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Seroprevalence of Enterovirus 71 Antibody Among Children in China: A Systematic Review And Meta-Analysis

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Abstract

Background—Hand, foot and mouth disease mostly affects children and carries a substantial disease burden in the Western Pacific region. Enterovirus 71 (EV71) is the most virulent causative agent, and a monovalent vaccine against EV71 will soon become commercially available in China. An improved understanding of EV71 epidemiology could aid policy decisions regarding childhood immunization in China.

Objective—We aimed to assess and summarize information to date from individual seroepidemiologic studies of EV71 in mainland China in order to determine patterns of the age-specific risk of infection.

Methods—A systematic review and meta-analysis of studies of children aged 0–15 years, published in English or Chinese, was conducted. Estimates of seroprevalence were summarized by age group. A mixed-effects regression model was used to explore factors co-varying with EV71 seroprevalence.

Results—We identified 42 published studies, including 15 in English. We found that an average of 78% of neonates were seropositive to EV71 infection but such maternally conferred immunity almost completely waned by 5 months. The seroprevalence of EV71 antibody increased directly with age among pre-school children, from 26% (95% CI, 18–33%) at 1 year to 70% (95% CI, 62–78%) at 5 years. Age of subjects, sample size, sampling year, sampling method, geographic latitude and publication language were associated with variations of individual seroprevalence estimates.

Conclusions—Seroprevalence of EV71 antibody gradually declined during the first five months in infants. Infection with EV71 was most likely to occur between 2 and 4 years. Our findings may be useful in informing population-based EV71 vaccination strategies.

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CONFLICTS OF INTEREST

The authors report no other potential conflicts of interest.

Keywords

enterovirus; seroprevalence; children; meta-analysis

INTRODUCTION

Hand, foot and mouth disease (HFMD) is a childhood disease causing substantial disease burden, particularly in Western Pacific Region [1–4]. Although HFMD is mainly a self-limited disease, severe complications may occur including brain-stem encephalitis, acute flaccid paralysis and aseptic meningitis [5, 6]. From 2008 through 2012, 7.2 million cases of HFMD were reported in mainland China, 2,457 were fatal [7]. Several serotypes of human enterovirus, including enterovirus 71 (EV71), Coxsackievirus A6 and A16 (CV-A6 and CV-A16), are considered to be common causative pathogens of HFMD [6]. In severe and fatal HFMD cases, EV71 is more commonly identified as the likely causative enterovirus type compared to the mild HFMD cases where CV-A16 was more commonly identified [6, 8, 9]. EV71 is therefore thought to be the most virulent serotype.

HFMD has been the most frequently reported notifiable infectious diseases and led to the largest number of deaths in children among pediatric notifiable diseases in mainland China since 2009 [10]. Severe EV71 cases develop to complications within 2 to 3 days after initially showed mild symptoms and the median time from diagnosis to death was 0.5 days for severe HFMD cases which were mostly caused by EV71 [10–14]. It is therefore necessary to develop a cost-effective vaccine particularly to prevent severe infections since currently no specific treatment is available for HFMD [6, 11]. Five manufactures from mainland China, Taiwan and Singapore have developed inactivated EV71 vaccines [11–15]. All three vaccines from mainland China were developed by the use of EV71 subgenotype C4a strains, while the Taiwanese and Singaporean vaccine used B4 and B2 strain, respectively [11, 12]. Phase III clinical trials have been finished on the three vaccines developed in mainland China. They all showed high efficacy (90.9–97.4%) in protecting against EV71-associated HFMD, and will likely be commercially available to the public soon [13–15]. Questions of the target population and immunization schedule are major considerations for Chinese policy makers when determining the potential impact and cost-effectiveness of population-based EV71 vaccination strategies [11].

The age-specific seroprevalence of EV71 antibody, estimated in the absence of vaccine, can provide a population profile of the risk of infection with the virus in different ages, contributing to decision making on vaccination implementation strategies. A number of serologic studies have been conducted in mainland China in different settings with various study designs. The objective of our study was to evaluate and summarize the information from these studies to guide vaccination policy decisions for mainland China. As part of our analysis, we explored potential factors that might affect the estimates of seroprevalence.

METHODS

Search Strategy

We searched for published studies on the prevalence of antibody against EV71 virus in children in mainland China. We searched PubMed for publications in any language, and the China National Knowledge Infrastructure (CNKI) and the Wanfang (WF) databases for publications in Chinese. The following keywords were used in the English literature search: ("hand foot and mouth disease" OR "HFMD" OR "enterovirus 71" OR "EV71" OR "HEV71") AND ("seroprevalence" OR "seroprevalent" OR "seronegative" OR "seropositive" OR "seroepidemiology" OR "seroepidemiological" OR "serologic" OR "serological" OR "antibody") AND ("China" OR "Chinese"). We used Chinese translations of the key words (Appendix Table 1) to search the Chinese databases.

