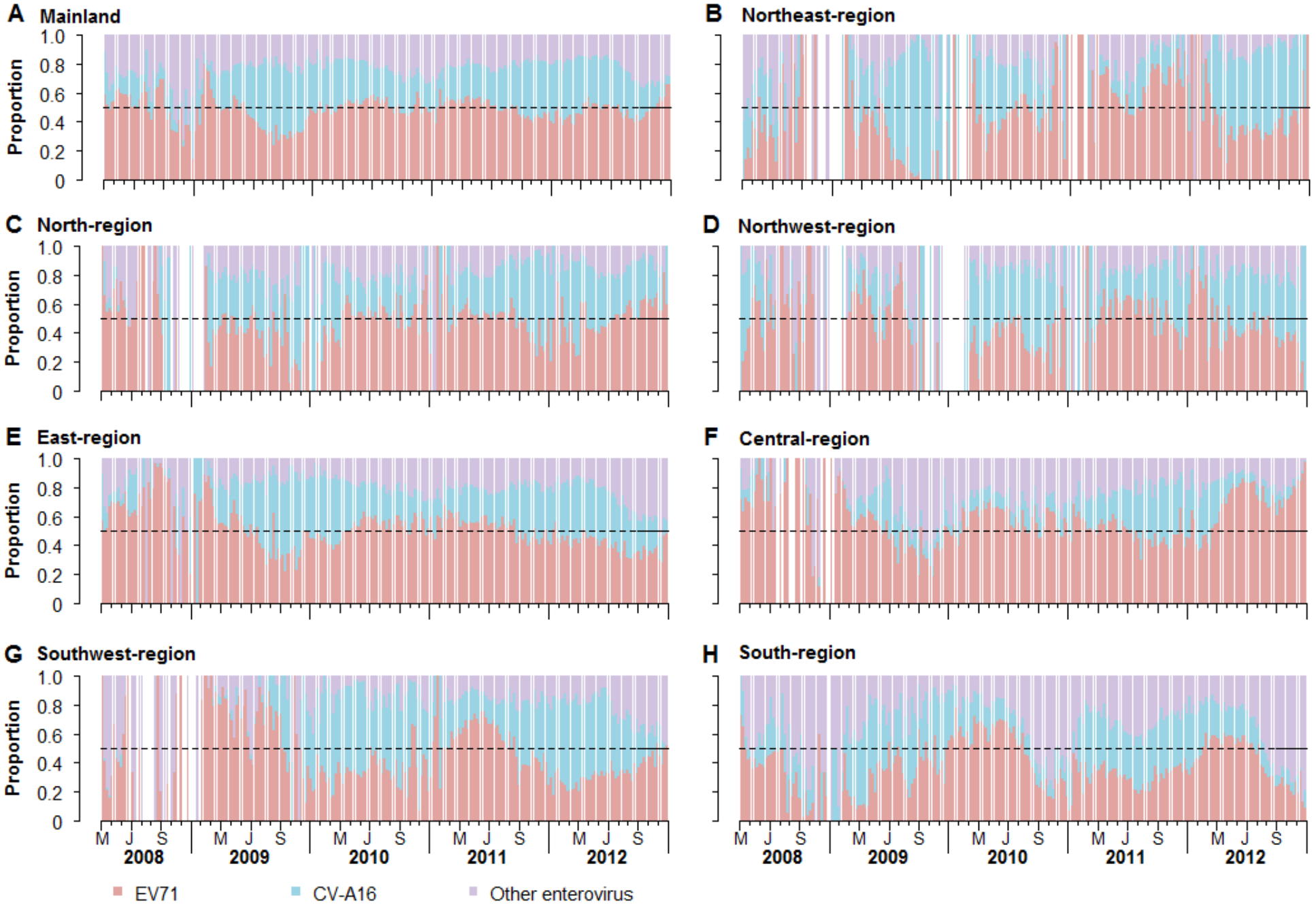
B



Appendix Figure 2. Map of the Chinese mainland provinces

31 Chinese mainland provinces were included in this analysis. The different colors represent the different climatic or geographic regions. (A) Geographic regions in Mainland China. Blue: mid-temperate region; green: warm-temperate region; black: cold region; red: sub-tropic region; turquoise: tropic region. (B) Geographic regions in Mainland China. Northeast: Liaoning, Jilin, Heilongjiang; North: Beijing, Tianjing, Hebei, Shanxi, Inner Mongolia; Northwest: Shaanxi, Gansu, Qinghai, Ningxia,

Xinjiang; East: Shanghai, Jiangsu, Zhejiang, Anhui, Fujian, Jiangxi, Shandong; Central: Henan, Hubei, Hunan; Southwest: Chongqing, Sichuan, Guizhou, Yunnan, Tibet; South: Guangdong, Guangxi, Hainan.


A Mainland
B Northeast-region
C North-region
D Northwest-region
E East-region
F Central-region
G Southwest-region
H South-region
Proportion
1.0
0.8
0.6
0.4
0.2
0
M J S
2008
2009
2010
2011
2012
EV71
CV-A16
Other enterovirus

Appendix Figure 3. Proportions of enterovirus serotypes among laboratory-confirmed HFMD cases by week of illness onset and by geographic region, 2008-12, China

EV71=enterovirus 71. CV-A16=Coxsackievirus A16.

