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REVIEW

Seroprevalence of coxsackievirus A6 and enterovirus A71 infection in humans: a systematic review and meta-analysis

Yingying Shi[1] · Peiqing Chen[1] · Yijing Bai[1] · Xuan Xu[1] · Yongjuan Liu[2]

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Abstract

Hand, foot, and mouth disease (HFMD) is a common infectious disease in children. Enterovirus A71 (EV-A71) is one of the main pathogens, and coxsackievirus A6 (CVA6) has gradually become the dominant pathogen of HFMD in recent years. This study was conducted mainly to assess the serological prevalence of EV-A71 and CVA6 antibodies in people of different ages, sexes, and regions through a systematic review and meta-analysis. A comprehensive study was performed based on the EV-A71 and CVA6 serological literature published before May 2022. Heterogeneity analysis (Cochrane's Q test and the I^2 statistic) and random effect models were adopted. Subgroup and meta-regression analyses were used to identify potential sources of heterogeneity in the data, and all analysis was performed using STATA version 16.0. This study included 71 studies involving 55,176 people from 13 countries that met the inclusion criteria. The serological prevalence of EV-A71 antibody in different studies was 4.31-88.8%, and that of CVA6 antibody was 40.8-80.9%. Meta-analysis results showed that the serum positive rate for EV-A71 antibody was 45.9% (95% CI: 37.6-54.1%). The rate in the Chinese population was 47.8% (95% CI: 42.4-53.2%), and in the other countries, it was 38% (95% CI: 23-55%). The serum positive rate for CVA6 antibody was 58.3% (95% CI: 46.5-70.2%). The rate in the Chinese population was 49.1% (95% CI: 38.3-59.9%), and in the other countries, it was 68% (95% CI: 51-83%). Subgroup analysis was also conducted. The seroprevalence of EV-A71 and CVA6 antibodies is related to age rather than gender or region. The rates of EV-A71 and CVA6 seropositivity are considerably lower in children younger than five years of age. However, the rates gradually increase with age. The findings of this study suggest that children under five years of age may be susceptible to EV-A71 and CVA6. Thus, safety education and vaccination should be strengthened accordingly. This study provides a basis for understanding the risk factors for EV-A71 and CVA6 infection in China and for deciding how to formulate standard preventive measures to prevent the spread of the virus.

Introduction

Hand, foot, and mouth disease (HFMD) is a common infectious disease caused by a variety of enteroviruses that mainly infects infants [1]. Most patients have mild symptoms, with fever and rash or herpes on the hands, feet, and mouth as the main clinical signs [2]. Aseptic meningitis, encephalitis, acute flaccid paralysis, neurogenic pulmonary oedema, and myocarditis may occur in a small number of patients, and the disease progresses rapidly in some children with severe symptoms, which may lead to death [3]. HFMD is an important public health problem that endangers children's health worldwide. Every year, the occurrence of HFMD in children results in economic losses for many families. In 2008, the disease was listed as a class C infectious disease by the Ministry of Health of China [4].

HFMD can be caused by a variety of enteroviruses [2]. Before 2012, enterovirus A71 (EV-A71) and coxsackievirus A type 16 (CVA16) were the most common pathogens [5]. Since 2013, the proportion of HFMD caused by CVA6 infection has increased significantly, and CVA6 has gradually become the dominant pathogen of HFMD in many countries worldwide [6].

EV-A71 virus particles are icosahedral with a diameter of 24-30 nm and contain a single plus-stranded RNA [7]. Since the virus was first reported in the 1970s, epidemics of

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✉ Yongjuan Liu
lyjquanzhiyuan_09@163.com

[1] Department of Immunology, School of Medicine, Jianghan University, Wuhan, Hubei, China

[2] Department of Central Laboratory, The Affiliated Lianyungang Hospital of Xuzhou Medical University, Lianyungang 222000, Jiangsu, China

EV-A71 have been reported in many countries and regions [8, 9]. Humans are the only natural hosts of EV-A71. The main sources of EV-A71 infection are patients and asymptomatic carriers of the virus. Most infected people are under 10 years old, with infection being most common in children under 5 years old, but adult cases have occasionally been reported [1].

CVA6 is one of the serotypes of enterovirus coxsackievirus group A [10]. Previous studies have shown that CVA6 was mostly associated with the occurrence of herpetic pharyngitis. Since the outbreak of HFMD caused by CVA6 in Finland was first reported by Osterback et al. in 2009, attention has been given to HFMD caused by CVA6 worldwide [10]. Analysis of the epidemiology and aetiology of HFMD in China from 2013 to 2017 showed that CVA6 has become the dominant pathogen of HFMD in most regions of China [11]. The epidemiology of CVA6 has also been reported in some countries in recent years. However, due to the lack of continuous and systematic monitoring data for CVA6, the current incidence of CVA6 does not accurately reflect the true prevalence of CVA6. There is currently no effective vaccine against the virus, and targeted treatment is mainly achieved via pharmacotherapy. Therefore, it is of great importance to understand the characteristics of the seroepidemiological distribution of CVA6 for the formulation of intervention strategies for HFMD-susceptible populations.

Because HFMD infections can range from asymptomatic to fatal, despite surveillance efforts in numerous places, and given the large number of asymptomatic or subclinical cases, the actual number of people exposed or infected has been underestimated [12]. Serological screening is an important adjunct to PCR-based detection/diagnosis as well as an important means of assessing the cumulative rate of HFMD-associated virus infection. Such screening provides insight into the dynamic monitoring of specific antibody responses during and after transmission of the virus and can inform health authorities and policy-makers about seroprevalence at specific stages of an outbreak. The prevalence of specific serum antibodies to EV-A17 and CVA6 can provide reliable indicators of population exposure to the associated viruses and even indicate the immune status of an individual or population.

Serological analysis can help determine the susceptibility and immunity of people of different ages, sexes, and races, providing a reference and a theoretical basis for the prevention of virus-induced diseases as well as the implementation of immunization protocols. In previous serological studies of EV-A17 and CVA6, seropositivity rates have varied widely. In addition, due to differences in serological methods, sample sizes and regions among these studies, it is not possible to accurately assess the serological prevalence of EV-A17 and CVA6 infection. In this study, published studies on the seroepidemiology of EV-A71 and CVA6 antibodies were comprehensively collected and analysed. Accordingly, this study attempts to predict the immune dynamics of susceptible and general populations based on serological data to provide a theoretical basis for the prevention of virus-related diseases. It is hoped that this study can provide other researchers with reliable estimates of the true transmission rates of EV-A71 and CVA6, accurately assess the possible risk factors for the transmission of EV-A71 and CVA6, and provide a theoretical basis for virus prevention and control.

Methods

Search strategy

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines (Supplementary Table S3) [13]. During literature retrieval, the following four databases were utilized: China National Knowledge Infrastructure (CNKI), WanFang, PubMed, and the Web of Science (Supplementary Tables S1 and S2). Serology-related retrospective or cross-sectional studies of EV-A71 and CVA6 published before May 2022 were systematically searched in the corresponding databases. The following subject words or free words were used for the search: TS = (HFMD OR Hand, Foot, Mouth Disease) AND TS = (Coxsackievirus A6/Enterovirus 71) AND TS = (Seroprevalence OR Seroepidemiological Study). To avoid omissions, each database was also searched manually to ensure the integrity of the analysis.

Inclusion and exclusion criteria

The following inclusion and exclusion criteria were considered in the assessment of titles, abstracts, and texts of relevant literature. The inclusion criteria were as follows: (1) the research paper was published before May 2022; (2) the positive rate for EV-A71 or CVA6 antibodies was obtained accurately via direct or indirect methods; and (3) the study was a cross-sectional or retrospective study. The exclusion criteria were as follows: (1) the study was a review or conference paper and (2) the data were incomplete or contained obvious errors.