Study Selection

We screened in the title, abstract and full text of all articles obtained from the database search. We only included original studies investigating the seroprevalence of EV71 antibody in recovery phase in children ≤15 years of age in mainland China. We excluded studies recruiting clinical HFMD patients only and EV71 vaccine trials that did not provide pre-vaccined seroprevalence data [16, 17].

Data extraction

We extracted relevant information from each study onto a standardized form including the sampling period, sampling method, geographic location, age of subjects, assay used for antibody detection, study endpoint, threshold to define seropositivity, and the estimates of seroprevalence in different age groups. Seroprevalence is defined as the proportion of children testing positive for EV71 serum antibody among all children providing blood samples. Seropositivity was pre-defined in studies, and the endpoint and cut-off for determination of seroposivity was summarized for each study. Potential heterogeneity associated with varied definitions for seropositivity was further explored in later analyses. We estimated the 95% confidence interval (95% CI) of seroprevalence estimates using the Wilson method [18, 19].

Risk of bias

The study was conducted following the Preferred reporting items for systematic reviews and meta-analysis (PRISMA) statement (Appendix Table 2) [20]. Sampling methods, assay methods and the publication language of each study were used as proxies of risk of bias for individual studies [21]. Effects of the potential bias on seroprevalence estimates were examined in the meta-regression analysis.

Statistical analysis

Random-effects model was used to summarize the pooled mean of seroprevalence in each subgroup [22]. Cochran's Q test and the I^2 statistic were used to identify and quantify heterogeneity among included studies [20, 21]. An I^2 value more than 75% indicates high heterogeneity [24]. A linear mixed-effects meta-regression model was used to investigate

the effect of potential factors on the estimate of seroprevalence by weighting the estimates with the inverse of their variances. An omnibus test was used for the moderator test in the mixed-effects model [25]. The potential factors that were examined in the model included: language of publication, age of subjects, year of the study, season of sampling, sampling method, sample size, laboratory assay used for antibody detection and latitude of the study location. We divided China into two main epidemic regions – North and South – by the latitude of around 35°N according to previous epidemiological studies [7]. We defined the HFMD season according to the location of a study, i.e. the season of HFMD for studies conducted in northern China was from April to July while it was from April to October for studies in southern China, since HFMD occurs in the autumn in the south in addition to the main peak in the spring/summer observed both in the north and south of the country. We classified the sampling method used in each study as either (1) “random sampling”, referring to studies using a random or stratified random sampling method for selection of subjects; (2) “physical examination”, referring to studies using residual sera that were originally collected for the purpose of medical assessments; or (3) “trial cohort”, referring to studies using baseline sera collected from children participating in randomized controlled trials of EV71 vaccines. All analyses were conducted in R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We identified 42 articles that met the eligibility criteria [12, 13, 16, 17, 23–60], 15 studies in English and 27 in Chinese (Figure 1). Studies were conducted in 18 of the 31 provinces in mainland China, mostly (34/42) in the east and south (Figure 2). One study was conducted across 6 provinces [34], and we extracted separate data for each of the 6 provinces. Guangdong and Jiangsu were the most frequently studied provinces with 12 and 11 reports respectively. Table 1 summarizes the characteristics and methods used in each study.

Although serum samples were collected between 1999 and 2013, most were from 2010 (15 studies) or 2011 (10 studies). Ten studies, eight in Chinese, failed to describe their sampling methods explicitly. Neutralization assays (NTA) were used by most of the studies (32/42). Among studies using NTA, twenty-four studies set the endpoint value as the titer inhibiting 50% of cytopathogenic effect (CPE) and defined the positive cut-off as antibody titer ≥1:8. All ten studies applying the Enzyme-linked Immunosorbent Assay (ELISA) to detect EV71 antibody used the optical value density at 450nm (OD_{450}), while the cut-off for seropositivity was not reported in most (8/10) studies.

Seroprevalence among infants 0–12 months of age was reported in 23 studies. 9 reported seroprevalence at individual months of age, which mainly focused on infants aged 8 months and younger (Appendix Figure 1). In general, seroprevalence declined with age in younger infants, from 78% (95% CI, 72–85%) at birth to 10% (95% CI, 4–16%) at five months of age, and fluctuated at a generally low level (10–22%) from 6 months through 1 year of age (Figure 3, Appendix Figure 1). Limited estimates of individual month groups were available among infants ≥ 9 months, estimates based on studies on a broader age group however revealed a similarly low level of seroprevalence (Appendix Figure 1). Weighting seroprevalence estimates by population sizes of provinces involved into these studies did not

substantially change the pooled estimates of seroprevalence (data not shown). Seroprevalence estimates among infants were largely consistent across different studies within each one-month group (I^2 statistics range: 0–63.6%, $p>0.05$) except for the 2 and 7 months groups (I^2 statistics: 76.1% and 89.3% respectively, $p<0.01$).