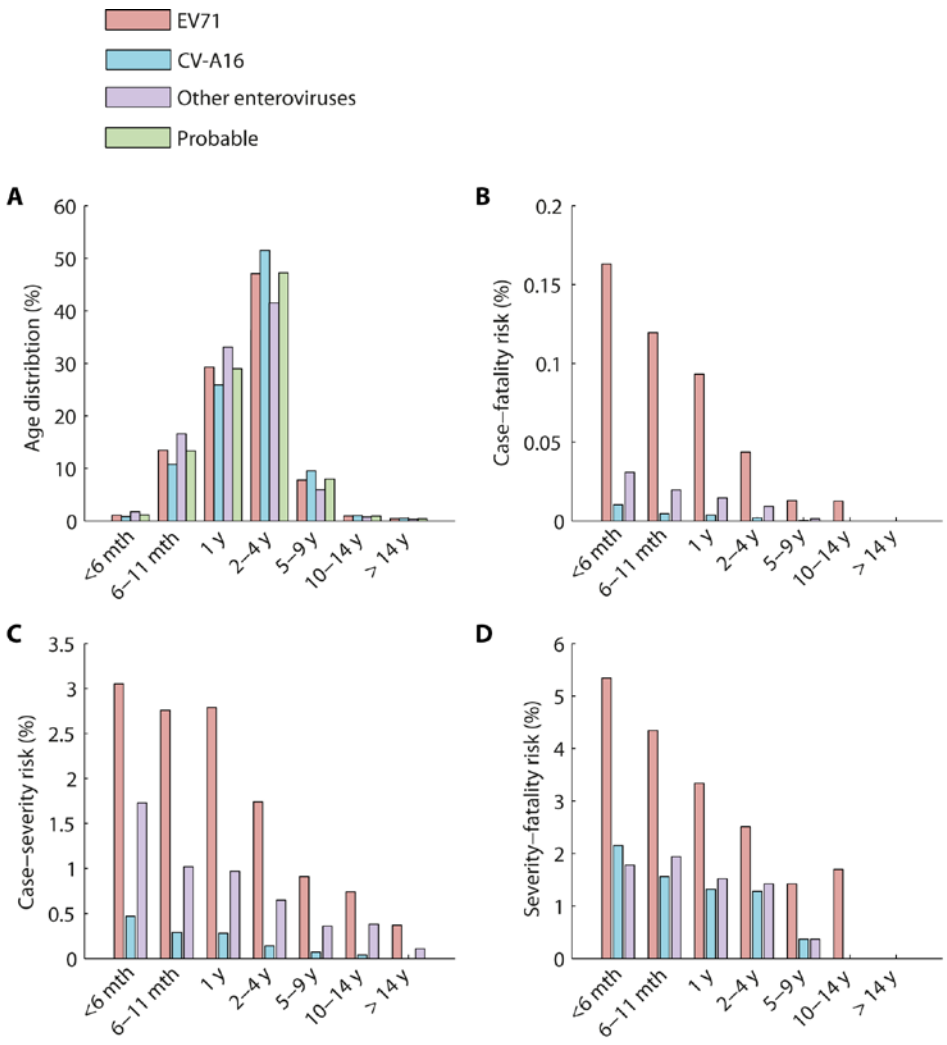


Figure 4. Age distribution and clinical severity of probable and laboratory-confirmed HFMD cases, 2010-12, China

(A) Age distribution of probable and lab-confirmed cases. (B) Risk of fatality among cases by age group and viral etiology. (C) Risk of severe illness among cases by age group and viral etiology. (D) Risk of fatality among severe cases by age group and viral etiology. EV71=enterovirus 71. CV-A16=Coxsackievirus A16. mth=months. y=years.