Literature screening and data extraction

By reading the corresponding studies, valid information from each study, including first author, sampling time, publication date, investigation area, sample size, test method, threshold for defining seropositivity, and age of the study population, was extracted into standardized tables. Seropositivity was defined as the proportion of people who tested positive for EV-A71 or CVA6 antibodies in the total

Table 1 Basic information about the literature included in this analysis

First author	Sampling year	Publication year	Country	Sample size	No. positive	Assay method	Positive threshold	Age range	Group factors	Language	AHRQ score
CVA6											
Li Wei Ang	2008-2010	2015	Singapore	700	439	Neutralization test	≥1:8	1-17y	Age	ENG	7
FanGao	2012	2016	China	180	75	CPE	≥1:8	6-35m	Age	ENG	7
Rui Zhu	2016	2018	China	515	203	Microneutralization test	≥ 1:16	5m-83y	Age	ENG	8
Fan Gao	2012-2014	2018	China	181	74	Neutralization test	≥1:8	6-35m	Age	CHN	8
Dan Song	2016~2019	2020	China	171	107	Neutralization test	≥1:8	All ages	Age	CHN	8
C. Q. Hoang	2014	2020	Southern Vietnam	336	106	Microneutralization test	≥1:8	All ages	Age	ENG	7
Fan Gao	2018	2021	China	488	298	Microneutralization test	≥1:8	2-83m	Age	ENG	7
Everlyn Kamau	2006, 2011, 2017	2021	United Kingdom	1508	1220	Microneutralization test	≥ 1:16	All ages	Age	ENG	7
EV-A71											
Ceyla M.O. CASTRO	1998-2001	2005	Brazil	389	222	Neutralization test	1:8	0-15y	Age	ENG	7
Fan Gao	2018	2021	China	401	127	Neutralization test	1:8	2-83 m	Age	ENG	7
Chun-Yi Lu	1994-1999	2002	China	1705	740	Microneutralization assay	1:8	All ages	Age, year	ENG	8
Luan-Yin Chang	1992	2002	China	539	242	Neutralizing antibody test	≥8	All ages	Age	ENG	9
Shili Zhou	1999- 2003	2007	China	584	207	ELISA	OD450	All ages	Age	CHN	7
Xuebin Guo	2005	2009	China	371	164	Neutralization test	≥8	1-6y	Age	CHN	7
Shu-Ting Luo	2006- 2008	2009	China	618	158	Neutralization test	≥8	0-6 m	Age	ENG	7
MAO Qun-ying	2007-2009	G	China	399	159	Neutralization test	≥8	0-6 m	Age	ENG	8
Dongxiao Zhang	2011	2011	China	382	257	ELISA	≥0.16	0-59y	Age	CHN	6
Shengcang Zhao	2009	2011	China	181	68	Neutralization test	≥8	1-6y	Age	CHN	6
Ruiling Guo	2009	2011	China	856	546	ELISA	OD450	0-15y	Age, sex	CHN	6
Qiang Ding	2010	2011	China	420	178	Neutralization test	≥8	0-15y	Age	CHN	6
Lu Kuang	2010	2011	China	819	239	ELISA	OD450/630	0-14y	Age	CHN	7

Table 1 (continued)

First author	Sampling year	Publication year	Country	Sample size	No. positive	Assay method	Positive threshold	Age range	Group factors	Language	AHRQ score
Haiyang Yu	2006–2007,2010	2011	China	472	247	Microneutralization assay	≥8	0-15y	Age, year	ENG	7
Hongbin Hou	2010	2012	China	436	152	ELISA	OD450	1m-28y	Age, sex	CHN	7
Jingmei Li	2012	2012	China	528	241	ELISA	A450	0-5y	Age	CHN	6
Huiling Deng	2010	2012	China	312	108	ELISA	A450	1-4y	Age	CHN	6
Feng-Cai Zhu	2007	2012	China	715	404	Neutralization test	≥8	0-38 m	Age	ENG	7
Mei Zeng	2010~2011	2012	China	614	122	Microneutralization assay	≥8	0-5y	Age	ENG	7
Hong Ji	2010	2012	China	680	233	Microneutralization assay	≥8	0-15y	Age	ENG	7
Wen-Chan Huang	2006~2007	2012	China	228	36	Neutralization test	≥8	2-5y	Age	ENG	7
Hongxia Ni	2011	2012	China	258	138	Neutralization test	≥8	All ages	Age, region	ENG	7
Menghua Xu	2010-2011	2012	China	201	111	Microneutralization assay	CPE	All ages	Age	CHN	8
Miaosen Cai	2012	2013	China	240	106	ELISA	≥0.16	3m-62y	Age	CHN	6
Wei Li	2007~2009,2010	2013	China	1458	742	Neutralization test	≥8	1-9y	Age, year	ENG	9
Wenguo Xu	2006	2013	China	252	83	Neutralizing antibody assay	≥8	1-5y	Age	CHN	7
Xiaoqin Chen	2010	2013	China	420	192	Neutralizing antibody assay	≥8	0-15y	Age	CHN	8
Ying Xiong	2010	2013	China	1144	855	Neutralizing antibody assay	≥8	all ages	Age	CHN	8
Xiang Wang	2012	2014	China	391	335	Neutralization tests	≥10	18-71y	Age, gender, region	ENG	9
Yuling Xu	2010-2012	2014	China	254	175	Neutralizing antibody assay	≥8	0-6y	Age	CHN	8
Juanjuan Gui	2008~2012	2015	China	549	274	Neutralization test	≥8	0-20y	Age, year	ENG	9
Xiaoming Tu	2010	2015	China	420	169	ELISA	S/N≥2.1	0-20y	Age, gender, region	ENG	9

Table 1 (continued)

First author	Sampling year	Publication year	Country	Sample size	No. positive	Assay method	Positive threshold	Age range	Group factors	Language	AHRQ score
Fan Gao	2012	2016	China	180	75	Cytopathogenic effect (CPE) method	CPE	6-35m	year	ENG	6
Paul F. Horwood	2000-2011	2016	China	1707	1516	Microneutralization assay	≥1:8	2–15y	Age, year, region	ENG	8
Jian-xing Wang	2010	2016	China	1378	1030	Microneutralization test	≥1:8	All ages	Age, gender, region	ENG	8
Chuanxi Fu	2013~2014	2016	China	224	185	Modified cytopathogenic effect assay	CPE	mother-infant	Age	ENG	7
Xingui Tian	2014	2016	China	96	71	Neutralization tests	≥1:16	20–49y	Age, gender	ENG	7
Dingmei Zhang	2014-2015	2017	China	197	117	Microneutralization test	≥1:8	1–5y	Age	ENG	7
Jiayu Wang	2014-2016	2018	China	1230	621	Microneutralization test	≥1:8	0–18y	Age, gender	ENG	8
Rui Zhu	2016	2018	China	515	194	Microneutralization test	≥1:16	5m-83y	Age, district	ENG	9
Xianglin Wei	2013-2018	2021	China	1066	705	Neutralization assays	≥1:16	2-36m	Age	ENG	9
Qunying Mao	2004	2009	China	349	128	Micro-CPE method	≥1:8	7-30m	Age	CHN	8
Zhen Zhu	2005	2010	China	900	288	Neutralization assays	≥1:8	5y	District	ENG	8
Jiameng Li	2009 2010	2011	China	1611	1076	Neutralization assays	≥ 1: 4	0-50y	Age	CHN	8
Wen Zhu	2011	2013	China	93	54	Microneutralization test	≥ 1: 8	0-8y	Age, gender	CHN	8
Hanna Honkanen	1994-2010	2013	Finland	5686	2	Neutralization assays	CPE	<11	Age	ENG	6
Sabine Diedrich	1997-2007	2009	Germany	436	263	Neutralization test	≥ 1: 4	10m-75y	Age	ENG	7
Holger F	2006	2010	Germany	696	30	Microneutralization test	≥1:10	≥1y	Age	ENG	8
Mahsa Javadi	2015	2021	Iran	547	310	Neutralization assay	≥1:16	All ages	Age	ENG	8
Sol Kim	2013-2018	2020	Korea	220	141	Neutralization test	≥1: 4	7m-15y	Age	ENG	8

Table 1 (continued)