We identified 108 estimates of seroprevalence for children of 1–15 years from 21 studies reporting seroprevalence for individual ages (Figure 3, Appendix Figure 2). The seroprevalence of EV71 antibody directly increased with age among pre-school children, from 26% (95% CI, 18–33%) at 1 year to 70% (95% CI, 62–78%) at 5 years. Among children ≥6 years of age, declining seroprevalence with age was reported in one study [57]. There was substantial heterogeneity in estimates of seroprevalence within each age group (I^2 statistics range: 70.1–94.6%, $p<0.001$). The results of a sensitivity analysis, weighting the estimates of seroprevalence with the population size of the underlying province, were similar with unweighted estimates (results not shown).

We conducted a meta-regression analysis on the seroprevalence of EV71 antibody reported for children 1–5 years. 76% of the heterogeneity can be explained by our model (Table 2). Except for the type of laboratory assays and the season of study period, factors included in the meta-regression model substantially affected estimates of seroprevalence (Ommibus test: $p <0.001$). After accounting for potential factors and biases, the estimates of seroprevalence still positively correlate with age (Table 2). Studies conducted in larger epidemic years or in the north of China were more likely to report higher estimates of seroprevalence. Sampling methods may introduce bias in estimation of seroprevalence as studies using non-random sampling methods tended to report lower estimates of seroprevalence compared with studies using randomly sampled sera (Table 2). “Trial cohort” was not included in the final meta-regression model since estimates from the vaccine clinical trials were all by grouped ages. Published language is another potential source of bias, noting studies published in English-language journals were likely to report lower estimates compared with those published in Chinese journals (coefficient: −0.133, 95%CI (−0.213, −0.053), $p < 0.05$).

DISCUSSION

We were able to identify important patterns in the risk of infection by age and geography. Our meta-analysis showed that, on average, 78% of neonates in China investigated were seropositive (Figure 3, Appendix Figure 1), somewhat higher than reported in studies conducted in Viet Nam, Thailand and Singapore (ranging from 44–67%) [61–63], suggesting a high level of maternal antibody against EV71 infection in China, perhaps due to higher historical incidence of infections among adults [17, 29, 41, 64]. In general, the titer and duration of maternal immunity of infants are associated with the level of mother’s immunity [32]. Maternal antibody waned at about 5 months, which is comparable with the waning time of maternal immunity to EV71 in other countries and regions and to maternal immunity to CV-A16 [65, 66]. The age pattern of seroprevalence to EV71 in infants observed in our study was consistent with the age distribution of confirmed HFMD cases infected with EV71 reported through national surveillance. During 2008–2012, cases under 6 months only accounted for around 2% of all reported EV71 cases, while 15% of reported EV71 cases were 6–11 months of age [7].

The mean seroprevalence of EV71 antibody increased from 24% at 1 year of age to 70% at 5 years of age, indicating a substantial incidence of infection (Figure 3, Appendix Figure 1). Studies in other regions also revealed similar trends suggesting that preschool children are at a high risk of infection with EV71 [61, 62, 67, 68]. Notifiable disease surveillance data in China showed that about 50% of probable and confirmed EV71-associated HFMD cases occur in children 2 to 4 years of age [7]. One factor possibly driving this high risk of EV71 infection in young children is admission to kindergarten at around 3 years of age in China. In children 6 to 15 years of age, EV71 seroprevalence decreased with age, although this observation is based on estimates from just one study [57]. Previous findings as well as the estimates reported in our included studies of broader age groups (Appendix Figure 3) show that seroprevalence tends to fluctuate after 6 years of age at a generally high level [61, 67], suggesting the possibility of the presence of a long-lasting immunity. However, the geometric mean titer (GMT) of EV71 antibody wanes after peaking at around 5 years of age [32, 44, 46, 50, 56]. The relation between the titer level and protection against re-infection is unknown [69], so immunity to EV71 infection in older children is not clear at present [11].

Substantial heterogeneity was not detected in estimates of seroprevalence among infants except for those at age of 2 and 7 months (Appendix Figure 1). We noticed that one study with a relatively large sample size conducted by Zhu et al. [26] reported very high estimates of seroprevalence for children at age of 2 and 7 months. It might lead to an overestimated mean seroprevalence in the 7-month group, which however is not likely to substantially affect our main findings. Relatively high heterogeneity was observed in estimates for 1–5 years while most variations can be explained by variables included in the meta-regression model. The unaccounted variability may be due to some unmeasured factors in studies, e.g. possible variations in timing of specimen collection, storage of sera or choice of virus strains in laboratory testing.