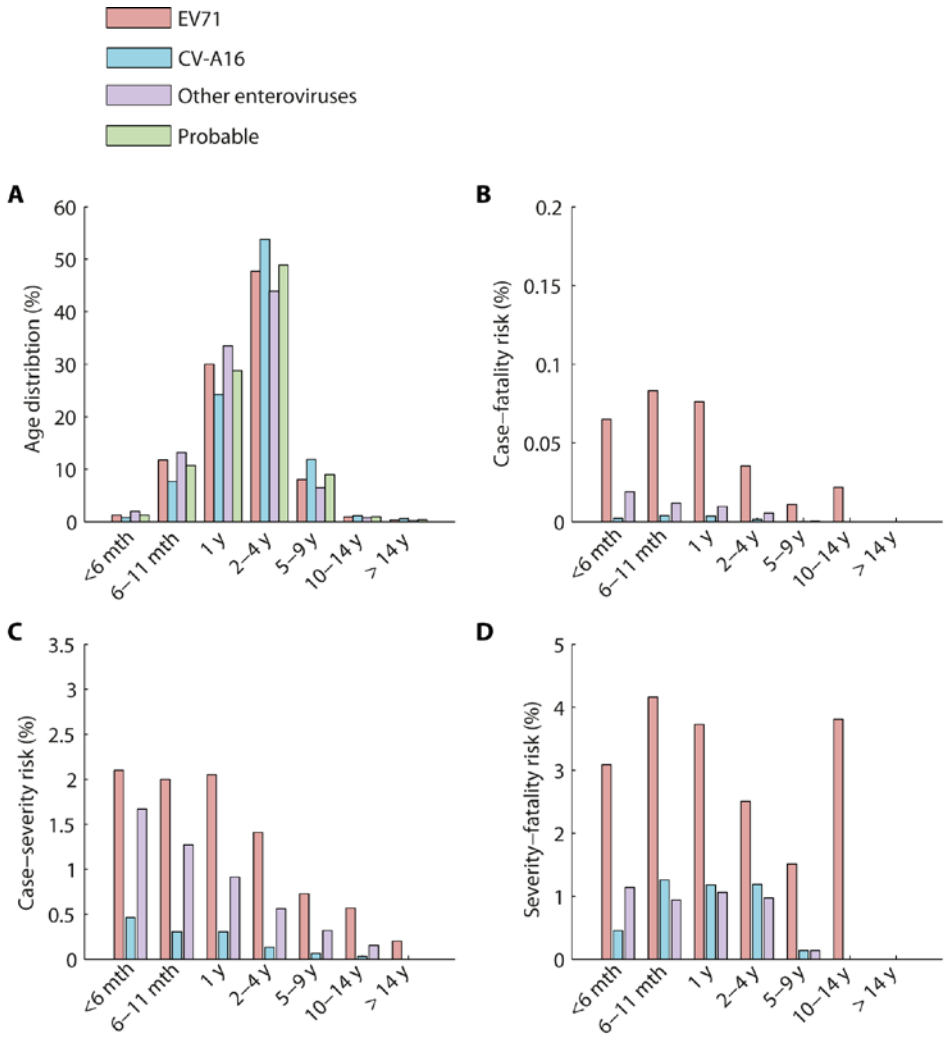
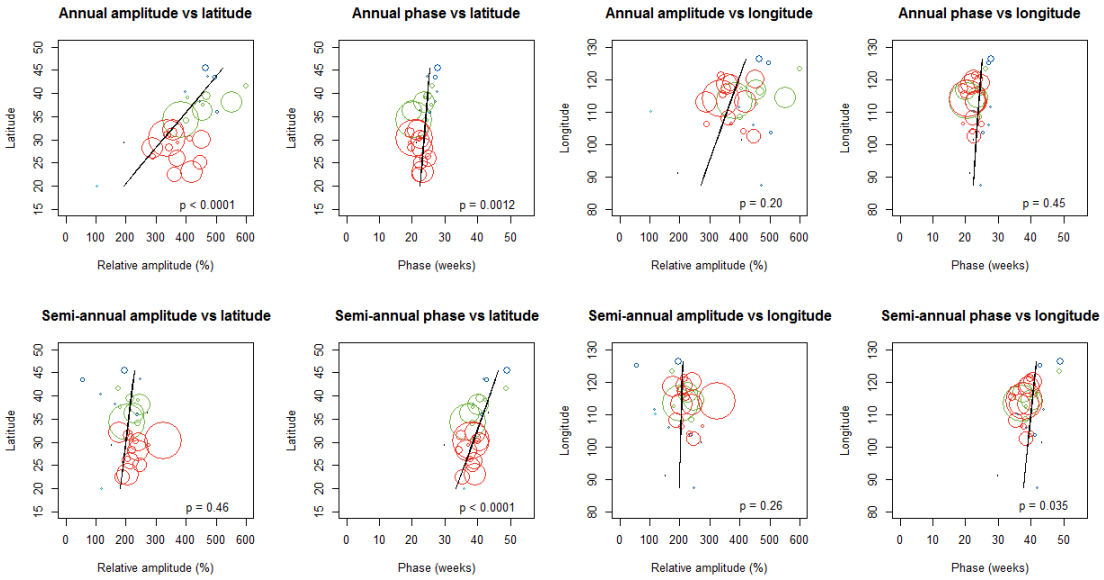


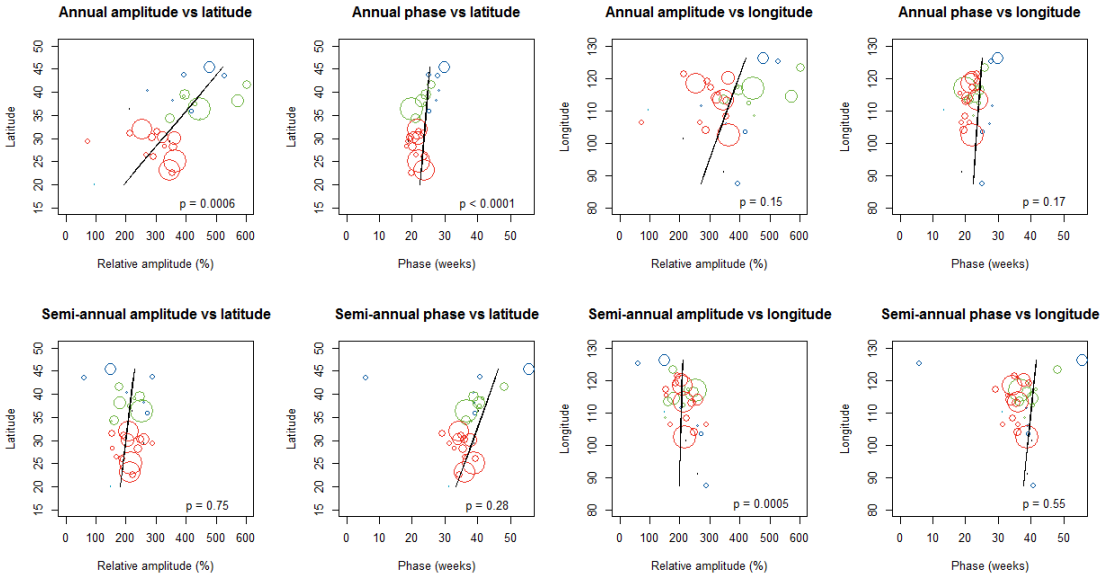
Figure 5. Age distribution and clinical severity of probable and laboratory-confirmed HFMD cases, 2012, China