First author	Sampling year	Publication year	Country	Sample size	No. positive	Assay method	Positive threshold	Age range	Group factors	Language	AHRQ score
NMN NikNadia	1995~2012	2016	Malaysia	1425	932	Neutralization test	≥1: 8	1-12y	Age, year	ENG	8
Jing Li	2017	2019	China	6513	4015	qRT-PCR	-	All ages	Age, year	ENG	8
Sabine M.G. van der Sanden	2010~2014	2016	Netherlands	122	50	Neutralization test	≥1:16	≥5 y	Age, year	ENG	8
Ludmila V. Akhmadishina	2007~2008	2014	Russia	826	241	Neutralization test	≥1:8	1-5y	Age	ENG	8
Eng-Eong Ooi	1996-1997	2002	Singapore	856	297	Neutralization test	≥1:8	≥12 y	Age	ENG	8
Li-Wei Ang	2008-2010	2011	Singapore	1883	526	Neutralization assay	≥ 8	1-17y	Age, gender	ENG	8
C. Q. Hoang	2014	2020	Southern Vietnam	84	40	Neutralization test	≥1:8	All ages	Age	ENG	7
Huang	2006-2007	2012	China (Taiwan)	228	36	Neutralization test	≥1:8	2-7y	Age, year	ENG	7
Fang-Lin Kuo	2012-2013	2020	China (Taiwan)	553	301	Neutralization test	≥1:8	All ages	Age	ENG	7
Piyada Linsuwanon	2009-2012	2014	Thailand	1050	92	ELISA	≥1:8	0-54y	Age	ENG	7
Hatairat Lerdsamran	2013	2018	Thailand	579	285	Microneutralization assay	≥1:10	0-60y	Age, year	ENG	9
Jiratchaya Puenpa	2015-2020	2020	Thailand	100	19	Microneutralization assay	≥1:16	0-4y	Age, year	ENG	9
Everlyn Kamau	2006,2011,2017	2021	UK	1558	1152	Neutralization Assays	≥1:8	All ages	Age, year	ENG	9
Chau Bich Nguyen Tran	2006-2007	2011	Viet Nam	1035	116	Neutralization assay	CPE	0-38y	Age	ENG	7

m, month; y, year; ENG, English; CHN, Chinese

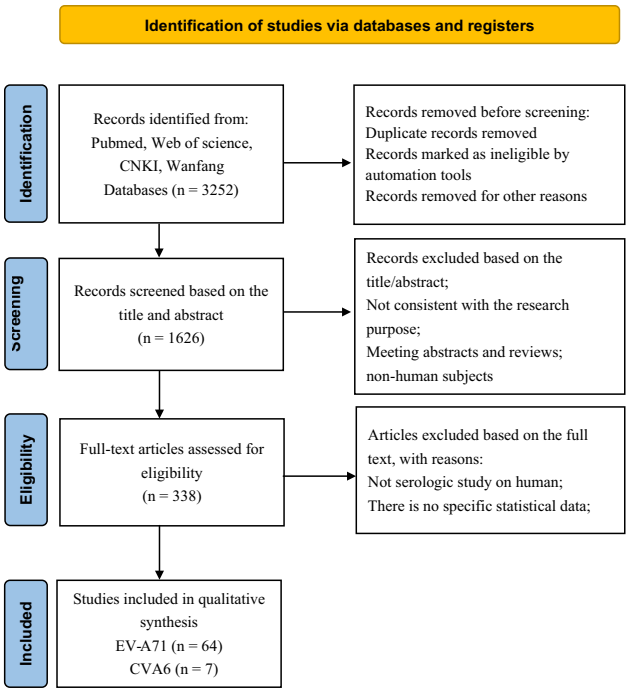

Fig. 1 Flow chart of the literature selection process

population who provided blood samples. For statistical information, we preclassified sampling methods. "Random sampling" refers to random selection of research samples. "Conditional sampling" refers to selection of samples meeting certain criteria or the use of residual serum samples collected for other research purposes. "Physical examination" refers to selection of serum samples based on physical examination. Two evaluators (SY and CP) preliminarily screened the titles and abstracts of the studies and excluded obviously irrelevant studies independently. Two researchers (BY and XX) further read the full texts, determined the final inclusion results, independently screened the valid studies, and extracted and verified the results independently. When two investigators disagreed on the data extraction, a third investigator (LY) was involved in the data extraction and decision-making (Table 1).

Quality assessment

In the literature quality evaluation, a cross-sectional study was conducted with reference to the evaluation criteria of the Agency for Healthcare Research and Quality (AHRQ). The standard includes a total of 11 items, and the literature was evaluated against this standard. The evaluation result was "yes" (score 1 point), "no" (score 0 point), or "unclear" (scored 0 point)", and the scores of each study were successively sorted. Scores of 0-3, 4-7, and 8-11 represented low-, medium-, and high-quality studies, respectively. In this study we used the Cochrane Risk of Bias Assessment tool. In the Cochrane Systematic review, risk of bias was assessed independently and repeatedly by two authors (SY and CP), both of which have a good understanding of the methodology. If the results of the two evaluations were different, a third author (LY) was consulted if there was still a difference of opinion after consultation.

Statistical analysis

STATA 16.0 was used for statistical processing. Descriptive analysis and single-group variable analysis were used. For data that did not conform to a normal distribution, the Freeman-Tukey double arcsine method was used for data conversion, whereas transformed data were used for the meta-analysis. Cochran's Q test was used to analyse the heterogeneity among the included results, and I^2 was used to judge heterogeneity quantitatively. If the results of Cochran's Q test were $P < 0.1$ and $I^2 \leq 50\%$, the fixed-effects model was chosen for the meta-analysis. Conversely, if statistical heterogeneity was noted between the results, the random-effects model was chosen. Seropositivity rates and 95% confidence intervals (CIs) were then calculated for the different subgroups. The relevant risk factors assessed in this study also included sampling methods, sample collection season, age, sex, and language. In the sensitivity analysis, the stability of the meta-analysis results was evaluated using a case-by-case exclusion study. Publication bias was evaluated by Egger's linear regression analysis and Begg's funnel plot analysis.

Results

Study search results

Two authors of this study searched two English-language databases and two Chinese-language databases using standard retrieval strategies (Supplementary Tables S1 and S2) [14–58]. Subsequently, the literature was screened independently with reference to pre-established inclusion and exclusion criteria, and valid data were extracted and verified. After excluding invalid and duplicate studies, 71 eligible studies were included in the meta-analysis [6, 8, 9, 59–73]. These 71 studies included a total of 55,176 samples, 23,297 of which were positive for EV-A71 neutralizing antibody. Thus, the total serum EV-A71 antibody positivity rate ranged from 37.6% to 54.1%. The serum CVA6 positivity rate was 58.3%. Among the 71 studies, there were 53 from China, three each from Singapore, Thailand, and Vietnam, two each from the United Kingdom and Germany, and one each from the Netherlands, Finland, Iran, Korea, and Russia (Table 1). Literature quality was also evaluated based on the evaluation criteria of AHRQ. In total, 36 of the included

Table 2 Seroprevalence in different groups (CVA6)

Variable	Ref (n)	n	Events	Transformation method	Seroprevalence (%)	95% CI (%)	I^2 (%)	Heterogeneity (*P*-value)	Begg's (*P*-value)	Egger's (*P*-value)
Total	8	2522	4079	Random-effects model	0.583	0.465, 0.702	98.6	<0.001	0.016	0.056
Age										
<1 y	5	192	406	Freeman-Tukey double arcsine	0.43	0.24, 0.63	95.01	<0.001		
1-3 y	4	269	582	Random-effects model	0.425	0.307, 0.542	88.1	<0.001		
3-5 y	2	143	207	Random-effects model	0.686	0.490, 0.882	89.9	<0.001		
<5 y	8	839	1564	Random-effects model	0.558	0.476, 0.640	90.4	<0.001		
>5 y	6	1989	2760	Freeman-Tukey double arcsine	0.72	0.61, 0.82	97.74	<0.001		
Gender										
Female	3	415	759	Random-effects model	0.554	0.425, 0.683	91.7	<0.001		
Male	3	334	627	Random-effects model	0.541	0.328, 0.754	96.5	<0.001		
Sampling method										
Physical examination	2	546	871	Fixed- effects model	0.627	0.595, 0.659	0.0	0.973		
Random sampling	2	501	1003	Random-effects model	0.502	0.290, 0.715	98.0	<0.001		
Conditional sampling	4	1475	2205	Random-effects model	0.595	0.421, 0.770	99.0	<0.001		
Seasonality										
Seasonal	4	362	868	Random-effects model	0.440	0.310, 0.571	93.7	<0.001		
Non-seasonal	1	439	700	Random-effects model	0.627	0.591, 0.663	-	-		
Unknown	3	1721	2511	Freeman-Tukey double arcsine	0.69	0.52, 0.84	98.75	<0.001		
Publication language										
English	6	2341	3727	Random-effects model	0.599	0.465, 0.733	98.9	<0.001		
Chinese	2	181	352	Random-effects model	0.517	0.305, 0.730	94.3	<0.001		
Ethnicity										
Chinese	5	757	1535	Random-effects model	0.491	0.383, 0.599	94.4	<0.001		
Other	3	1765	2544	Freeman-Tukey double arcsine	0.68	0.51, 0.83	98.78	<0.001		

