

Prevalence of recessive infection of pathogens of hand, foot, and mouth disease in healthy people in China

A meta-analysis

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Abstract

Background: To analyze the prevalence of latent infection of pathogens of hand, foot, and mouth disease (HFMD) in Chinese healthy population and its influencing factors, so as to provide reference for the prevention and control of HFMD.

Methods: A systematic literature searching about the incidence of latent infection of HFMD was conducted in Chinese and English databases. The inclusion and exclusion criteria of the retrieved literature were established. The qualified literatures were screened and the data were extracted. The pooled rate and its 95% confidence interval was used to assess the latent infection rate of HFMD pathogens in healthy Chinese population, and subgroup analysis was conducted based on gender and age. All statistical analyses were performed using the STATA version 12.0 software.

Results: A total of 31 literatures were included in this meta-analysis. The recessive infection rate of HFMD pathogens reported in the literature of Chinese healthy people ranged from 4.59% to 44.12%. The results of meta-analysis showed that the latent infection rate of human enteroviruses (HEVs) in healthy Chinese population was 17.5% (14.9–20.1%), among which, the latent infection rates of EV-A71, CV-A16, and other HEVs were 3.3% (2.2–4.4%), 1.7% (1.0–2.5%), and 15.1% (11.1–17.1%), respectively. The latent infection rates of HEVs in healthy men and women in China were 16.7% (12.9–20.4%) and 14.4% (10.8–18.0%), respectively. The latent infection rates of HEVs in the healthy population aged 0 to 5 years and over 5 years were 24.4% (20.4–28.5%) and 9.4% (6.5–12.2%), respectively. Meta regression showed that the factors affecting the latent infection rate of HEVs in Chinese healthy population included sampling period, sampling area, and study population.

Conclusion: The latent infection rate of HEVs is high in healthy people in China, but it is mainly caused by other enteroviruses. The latent infection rate of HEVs in male was higher than that of female and was greater in people aged 0 to 5 than that of aged over 5 years. Limited by the quantity and quality of the included studies, more high-quality studies are needed for further verification in the future.

Abbreviations: Cox A16 = coxsackievirus group A, EV71 = enterovirus 71, HEVs = human enteroviruses, HFMD = hand, foot, and mouth disease, RT-PCR = reverse transcription polymerase chain reaction.

Keywords: foot and mouth disease, hand, human enterovirus, inapparent infection, meta-analysis

1. Introduction

Hand, foot, and mouth disease (HFMD) is a common acute infectious disease in children, mostly under 5 years of age, caused

by several human enteroviruses (HEVs). The main clinical manifestations of HFMD are skin herpes of hand and foot and oral mucosal rash. In a few cases, there are sterile meningial

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encephalitis, brainstem encephalitis, neurogenic pulmonary edema, and cardiac injury.^[1] The pathogens that can cause HFMD clinically includes coxsackievirus type 2, 4, 5, 7, 9, and 10 in coxsackievirus group A, ECHO and enterovirus 71 (EV71) in group B, among which coxsackievirus group A (Cox A16) and EV71 are the most common.^[2] Cox A16 was the main pathogen prevalent in the early stage of HFMD, but it did not attract wide attention due to its mild symptoms and small number of patients.^[3] In recent years, in the outbreak or epidemic of Cox A16 and EV71 in mainland China and Taiwan, it has been found that coincident or alternating epidemic transmission of Cox A16 and EV71 is easy to cause severe disease or even death, and the infection rate of EV71 is increasing year by year.^[4,5]

Humans are the only natural host of enterovirus, the infection sources of HFMD include patients, recessive infection.^[6] HFMD virus was mainly transmitted by digestive tract (fecal–oral route) and respiratory tract (droplet, cough, sneeze). Hands, towels, toothbrushes, toys, bowls and chopsticks, milk products, and medical devices contaminated with human feces, herpes fluid, and respiratory secretions can also be spread. Among them, the contaminated hand is the key medium of transmission. Although it is not clear whether water and food are involved in transmission, studies have shown the potential for waterborne transmission of enterovirus if water is not treated effectively.^[7] People are generally susceptible to enterovirus, especially infants and children.