(A) Age distribution of probable and lab-confirmed cases. (B) Risk of fatality among cases by age group and viral etiology. (C) Risk of severe illness among cases by age group and viral etiology. (D) Risk of fatality among severe cases by age group and viral etiology. EV71=enterovirus 71. CV-A16=Coxsackievirus A16. mth=months. y=years.

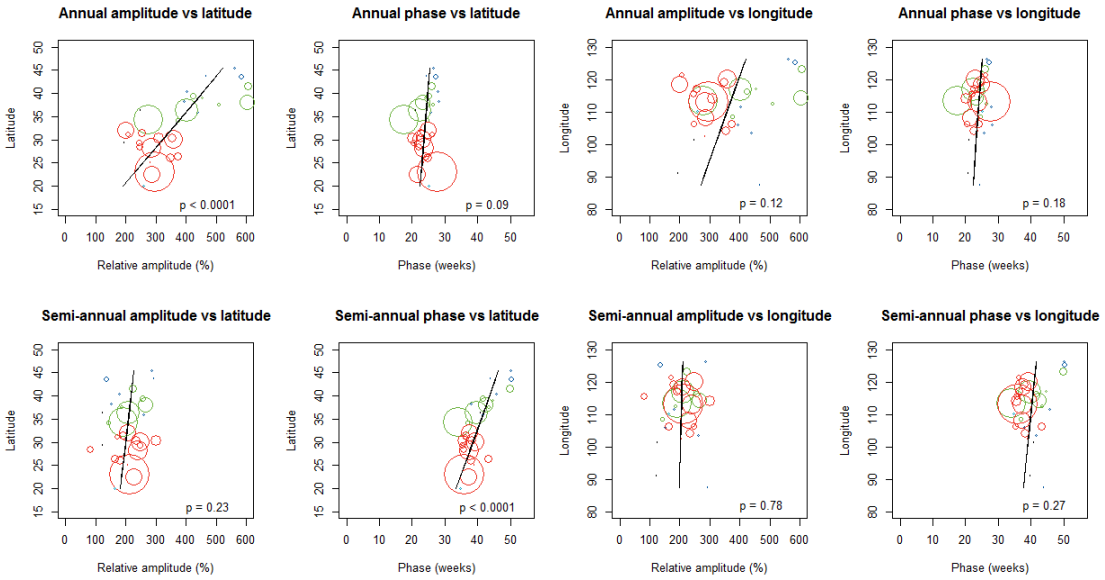
EV71 epidemics



CV-A16 epidemics

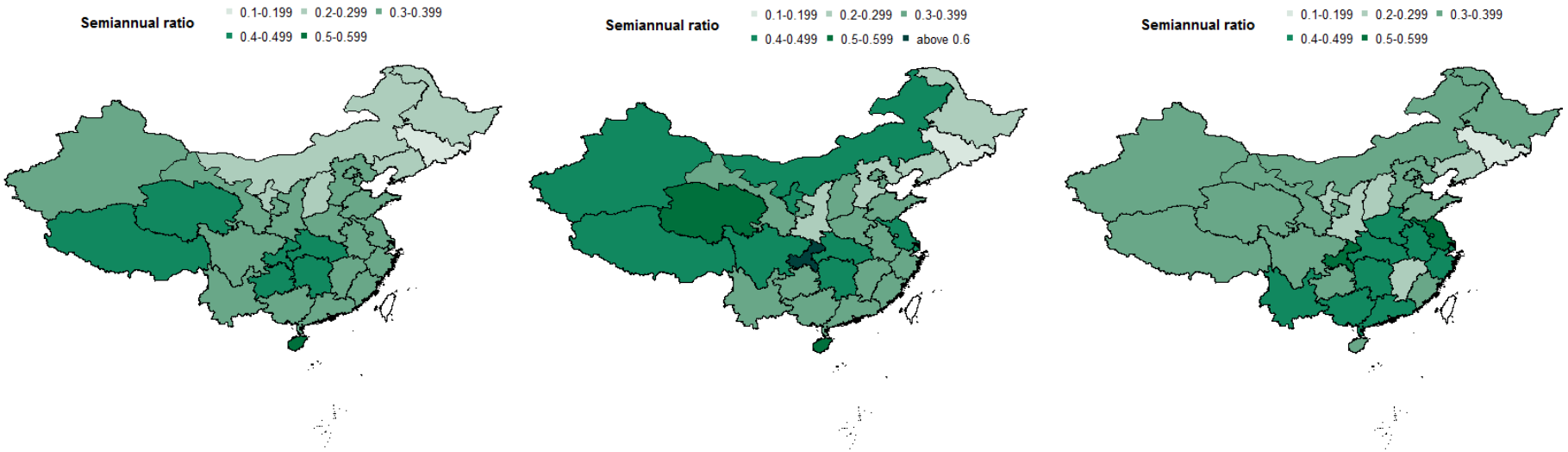


Other enterovirus epidemics



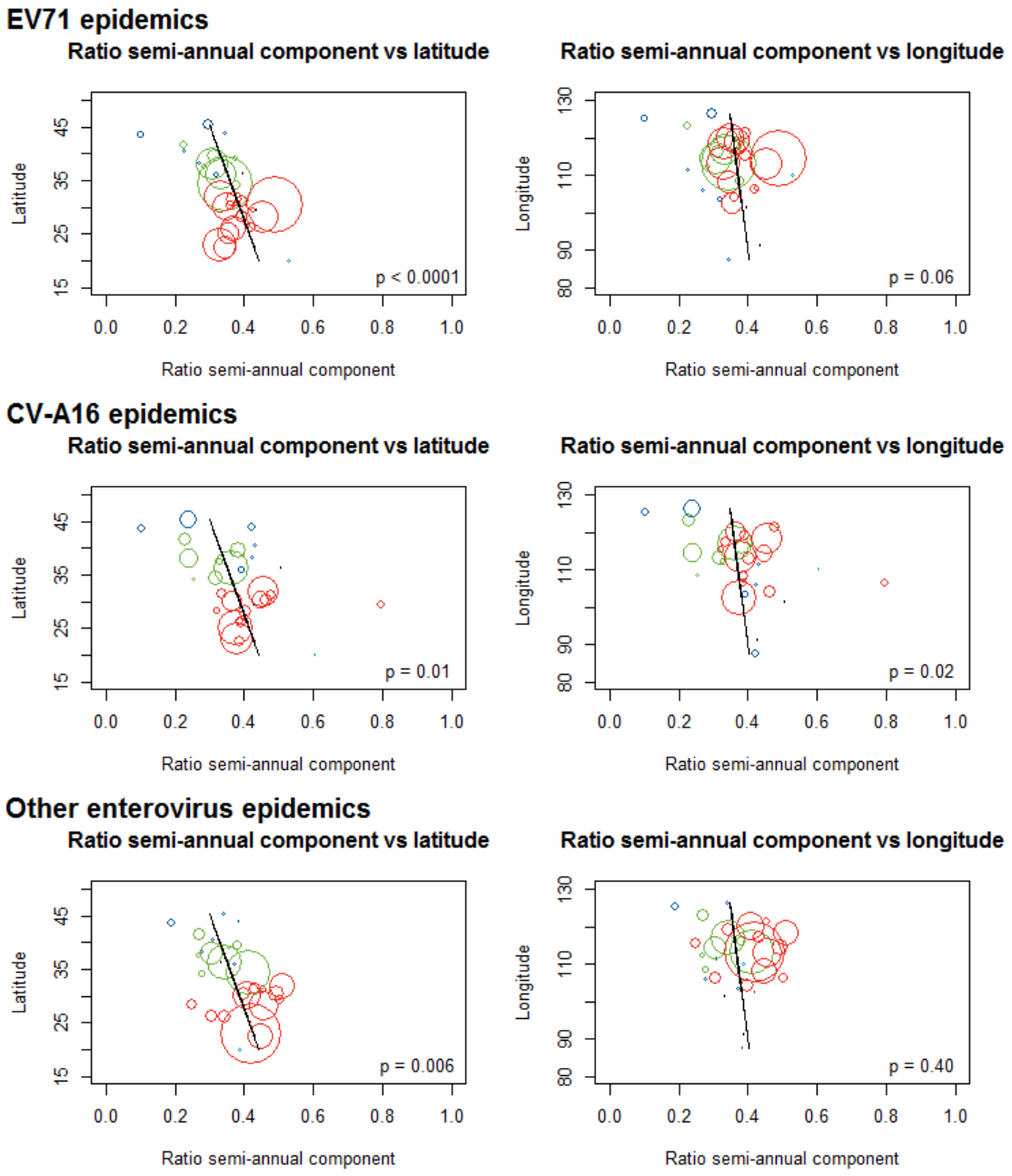
Appendix Figure 6. Latitudinal and longitudinal gradients in seasonality of EV71, CV-A16 and other enterovirus

Figure shows that all enteroviruses exhibited latitudinal gradients in the amplitude of annual and semiannual epidemics. Annual peak timing of CA-V16 activity occurred earlier than for other enteroviruses (p=0·0148). Southern provinces experienced earlier spring and fall epidemics of EV71 and other enterovirus than Northern provinces (difference between the annual peak timing of epidemics in Northern and that in Southern in EV71: p=0·031, in other enterovirus: p=0·21; difference between the semiannual peak timing of epidemics in Northern and that in Southern in EV71: p= 0·0008, in other enterovirus: p= 0·0008). The timing of fall EV71 epidemics was also associated with longitude, with later occurrence in coastal provinces. EV71=enterovirus 71. CV-A16=Coxsackievirus A16.