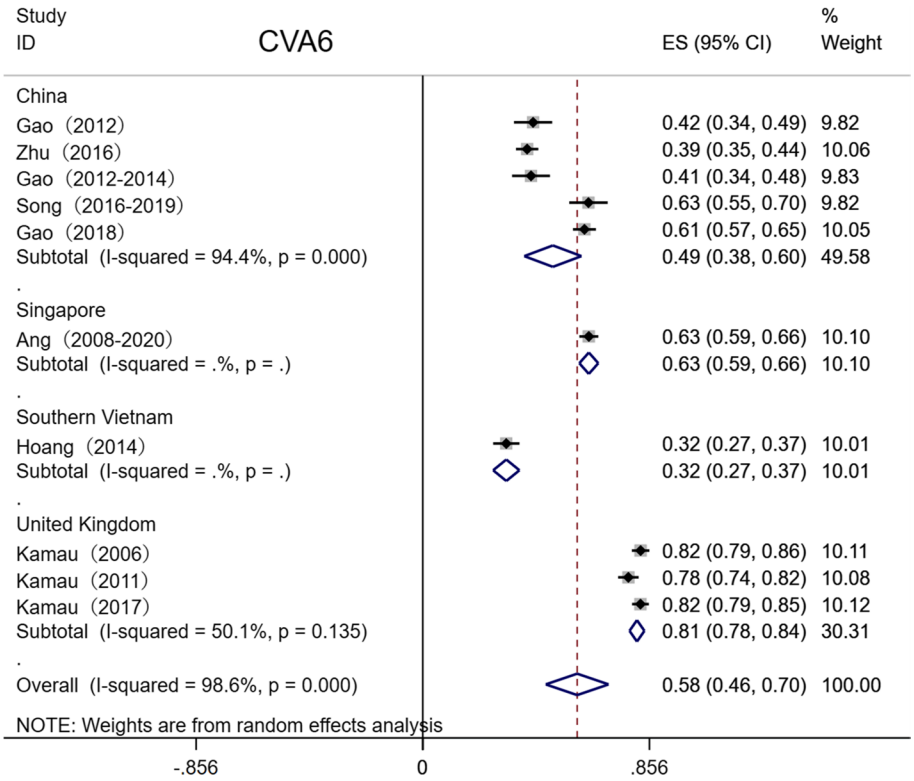

Fig. 2 Forest plots for the seroprevalence of CVA6 antibody in the overall population

studies were found to be of medium quality, and 35 were of high quality (Fig. 1).

Seroprevalence of EV-A71 antibody

Total seropositivity rates were analysed and stratified by age, sex, sampling method, seasonality, publication language, and ethnicity (Tables 1, 2, 3). Forest plots of the total seropositive rate of CVA6 are shown in Figure 2, and an overall summary of the results is shown in Table 2. The seroprevalence of EV-A71 antibody was 45.9% (95% CI: 37.6%-54.1%) in the overall population, 47.8% (95% CI: 42.4%-53.2%) in the Chinese population, and 38% (95% CI: 23%-55%) in other countries (Table 3). The seroprevalence of CVA6 antibody was 58.3% (95% CI: 46.5%-70.2%) in the overall population, 49.1% (95% CI: 38.3%-59.9%) in the Chinese population, and 68% (95% CI: 51%-83%) in other countries (Table 2). Moreover, age-based subgroup analysis showed that the seroprevalence of EV-A71 antibody was 28 % (22%-35%) in the < 1-year-old age group, 32.7 % (27.1%-38.2%) in the 1- to 3-year-old age group, 36.3% (95% CI: 32%-40.6%) in the 0- to 5-year-old age group, and 62 % (95% CI: 56%-68%) in the 5 years and older age group (Tables 3, 4, Figs. 4, 5, 6, 7). The seroprevalence of CVA6 antibody was 43% (24%-63%) in the < 1-year-old age group, 42.5 % (30.7%-54.2%) in the 1- to 3-year-old age group, 55.8% (95% CI: 47.6%-64%) in the 0- to 5-year-old age group, and 72 % (95% CI: 61%-82%) in the 5 years and older age group (Table 2, Fig. 3). Subgroup analysis based on sex showed that the seroprevalence of EV-A71 antibody was 44% (95% CI: 36.5%-51.6%) in males and 43.9% (95% CI: 33.8%-54%) in females (Fig. 8). The seroprevalence of CVA6 antibody was 54.1% (95% CI: 32.8%-75.4%) in males and 55.4% (95% CI: 42.5%-68.3%) in females.

Sensitivity analysis and publication bias

Univariate analysis showed that age and seasonality significantly affected the heterogeneity of the meta-analysis results (Tables 3, 4). A sensitivity analysis was also conducted on the serum prevalence of the CVA6 and EV-A71 antibodies (Supplementary Figs. S1-S2). Based on Begg's and Egger's tests, a publication bias plot was drawn, which demonstrated that the distribution of the prevalence in each study was not symmetrical (Figs. 9-10). Furthermore, the results indicated a possibility of publication bias.

Discussion

HFMD caused by enterovirus infection is a disease that threatens children's health [8]. In the past decade, numerous outbreaks have occurred worldwide, especially in the Asia-Pacific region. EV-A71 and CVA6 are the main pathogens of HFMD and are occasionally associated with severe neurological complications [2]. Since the approval of an

Table 3 Seroprevalence in different groups (EV-A71)

Variable	Ref(n)	n	Event	Transformation Method	Seroprevalence (%)	95% CI (%)	I^2 (%)	Heterogeneity (*P*-value)	Begg's (*P*-value)	Egger's (*P*-value)
Total	78	52654	23297	Random-effects model	0.459	0.376,0.541	99.9	< 0.001	0.0001	0.271
Age										
<1 yrs	36	5980	1716	Freeman-Tukey double arcsine	0.28	0.22,0.35	95.80	< 0.001		
1-3 yrs	34	5570	1766	Random-effects model	0.327	0.271,0.382	96.2	< 0.001		
3-5 yrs	29	3580	1601	Random-effects model	0.502	0.436,0.568	94.6	< 0.001		
<5 yrs	56	6713	19777	Random-effects model	0.363	0.320,0.406	98.1	< 0.001		
>5 yrs	40	8866	14387	Freeman-Tukey double arcsine	0.62	0.56,0.68	98.07	< 0.001		
Gender										
Female	17	2511	5026	Random-effects model	0.439	0.338,0.540	98.5	< 0.001		
Male	17	2712	5640	Random-effects model	0.44	0.365,0.516	97.3	< 0.001		
Sampling method										
Physical examination	9	2380	6961	Random-effects model	0.367	0.299,0.434	97.4	< 0.001		
Random sampling	41	12542	24843	Random-effects model	0.478	0.397,0.559	99.6	< 0.001		
Conditional sampling	13	3428	12912	Freeman-Tukey double arcsine	0.43	0.21,0.67	99.84	< 0.001		
Seasonality										
Season	35	11141	21373	Random-effects model	0.476	0.393,0.558	99.5	< 0.001		
Non-season	8	3094	8673	Freeman-Tukey double arcsine	0.36	0.21,0.52	99.58	< 0.001		
Unknown	19	4115	14670	Random-effects model	0.450	0.300,0.599	99.8	< 0.001		
Publication language										
English	46	18649	43952	Random-effects model	0.442	0.347,0.536	99.9	< 0.001		
Chinese	19	4940	9453	Random-effects model	0.502	0.429,0.575	98.2	< 0.001		
Ethnicity										
Chinese	49	18256	33962	Random-effects model	0.478	0.424,0.532	99.2	< 0.001		
Others	17	5041	18692	Freeman-Tukey double arcsine	0.38	0.23,0.55	99.79	< 0.001		

Table 4 The results of meta-regression (EV-A71)