In our study, EV71 seroprevalence in China and in larger epidemic years was higher than estimates from other Asian areas (ranging from 27.8% to 60.9%) [61, 63, 67, 70]. Our analysis revealed an increasing trend in seroprevalence with a more recent year of sampling except for the year of 2009, which is consistent with the causative pathogens in China HFMD epidemics. Similar findings were also reported in Japan. It has not been determined whether the seroprevalence of EV71 antibody is a reliable predictor for the HFMD incidence in the future [71, 72]. Studies conducted in northern China reported a higher EV71 seroprevalence although the southern areas tend to have longer periods of EV71 activity with two epidemics in some years [7]. However several large outbreaks of HFMD have been reported in northern China recently [73, 74]. Russia, a high-latitude country, also reported a relatively higher seroprevalence (83% in children 5 years) but with lower HFMD incidence in a recent study [73]. Although higher environmental temperatures and humidity are associated with the higher HFMD incidence in China [75–77], HFMD occurrence is likely to be driven by a range of factors not limited to environmental conditions, such as population structure, birth rates, urbanization and the prevalent circulating strains [63, 75, 78]. Due to limited data, we were not able to further compare the age-specific risk of EV71 infection between northern and southern China.

The analysis results (Table 2) suggest that biases in seroprevalence estimates could be introduced by sampling methods and language of publication. Studies using residual sera originally collected for physical examination were likely to report lower estimates compared with those studies using random sampling because some “physical examination” studies reported that sera from subjects who reported previous history of HFMD or fever were excluded from the analysis and therefore less likely to be infected by EV71. Higher estimates of seroprevalence of EV71 antibody were reported in studies published in Chinese-language journals suggesting the possibility of selective reporting by local literature. Similar local literature bias was suggested in a previous study for 13 different diseases [80]. Laboratory testing assays were not suggested to be a factor partially explaining the heterogeneity across the studies, which is consistent with a previous study on IgM antibody against EV71 [80].

Our study shows that infants become susceptible to EV71 infection after 5 months of age, and a substantial fraction of infections occur between 2 and 4 years of age. Therefore, it was proposed that the forthcoming 2-dose EV71 vaccination should be given to children at 6 and 7 month of age respectively with a 4-week interval between two dosages [12]. A booster dose might be given at ages of 18–24 months [12] given the undetermined duration of vaccine derived immunity and protective antibody [12–14]. Vaccine-derived EV71 antibody titer experienced a slightly decrease in the first 6–7 months after injection of the second dose (two-dose vaccines) and stayed relatively stable in the following 6 months [13, 15]. Currently we lack information on the long-term duration of vaccine derived EV71 antibody which however tends to be shorter than the natural immunity for other vaccine-preventable disease [83]. Further evidence is needed to determine the optimal time period for a booster dose to avoid a potential window of EV71 susceptibility. Population at high risk of EV71 infection may shift to older age groups after implementation of childhood EV71 mass vaccination because of waning of the vaccine-derived immunity and declined risk of infection in whole population. Low incidence of EV71 infection in post-vaccination period would also decrease the natural immunity in women of childbearing age, which as a result would lead to a lower level and possibly shorter duration of maternal immunity in neonates and infants [32, 84, 85].

There are some limitations of our study. First, estimates included in our analysis were mainly from the east and south of China which are more densely populated and economically developed and may not represent the population in other areas of China. Second, we found relatively high heterogeneity among the seroprevalence estimates which could not be entirely explained in the meta-regression (Table 2). There are likely to be other factors affecting seroprevalence that were not considered in this review.

CONCLUSION

Data from 42 published studies of the seroprevalence of EV71 antibody in Chinese children suggested that younger infants are likely to be protected by maternal antibody against EV71 infection and become susceptible to the infection since 5 months of age, and that EV71 infection is more likely to occur in young children at 2–4 years of age. Incidence of infection