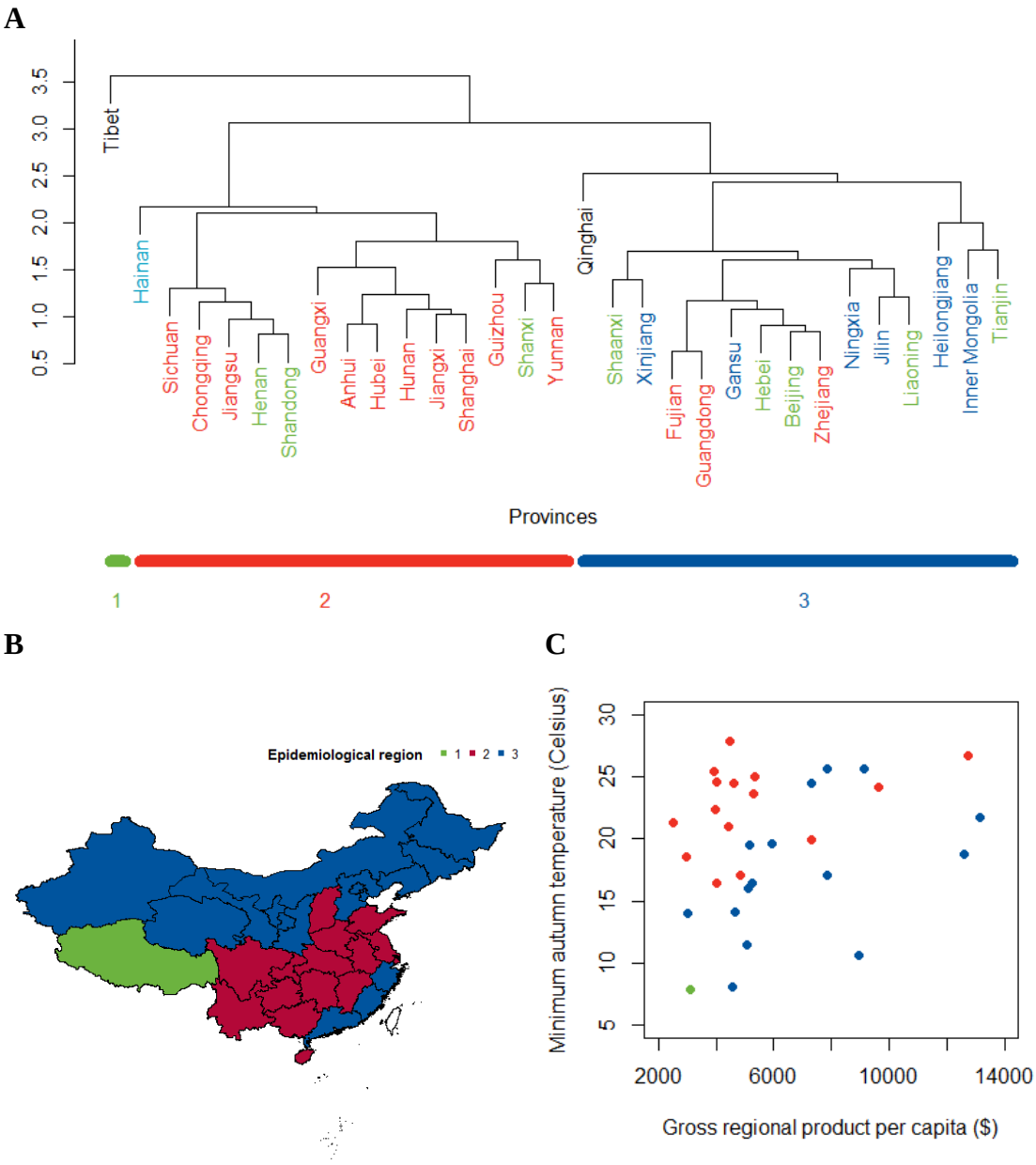
Appendix Figure 7. Periodicity of EV71, CV-A16 and other enterovirus epidemics in China

Importance of the semiannual periodicity, measured by the ratio of the amplitude of the semiannual cycle to the sum of the amplitudes of annual and semiannual cycles. EV71=enterovirus 71. CV-A16=Coxsackievirus A16.