Covariate	Coefficient	95% CI	*t*	*P*	Adjusted R^2 (%)
Age					29.58%
<1 y	-	-	-	-	
1-3 y	-.1894914	-.2742778 -.104705	-4.40	0.000	
3-5 y	-.1741073	-.2596863 -.0885282	-4.01	0.000	
< 5 y	-.13795	-.2150069 -.0608931	-3.53	0.001	
>5 y	.1171723	.036483 .1978616	2.86	0.005	
Gender					-1.53%
Female	-	-	-	-	
Male	-.0013413	-.1044165 .1017338	-0.03	0.979	
Sampling method					1.39%
Physical examination	-	-	-	-	
Random sampling	.1104207	-.0156826 .2365241	1.75	0.085	
Conditional sampling	.0836351	-.0691683 .2364385	1.09	0.279	
Seasonality					0.47%
Unknown	-	--	-	-	
Seasonal	.1089901	-.0323517 .2503319	1.54	0.129	
Non-seasonal	.0829203	-.072398 .2382386	1.06	0.291	
Publication language					0.47%
English	-	-	-	-	
Chinese	.0610441	-.0415077 .163596	1.19	0.240	
Ethnicity					1.28%
Others	-	-	-	-	
Chinese	.0723176	-.0303551 .1749904	1.40	0.165	

The first line of every covariate represented reference; adjusted R^2 was used to indicate the degree of heterogeneity explained by study characteristics.

inactivated EV-A71 vaccine in China in 2016, the number of deaths from HFMD in China has decreased significantly, and the EV-A71 epidemic has been effectively controlled [74]. In this meta-analysis, most of the EV-A71 studies assessed the prevalence of the virus before 2016 [5]. Since the EV-A71 vaccine was widely administered in 2016, other enterovirus serotypes have replaced EV-A71 as the main cause of severe HFMD for the first time [75]. Due to the lack of cross-protection by inactivated monovalent EV-A71 vaccine against other enteroviruses, enteroviruses such as CVA16 and CVA6 remain relatively common, highlighting the need for attention to be given to these enteroviruses in future epidemic prevention and control strategies. In addition, serotype identification and vaccine development for other enteroviruses should be strengthened.

In a number of studies performed in China, the prevalence of HFMD showed obvious seasonality, with two main epidemic peaks: one in late spring and early summer and one in late autumn and winter. The factors causing this epidemic and seasonal pattern may be related to geographical location, population density, and environmental conditions, which need to be evaluated further [1].

Maternal antibodies play an important role in protecting children from HFMD. In a study in Thailand, serum samples were collected from children at birth (0 months of age) and 2, 7, 18, 24, 36, and 48 months of age, and the titre of neutralizing antibodies against EV-A71 was measured. The results showed that the serum protection rates (NT antibody 1:16) of children at 0, 2, 7, 18, 24, 36, and 48 months were 81.0%, 60.0%, 9.0%, 10.0%, 13.0%, 17.0%, and 37.1%, respectively. These findings indicated that the antibody titre was very high at birth. However, the antibody titre decreased significantly in the first year of life and reached its lowest value at approximately 7 months of age [70]. In another sero-epidemiological investigation of enteroviruses in 488 healthy subjects aged 2-83 months, maternally derived neutralizing antibodies to EV-A71 and CVA6 in neonates decreased to their lowest levels (11.11% and 10.14%) at approximately 6 months of age and increased thereafter [72]. In China, antibody levels in preschool children are low, and kindergarten children have a significantly increased risk of contracting the virus. A separate report detailing the seroepidemiology of EV-A71 in children in Singapore also noted that most infections occurred in preschool children at an age when children are concentrated in classrooms, sharing toys and teaching tools [43].

The seroprevalence rate of HFMD virus varies greatly in different countries and regions. In a prospective study of

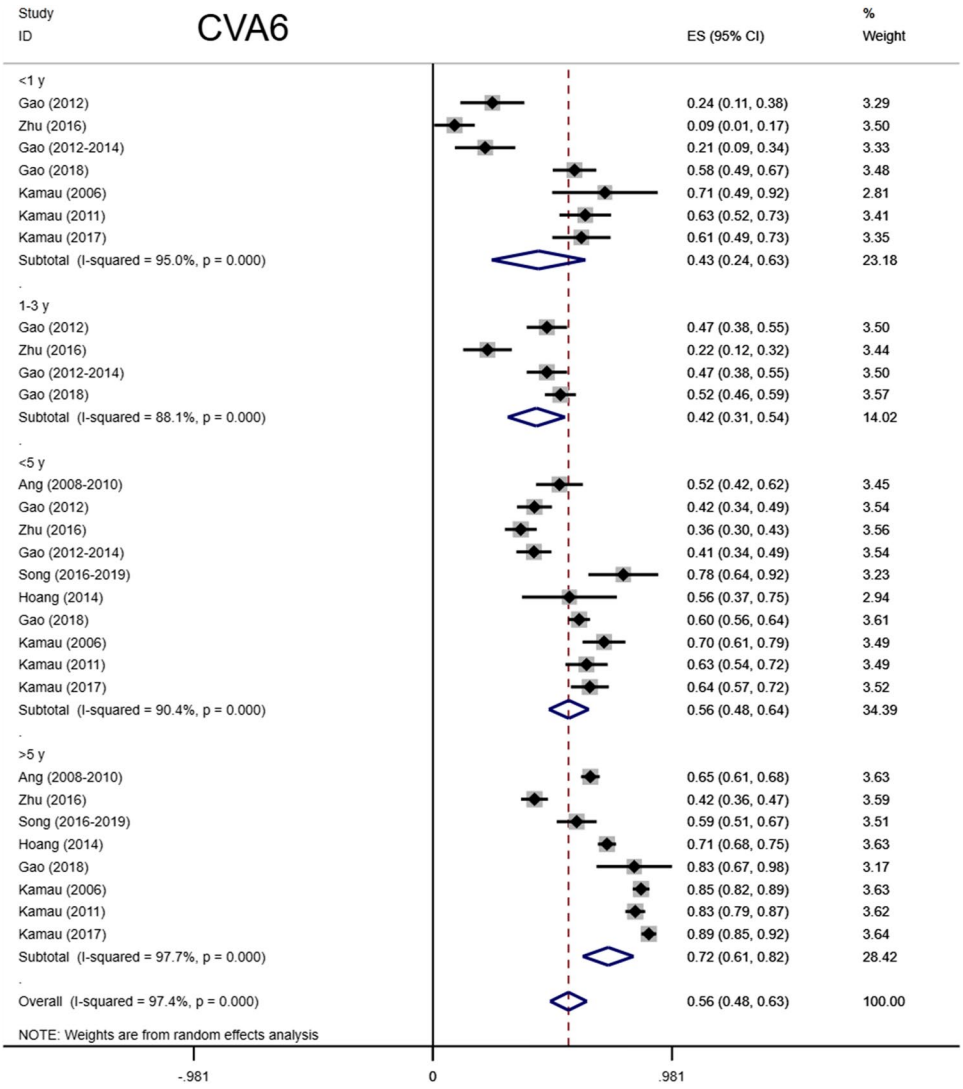

Fig. 3 Forest plots for the seroprevalence of CVA6 antibody in different age groups

healthy children in Finland, EV-A71 was detected in only 0.3% of fecal samples and two serum samples, and the positive rate for neutralizing antibodies was only 1.6% [76]. In a Norwegian study of 1,255 stool samples tested by RT-PCR, the rate of EV-A71 infection was only 1.4% [77]. In Germany, 27% of healthy children under 4 years of age had neutralizing antibodies against EV-A71, and 75% of people aged 20-40 years had neutralizing antibodies against EV-A71 [50, 53]. A study in the United Kingdom found that the seroprevalence of EV-A71 and CVA6 increased from 32% and 54% at 6-11 months of age to >75% at 10 years of age. EV-A71 was most commonly found in stool, followed by cerebrospinal fluid, respiratory tract samples, vesicle or skin swabs, and blood. CVA6 was most frequently detected in vesicles or skin swabs, followed by respiratory specimens, stool, cerebrospinal fluid, and blood [8]. In this study, the lowest serum positive rate of EV-A71 in children less than 1 year old was 28%, and the serum positive rate gradually increased with age. The positive rate of EV-A71 was 32.7% in the 1- to 3-year-old group, 50.2% in the 3- to 5-year-old group, and 62% in the over 5-year-old group. The serological survey of CVA6 showed a similar trend. In another serological meta-analysis, the seropositivity of CVA16 was lowest in children under one year of age and increased with age.