was relatively higher in the north of China compared to the south. EV71 vaccines should be administered in the window between 5 months and 1 year of age.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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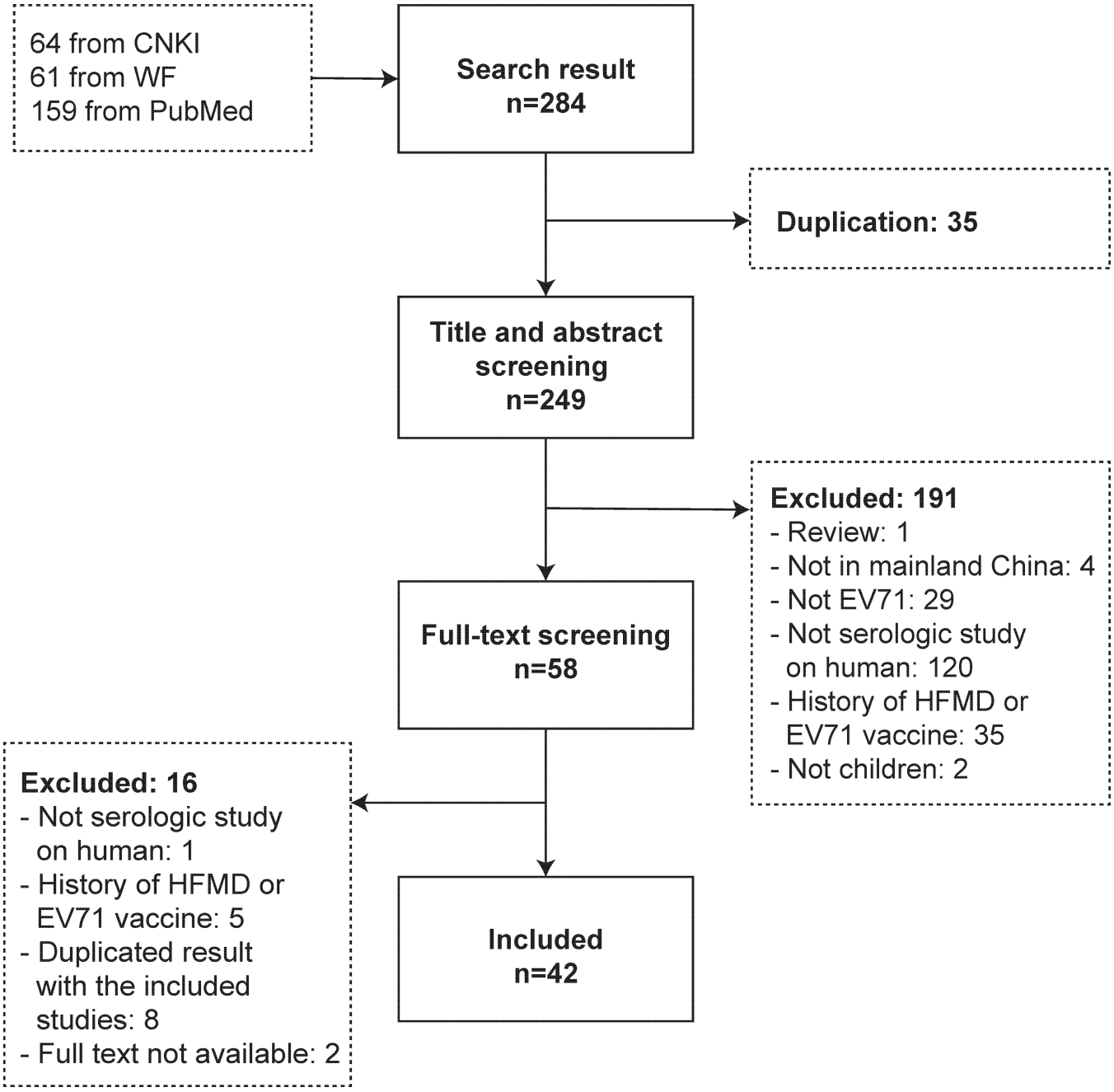