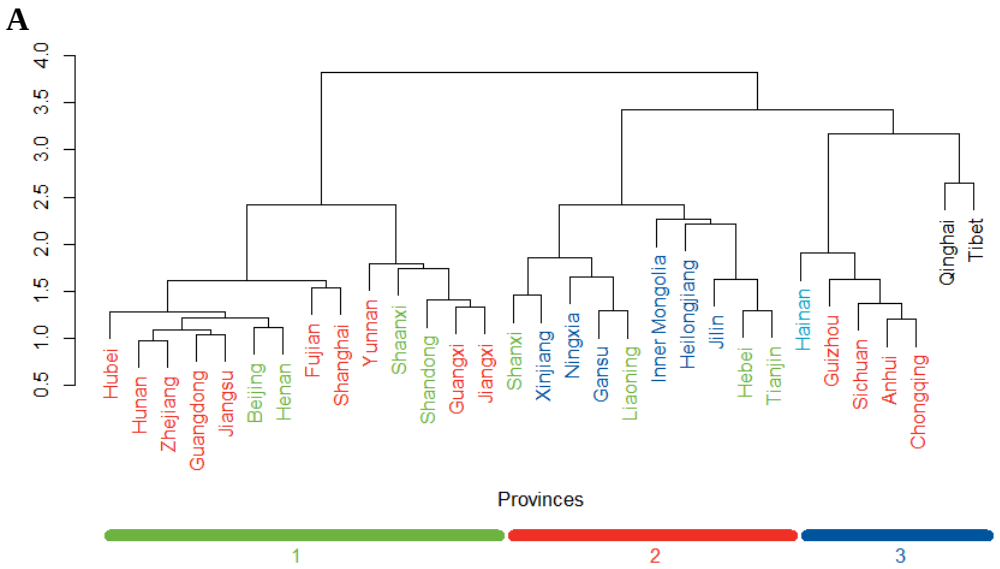
Appendix Figure 8. Importance of semiannual seasonality of EV71, CV-A16 and other enterovirus

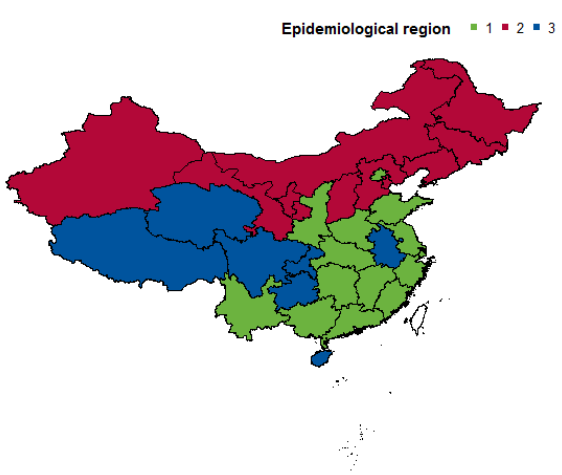
Importance of the semiannual periodicity, measured by the ratio of the amplitude of the semiannual cycle to the sum of the amplitudes of annual and semiannual cycles. EV71=enterovirus 71. CV-A16=Coxsackievirus A16.

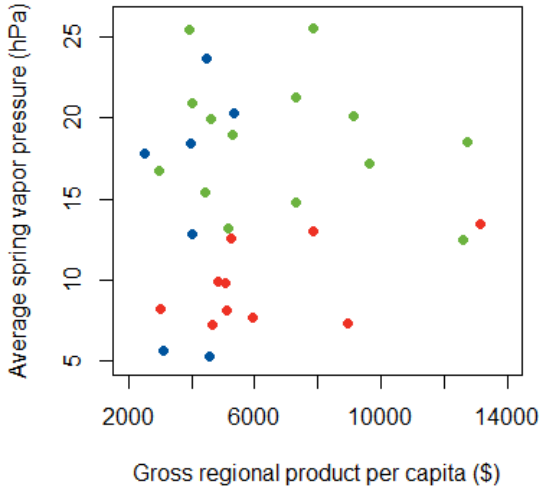


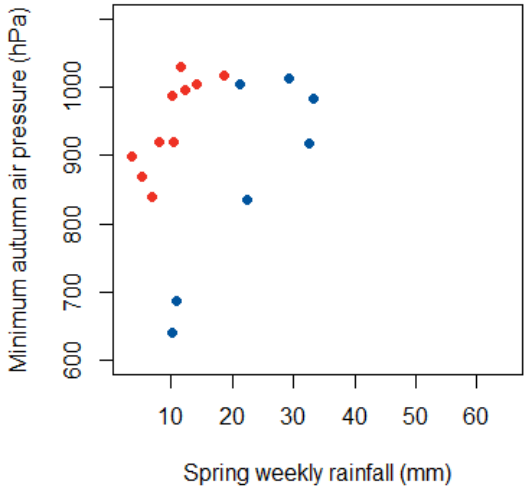
Appendix Figure 9. EV71 epidemiological regions and predictors

(A) Identified epidemiological regions based on hierarchical clustering, using the Euclidian distance between weekly standardized EV71 time series. Provinces are color-coded by climatic region (see legends of appendix figure 1). (B) Map of the three epidemiological regions identified in panel A. (C) Predictors of the two main clusters identified in panel A, based on stepwise discriminant analysis.