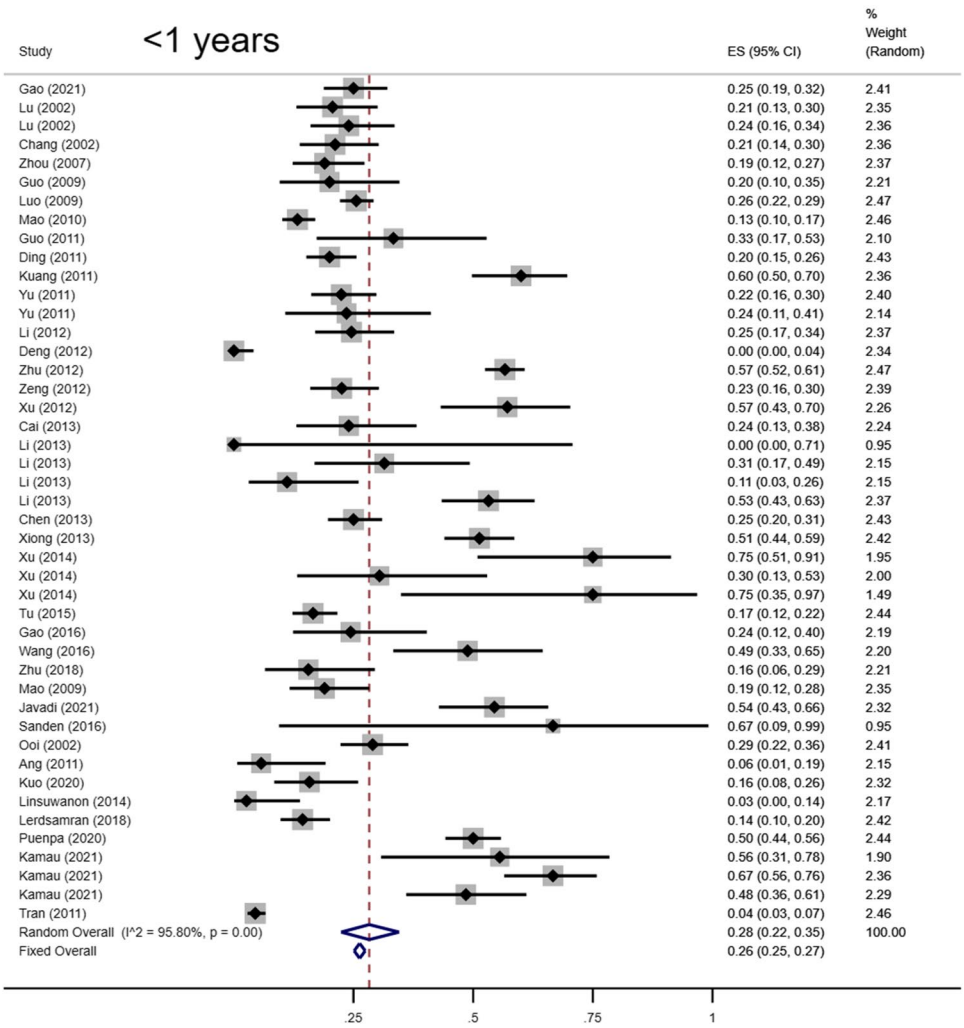

Fig. 4 Forest plots for the seroprevalence of EV-A71 antibody in the less-than-one-year age group

No significant differences were observed between males and females in the serum positivity rates for EV-A71 and CVA6. The EV-A71 positivity rate in China was higher than that in other countries, whereas the CVA6 positivity rate in China was lower than that in other countries [78].

In previous studies, most HFMD cases occurred in children, with a lower proportion of symptomatic infections in adults. However, a survey in Vietnam involving household contact transmission by subjects of all ages showed that the EV-A71 infection rate by household contact was 47.6%, the CV-A6 infection rate was 31.5%, and only 6.20% of household contacts developed symptoms. Symptoms of EV-A71 infection occurred in less than 10% of the 25-29, 30-34, and 45-49 age groups, and symptoms of CV-A6 infection occurred in less than 10% of the 25-29, 50-54, and 60-plus age groups. These results suggest that serologically detectable infections continue to occur in middle-aged and older adults at rates similar to those in children, but only a few individuals develop clinical symptoms. Therefore, specimen collection from family members of positive patients is strongly recommended, and the home environment is one of the targets of HFMD intervention [69]. At the same time, in the field of basic research, we should further clarify the main causes of susceptibility differences between children and adults and for symptoms after infection [69]. Molecular epidemiological surveillance of epidemic strains should be strengthened to prevent large-scale epidemics of mutant strains.

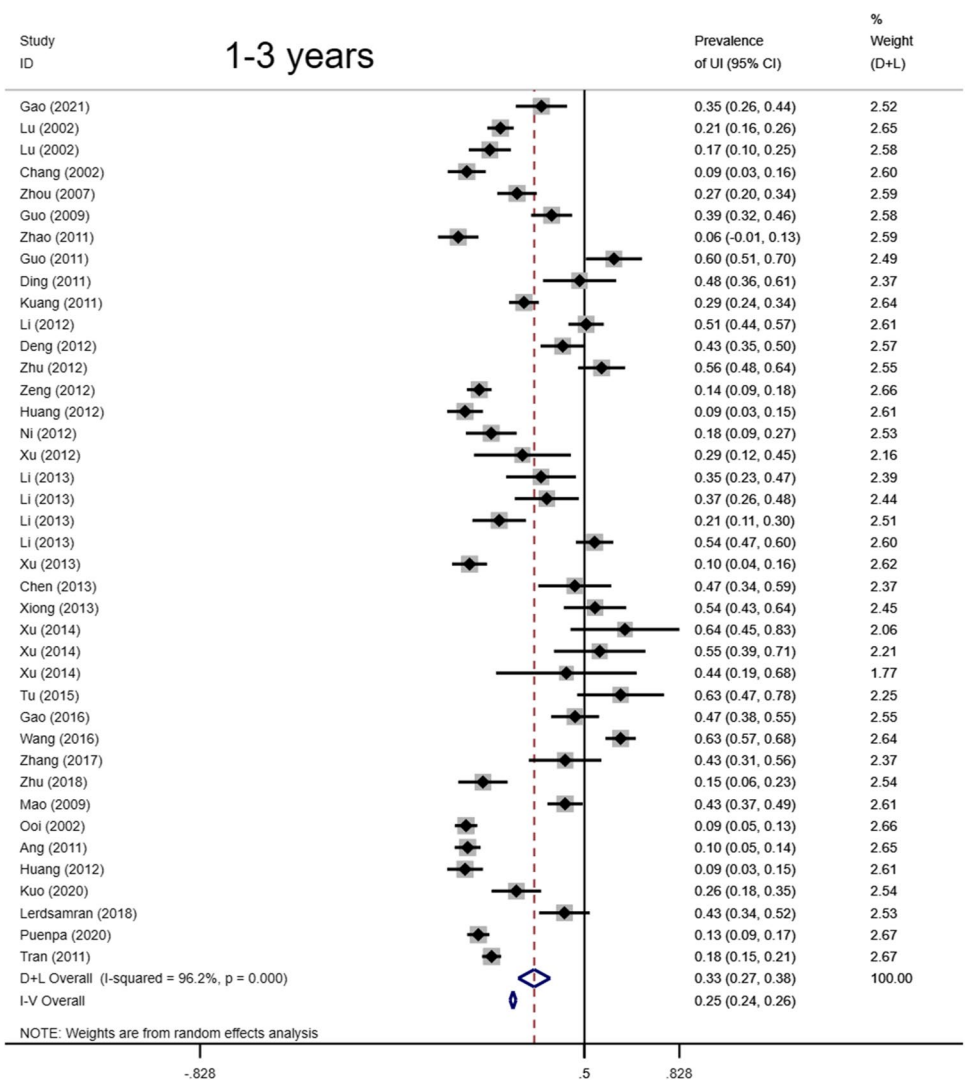

Fig. 5 Forest plots for the seroprevalence of EV-A71 antibody in the 1-3 years age group

Given the increase in the incidence and severity of cases of HFMD caused by CVA6, it is necessary to strengthen the monitoring of HFMD and research on related vaccines and to actively educate the population about HFMD prevention and control before the epidemic peak years and peak seasons to improve public awareness. In view of the prevalence of HFMD, the accuracy of virus detection needs to be improved to prevent missed diagnosis and misdiagnosis. Early detection, diagnosis, and treatment are necessary to reduce the number of severe cases and deaths.

Most of the data in this study showed relatively high heterogeneity. Thus, for most of the analyses, random effects models were used or double-arcsine transformation was performed, and most of the variation could be explained by variables contained in the meta-regression model. The unexplained variability may be due to some other unmeasured factors in the study, such as possible differences in the time of specimen collection, serum storage time and methods, or virus strains selected in laboratory tests.