Figure 1. Flowchart of study selection

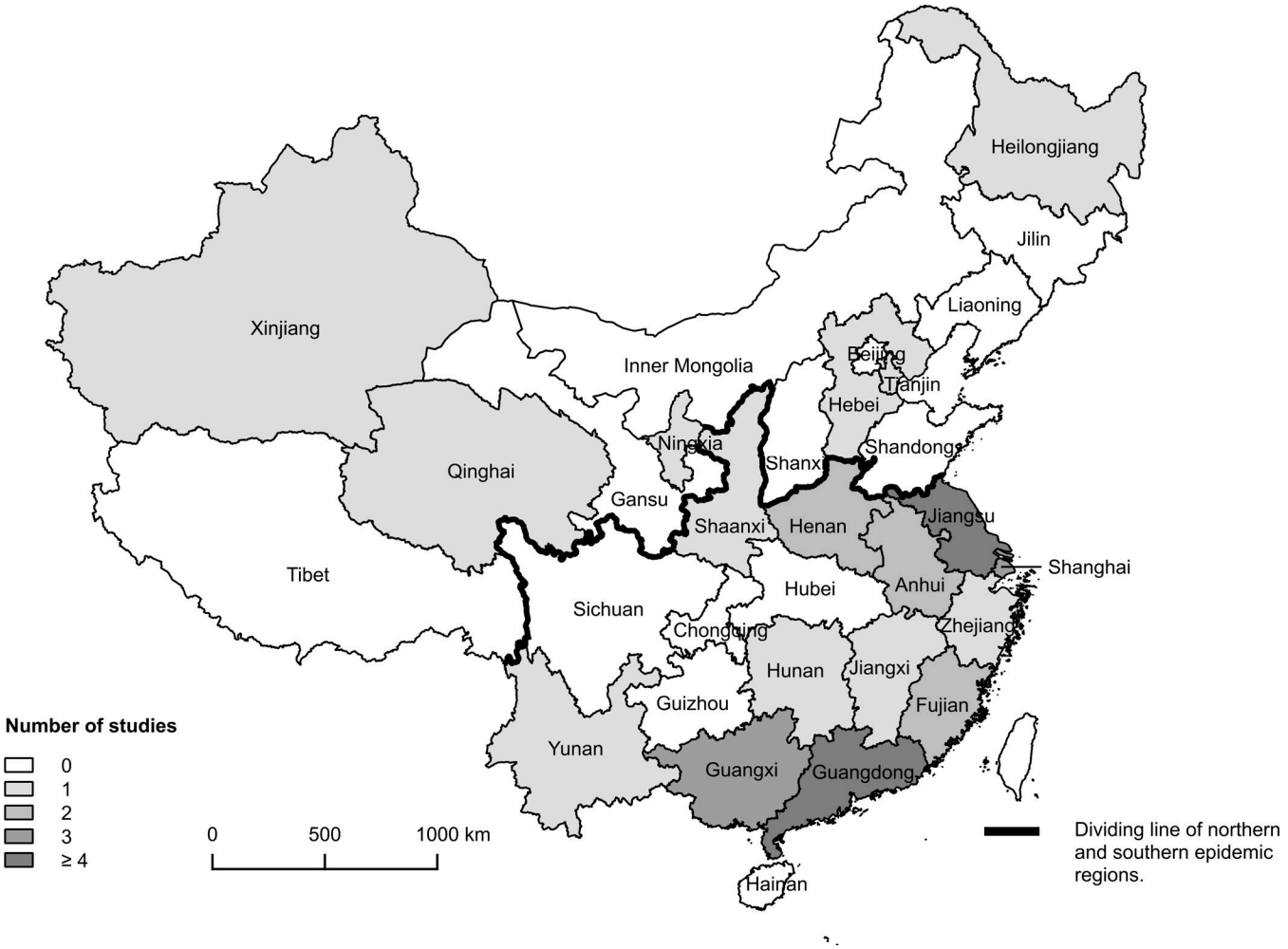

Figure 2. Number of studies included in mainland China

The Study that collected sera from more than one province counted separately for each involved province.

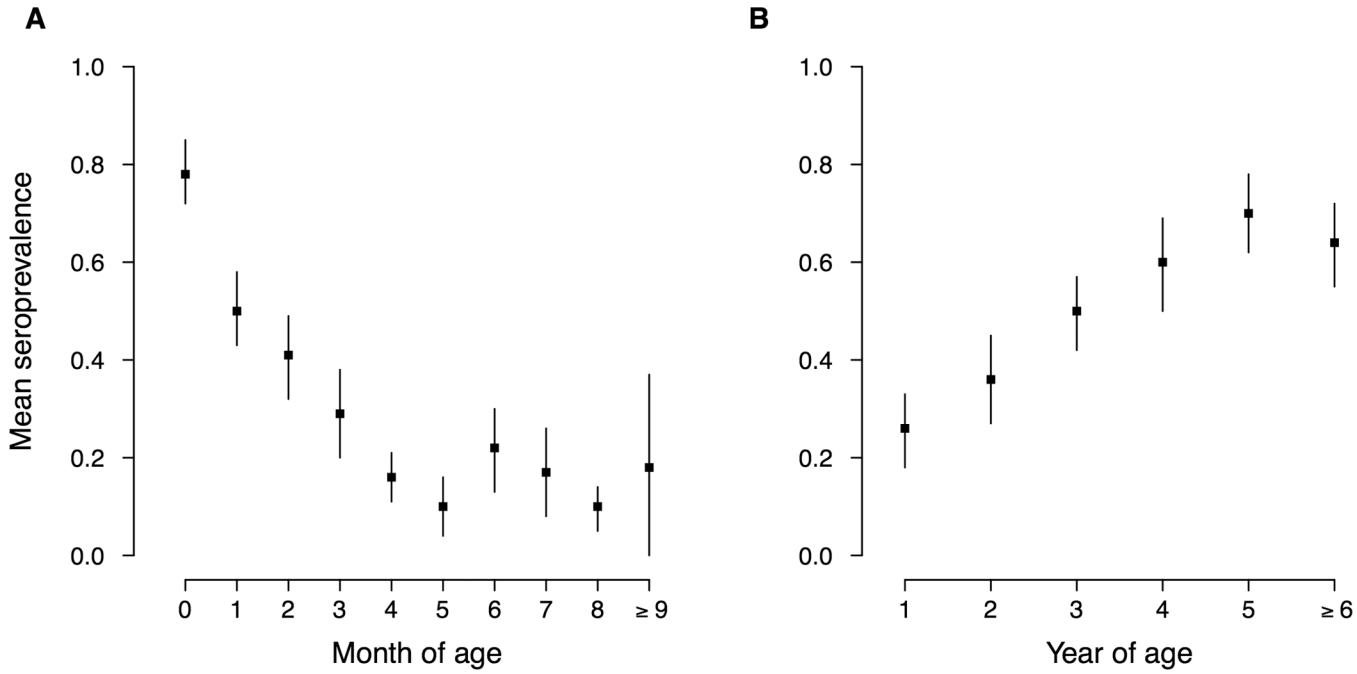

Figure 3. Mean seroprevalence of EV71 antibody among infants and children

Panel A: Mean seroprevalence of EV71 antibody among infants. Panel B: Mean seroprevalence of EV71 antibody among children. Estimates are the pooled mean seroprevalence derived from random-effects models for each age group.