A
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0
Hubei
Hunan
Zhejiang
Guangdong
Jiangsu
Beijing
Henan
Fujian
Shanghai
Yunnan
Shaanxi
Shandong
Guangxi
Jiangxi
Shanxi
Xinjiang
Ningxia
Gansu
Liaoning
Inner Mongolia
Heilongjiang
Jilin
Hebei
Tianjin
Hainan
Guizhou
Sichuan
Anhui
Chongqing
Qinghai
Tibet
Provinces
1
2
3

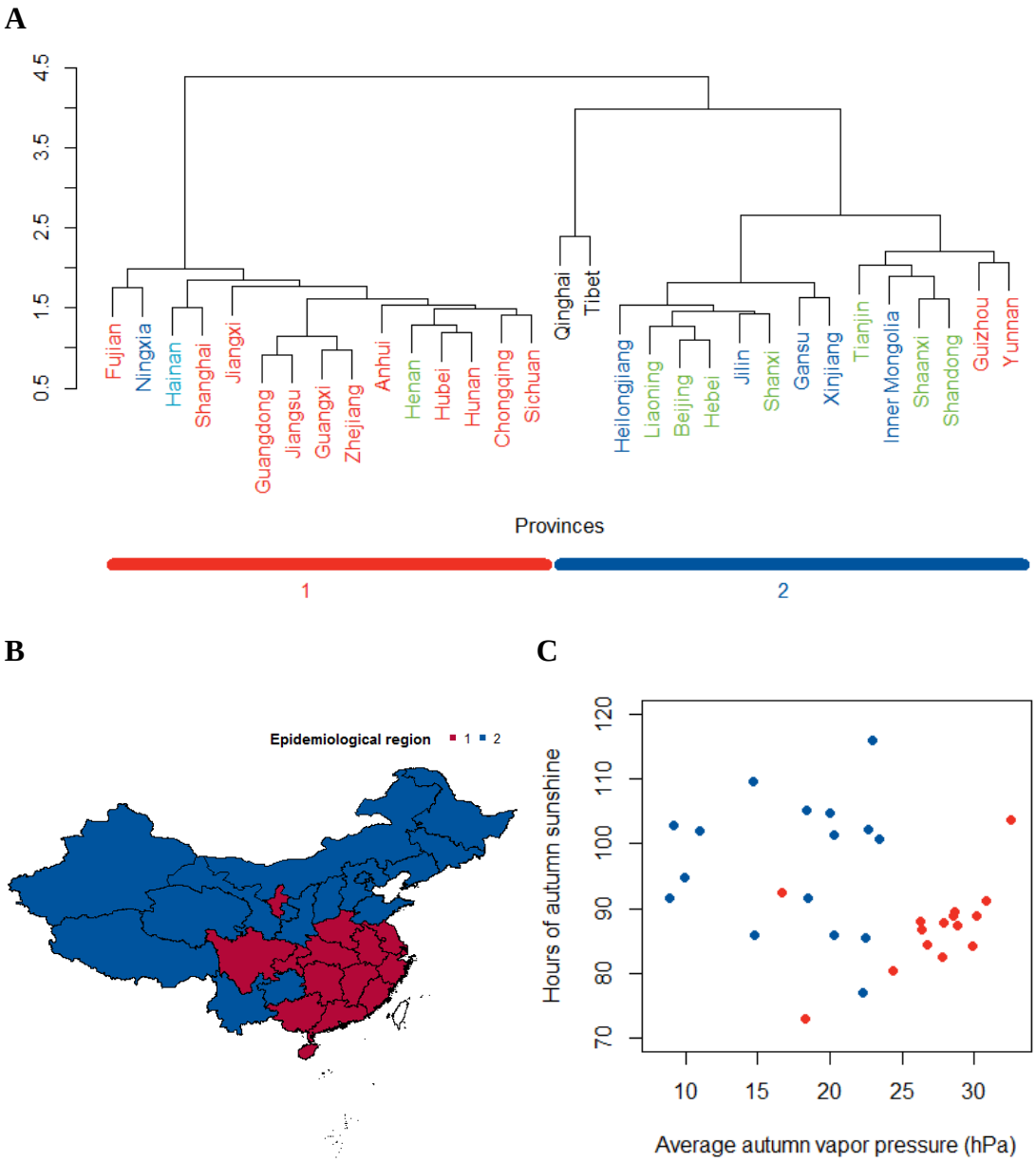

B
Epidemiological region 1 2 3


C
Average spring vapor pressure (hPa)
5 10 15 20 25
2000 6000 10000 14000
Gross regional product per capita ($)


D
Minimum autumn air pressure (hPa)
600 700 800 900 1000
10 20 30 40 50 60
Spring weekly rainfall (mm)

Appendix Figure 10. CV-A16 epidemiological regions and predictors

(A) Identified epidemiological regions based on hierarchical clustering, using the Euclidian distance between weekly standardized CV-A16 time series. Provinces are color-coded by climatic region (see legends of appendix figure 1). (B) Map of the three epidemiological regions identified in panel A. (C) Climate predictors of the two main clusters identified in panel A, based on stepwise discriminant analysis. (D) Climate predictors of the two sub-clusters identified in panel A, based on stepwise discriminant analysis.



Appendix Figure 11. Other enteroviruses epidemiological regions and predictors

(A) Identified epidemiological regions based on hierarchical clustering, using the Euclidian distance between weekly standardized other enterovirus time series. Provinces are color-coded by climatic region (black: cold-temperate, blue: mid-temperate, green: warm temperate, orange: subtropical, red: tropical). (B) Map of the three epidemiological regions identified in panel A. (C) Climate predictors of the two main clusters identified in panel A, based on stepwise discriminant analysis.