Our study has some limitations. First, the estimates we obtained were mainly obtained from China and other Southeast Asian countries, which are densely populated. Therefore, these findings may not objectively reflect the global seroepidemiological situation. Second, a standardized protocol for the detection of neutralizing antibodies is lacking, and laboratory techniques and reagents used by different

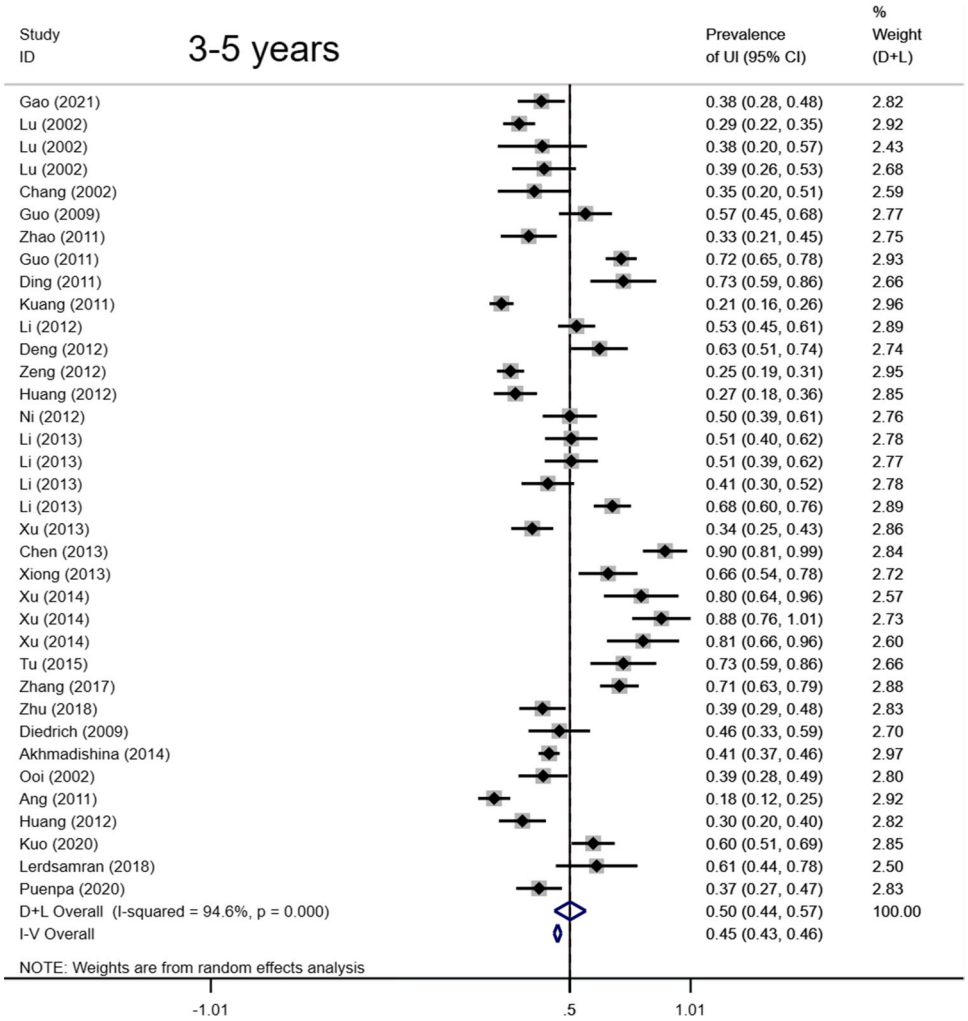

Fig. 6 Forest plots for the seroprevalence of EV-A71 antibody in the 3-5 years age group

laboratories differ greatly, which may lead to differences in the titre and serological prevalence of neutralizing antibodies. Third, relatively high heterogeneity was observed among the seroprevalence estimates in this study, which could not be fully explained in the meta-regression. Thus, there may be other factors influencing seroprevalence that were not analysed in this meta-analysis. Numerous retrospective and cross-sectional studies were included in this meta-analysis, and a number of studies used previously preserved serum samples. Antibody titres may decrease over time during storage, leading to deviations in the test results.

Conclusions

EV-A71 is the main pathogen of HFMDin infants and young children and is capable of causing neurological complications. Due to widespread vaccination with the EV-A71 vaccine, CVA6 has gradually become the dominant virus causing HFMD. Because only a subset of EV-A71- and CVA6-infected individuals experience clinical symptoms, the available data do not accurately reflect the prevalence of the virus. In this study, the seroepidemiology of EV-A71 and CVA6 was analysed systematically. The results showed that infants and young children had higher antibody levels at

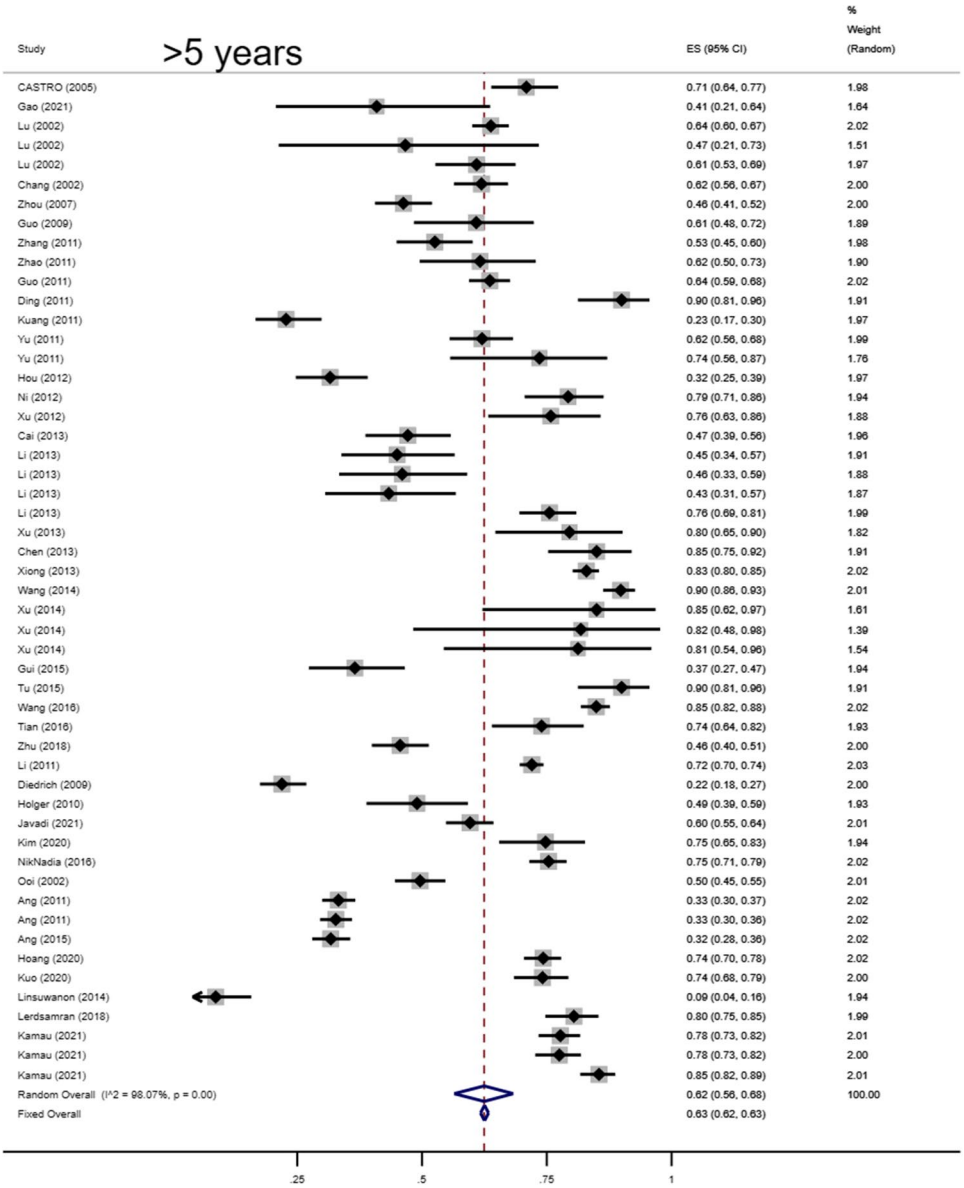

Fig. 7 Forest plots for the seroprevalence of EV-A71 antibody in the older-than-five-years age group