Table 1

Detailed information on the 42 included studies.

Reference	Province	Location	Sampling year	Sampling period	Sampling method	Age range	Sample size	Assay method	Endpoint	Positive threshold	Language
Bai Y. 2012	Ningxia	Yinchuan	2011	Unknown	Physical examination	0–65 yrs	204	ELISA	OD_{450}, OD_{680}	0.1+ ODc	CHN
Cai M. 2013	Guangdong	Shenzhen	2012	Unknown	Unknown	0–62 yrs	240	ELISA	OD_{450}	0.16	CHN
Chen R. 2013	Guangdong	Futian	2011–12	Unknown	Random sampling	all ages	471	NTA	50% CPE	1:256	CHN
Chen X. 2012	Guangxi	Mengshan	2010	Aug	Random sampling	0–15 yrs	519	NTA	Unknown	1:8	CHN
Chen X. 2013	Jiangsu	Donghai	2010	Aug	Random sampling	0–15 yrs	420	NTA	Unknown	1:8	CHN
Deng H. 2012	Shaanxi	Xi'an	2010	Sep	Unknown	1–4 yrs	312	ELISA	OD_{450}	Unknown	CHN
Ding Q. 2011	Jiangsu	Ganyu	2010	Aug	Random sampling	0–15 yrs	400	NTA	50% CPE	1:8	CHN
Guo R. 2011	Hebei	Handan	2009	Unknown	Random sampling	0–15 yrs	856	ELISA	OD_{450}	Unknown	CHN
Guo X. 2009	National	Unkown	2005	Sep	Random sampling	1–6 yrs	371	NTA	50% CPE	1:8	CHN
Hou H. 2012	Guangdong	Futian	2010	Jan–Jun	Physical examination	0–28 yrs	436	ELISA	OD_{450}	Unknown	CHN
Hu Y. 2013	Jiangsu	Jurong	2012–13	Jan12–Mar13	Random sampling	0.5–5 yrs	1400	NTA	50% CPE	1:8	ENG
Huang X. 2010	Henan	Zhengzhou, Kaifeng, Nanyang, Anyang, Sanmenxia	2010	Jan–Feb	Unknown	0–12 yrs	103	NTA	No CPE	1:8	CHN
Ji H. 2012	Jiangsu	Ganyu, Donghai	2010	Aug	Random sampling	0–15 yrs	840	NTA	50% CPE	1:8	ENG
Kuang L. 2011	Guangdong	Guangzhou	2010	Jan–Mar	Physical examination	0–14 yrs	819	ELISA	OD_{450}, OD_{630}	$A_{max(YOUDEN\ index)}$	CHN
Li J. 2011	Tianjin	Tianjin	2009–10	Jan09–Nov10	Random sampling	0–50 yrs	1611	NTA	50% CPE	1:4	CHN
Li J. 2012	Guangdong	Longgang	2012	Jun	Unknown	0–5 yrs	528	ELISA	OD_{450}	0.1+ OD_c	CHN
Li W. 2013a*	Guangdong	Guangdong	2007–09	Unknown	Random sampling	0–9 yrs	715	NTA	50% CPE	1:8	ENG
Li W. 2013b*	Guangdong	Guangdong	2010	Jan, Aug	Random sampling	all ages	707	NTA	50% CPE	1:8	ENG
Li Y. 2012	Guangxi	Mengshan	2010	Dec10–Jun11	Trial cohort	0.5–11 yrs 18–49 yrs	168	NTA	50% CPE	1:8	ENG
Li Z. 2013	Guangxi	Lipu	2010	Aug	Random sampling	0–15 yrs	445	NTA	50% CPE	1:8	CHN
Liao Y. 2012	Fujian	Longyan	2006, 2009	Unknown	Random sampling	all ages	250	NTA	No CPE	1:8	CHN
Lin X. 2007	Guangdong	Shantou	2001	Unknown	Physical examination	0–90 yrs	380	NTA	No CPE	1:10	CHN
Liu F. 2013	Guangdong	Longgang	2011–12	Jan–Jun	Physical examination	0–6 yrs	464	ELISA	OD_{450}	Unknown	CHN
Mao Q. 2009	Henan	Kaifeng	2004	Unknown	Unknown	0.5–2.5 yrs	349	NTA	Unknown	1:8	CHN
Mao Q. 2010	Jiangsu	Jiangsu	2007–09	Sep07–Jul09	Physical examination	2, 7 mons	133	NTA	50% CPE	1:8	ENG

Reference	Province	Location	Sampling year	Sampling period	Sampling method	Age range	Sample size	Assay method	Endpoint	Positive threshold	Language
Ni H. 2012	Zhejiang	Cixi	2011	Apr	Unknown	all ages	258	NTA	50% CPE	1:8	ENG
Xiong Y. 2013	Jiangxi	Nanchang	2010	Jan–Feb	Random sampling	all ages	1144	NTA	50% CPE	1:8	CHN
Xu M. 2012	Shanghai	Shanghai	2010–11	Jul10–Jan11	Physical examination	children	164	NTA	50% CPE	1:8	CHN
Xu W. 2013	Jiangsu	Changzhou	2006	Unknown	Unknown	1–5 yrs	252	NTA	50% CPE	1:8	CHN
Yang X. 2010	Fujian	Fujian	2006, 2009	Unknown	Random sampling	0–68 yrs	2374	NTA	50% CPE /No CPE	1:8/1:4	CHN
Yu H. 2011	Anhui	Luan	2005–08, 2010	Dec05–Mar08; Mar–Apr10	Unknown	0–15 yrs	555	NTA	50% CPE	1:8	ENG
Zeng M. 2012	Shanghai	Shanghai	2010–11	Nov10–Apr11	Physical examination	0–5 yrs	614	NTA	50% CPE	1:8	ENG
Zhang D. 2011	Guangdong	Futian	2011	Unknown	Random sampling	0–59 yrs	382	ELISA	OD_{450}	0.16	CHN
Zhao S. 2011	Qinghai	Xining	2009	May-Aug	Unknown	1–6 yrs	181	NTA	50% CPE	1:8	CHN
Zhou S. 2007	Guangdong	Shenzhen	1999–2003	Unknown	Unknown	all ages	584	ELISA	OD_{450}	Unknown	CHN
Zhu F. 2012a*	Jiangsu	Jiangsu	2007–09	Nov07–Aug09	Random sampling	2, 7, 12 mons	975	NTA	50% CPE	1:8	ENG
Zhu F. 2012b*	Jiangsu	Donghai	2011	Mar–Jun	Trial cohort	0.5–5 yrs	332	NTA	50% CPE	1:8	ENG
Zhu F. 2013c*	Jiangsu	Donghai	2011	Aug–Sep	Trial cohort	0.5–3 yrs	1106	NTA	50% CPE	1:8	ENG
Zhu F. 2013d*	Jiangsu	Donghai, Pizhou, Baoying	2012–13	Jan12–Mar13	Trial cohort	0.5–3 yrs	1219	NTA	50% CPE	1:8	ENG
Zhu F. 2014e*	Jiangsu	Ganyu, Taixing, Sheyang	2012	Jan	Trial cohort	0.5–3 yrs	1150	NTA	50% CPE	1:8	ENG
Zhu W. 2013	Shanghai	Shanghai	2011	Jul–Aug	Physical examination	0–8 yrs	93	NTA	50% CPE	1:8	CHN
Zhu Z. 2010	Anhui, Guangdong, Heilongjian, Hunan, Xinjiang and Yunnan	Anhui, Guangdong, Heilongjiang, Hunan, Xinjiang and Yunnan	2005	Aug	Random sampling	0–5 yrs	900	NTA	50% CPE	1:8	ENG

* Li W. 2013a [40]; Li W. 2013b [42]; Zhu F. 2012a [26]; Zhu F. 2012b [37]; Zhu F. 2013c [35]; Zhu F. 2013d [13]; Zhu F. 2014e [14]

Table 2

Meta-regression of factors correlated with seroprevalence among children 1–5 years of age.

Characteristic	Number of estimates	β coefficient	95% CI
Total	98	0.002	(0.001, 0.003)**
Age			
1 year	25	ref	
2 years	21	0.083	(0.002, 0.164)*
3 years	20	0.226	(0.142, 0.310)**
4 years	20	0.312	(0.230, 0.395)**
5 years	12	0.386	(0.285, 0.487)**
Sampling year			
2008 or before	23	ref	
2009	15	−0.176	(−0.315, −0.037)*
2010	42	0.186	(0.012, 0.360)*
2011 or after	18	0.261	(0.092, 0.431)**
Sampling method			
Random sampling	62	ref	
Physical examination	9	−0.437	(−0.573, −0.302)**
Unknown	27	−0.227	(−0.310, −0.145)**
Assay method			
ELISA	18	ref	
NTA	80	0.067	(−0.030, 0.164)
Seasonality			
Season	46	ref	
Non-season	17	−0.045	(−0.152, 0.062)
Unknown	35	0.082	(−0.061, 0.225)
Epidemic region			
North	10	ref	
South	84	−0.203	(−0.376, −0.029)*
Published language			
Chinese	60	ref	
English	38	−0.133	(−0.213, −0.053)**

* $p < 0.05$;

** $p < 0.01$.

I^2 = 79.9% (95%CI, 72.0–85.9%; $p < 0.001$). QM (df=15) = 240.80; $p < 0.001$.