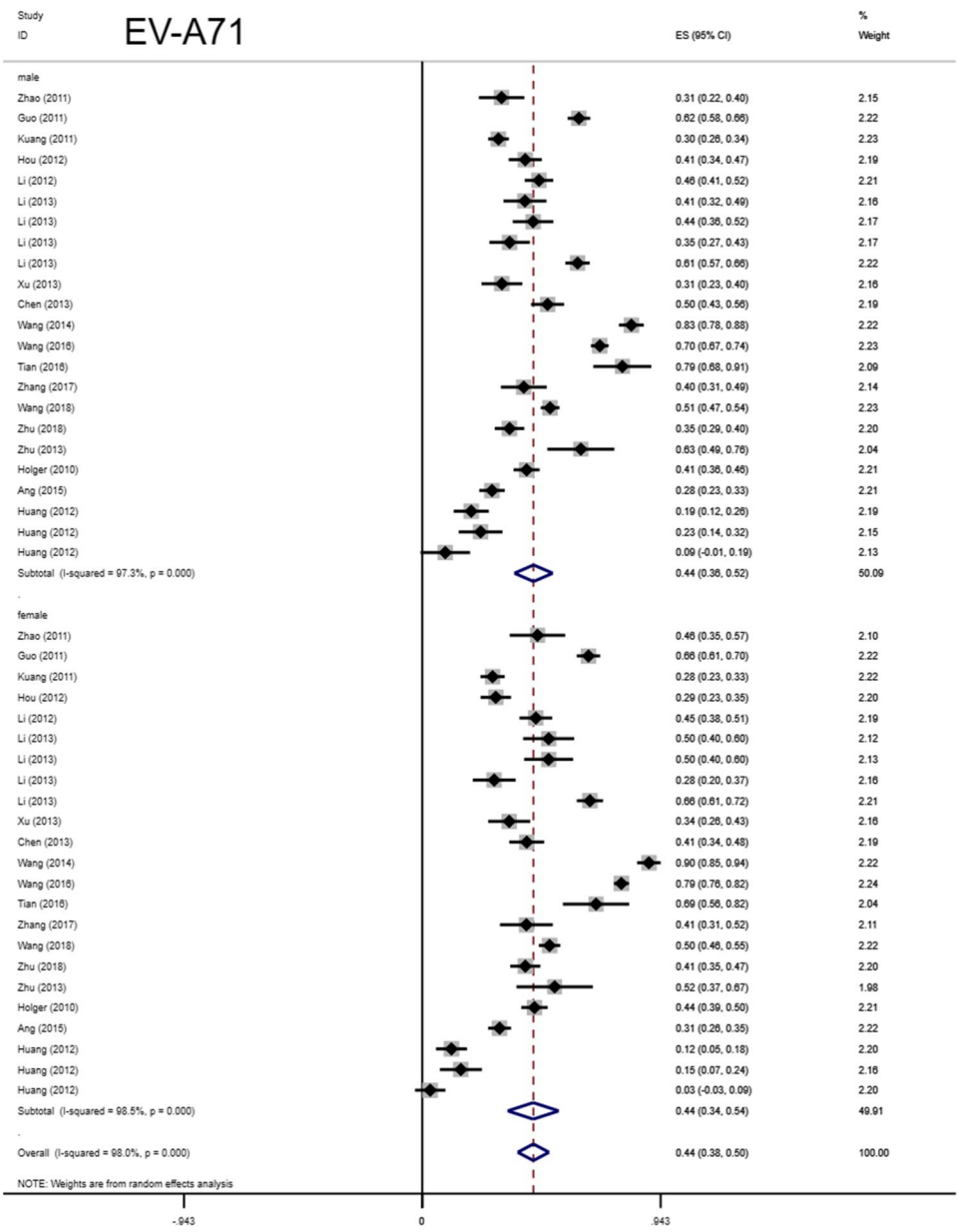

Fig. 8 Forest plots for the seroprevalence of EV-A71 antibody in males and females

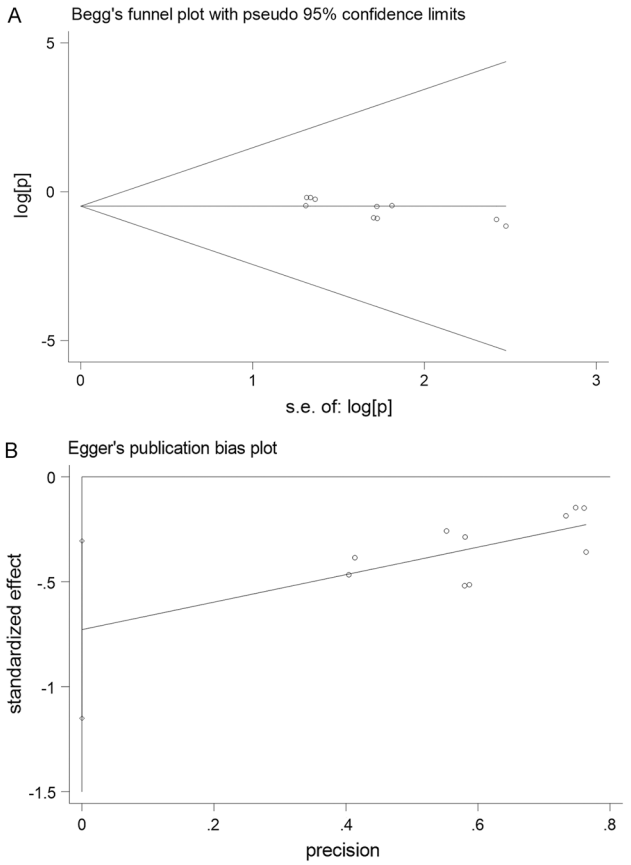

Fig. 9 Begg's and Egger's publication bias plot for the seroprevalence of CVA6 antibody

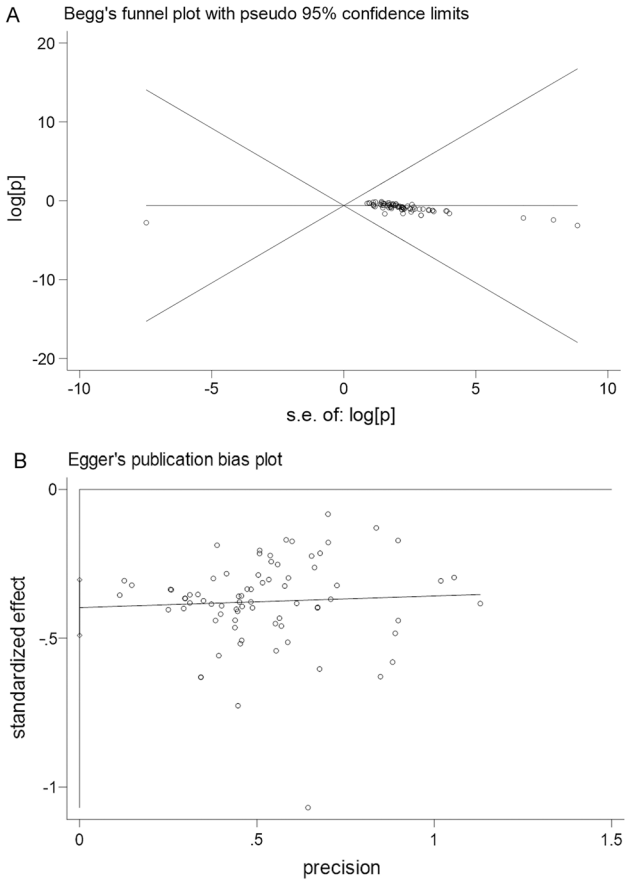

Fig. 10 Begg's and Egger's publication bias plot for the seroprevalence of EV-A71 antibody

birth, which decreased to the lowest level within the first year and then gradually increased thereafter. EV-A71 and CVA6 serum antibody levels were correlated most closely with age, followed by sampling season and sampling method.

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Author contributions Yingying Shi and Yongjuan Liu designed the project. Yingying Shi, Peiqing Chen, Yijing Bai, and Xuan Xu performed statistical analysis. Yingying Shi, Peiqing Chen, Yijing Bai, Xuan Xu, and Yongjuan Liu interpreted and wrote the manuscript. All authors approved the final manuscript.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflicts of interest relevant to this study.

Ethical approval Ethical approval was not needed because this is a meta-analysis.

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