HFMD is a public health problem that seriously affects children's health, and the number of cases is increasing year by year. At present, the related researches on HFMD mainly focus on the epidemic characteristics and pathogenesis characteristics of the diagnosed population, risk factors of severe cases, and other related aspects. However, the main transmission group of HFMD is the recessive infected person. Since most people show recessive status after human enterovirus infection, it is difficult to be detected clinically, so it is difficult to carry out effective isolation measures, which may easily lead to further spread of the epidemic.

The investigation of the latent infection rate of enterovirus in healthy people is of great significance for the effective prevention of HFMD transmission among children, which is conducive to the understanding of the current situation of the latent infection group of HFMD. Therefore, meta-analysis was adopted in this study to comprehensively analyze the published literature on the recessive infection rate of HFMD pathogens, and to evaluate its influencing factors, so as to provide scientific basis for the prevention and control of HFMD in the future.

2. Materials and methods

2.1. Literature retrieval

The electronic databases were searched by computer for retrieval articles about the prevalence of latent infection of pathogens of hand, foot, and mouth disease in healthy people in China. The China National Knowledge Infrastructure (CNKI) and Chinese WanFang database were used for retrieval in Chinese, and PubMed was used for literature retrieval in English with the search terms of (“HFMD” OR “hand foot and mouth disease”) and (“recessive” OR “inapparent” OR “silent”). Relevant literatures published from January 1, 1996 to April 30, 2020 were searched in the above databases. Retrospective search was conducted on the references cited by relevant literature after

screening to prevent the omission of literature search in the above databases.

2.1.1. Ethical approval. This study is a meta-analysis and does not involve patient and animal experiments so the ethical approval is not necessary.

2.2. Inclusion and exclusion criteria

Inclusion criteria:

- (1) the study type is cross-sectional study;
- (2) the research area is mainland China;
- (3) the sample size and the positive number or positive rate of hand, foot, and mouth enterovirus were available;
- (4) the detection method of enterovirus recessive infection was reverse transcription polymerase chain reaction (RT-PCR);
- (5) there are repeated published data in different literatures, and literatures with large sample size are selected.

Exclusion criteria:

- (1) literature on the analysis of recessive infection rate by antibody detection;
- (2) the latent infection rate of medical personnel and other special groups;
- (3) case reports, literature reviews, meeting abstracts, and other research literature;
- (4) repeated publication.

2.3. Literature screening and data extraction

The retrieved articles were imported into the Note Express 3.2 document management software for sorting and deduplication. The 2 researchers screened the literatures according to the inclusion and exclusion criteria. The literature finally included by the 2 researchers was compared with each other, and the inconsistencies were decided to be included or excluded through group discussion. The 2 researchers extracted information from the included literatures, including the first author, the year of publication, the study province, the positive number of enterovirus, the sample size, the type of specimen, whether the sampling period was in epidemic period, the sampling area, the study population, etc.

2.4. Quality evaluation

The methodological Quality of the included studies was assessed using 11 checklists recommended by the Agency for Healthcare Research and Quality (AHRQ).^[8] If a item is answered “no” or “unclear,” the project scores “0”; if the answer is “yes,” then the item gets a score of “1.” The quality evaluation score criteria are as follows: low quality=0 to 3, medium mass=4 to 7, high quality=8 to 11.

2.5. Statistical analysis

The point assessment of the recessive infection rate and the 95% confidence interval of each study were combined for the pooled rates, and the DerSimonian and Laird method (D–L method) of random effect model or the Mantel–Haenszel method (M–H method) of fixed effect model was selected according to the heterogeneity.^[9] Cochran's Q test was used for qualitative evaluation of the heterogeneity. If $P < .1$ for Cochran's Q test,

heterogeneity was indicated and random effect model was used for meta-analysis. If $P \geq .1$ for Cochran's Q test, the fixed effect model was selected for meta-analysis. The heterogeneity was quantified by I^2 test, and those with I^2 values of 0% to 25%, 26% to 50%, and 51% to 100% were considered as low, medium, and high heterogeneity, respectively.^[9]

Meta-regression analysis was used to explore the sources of heterogeneity. Potential publication bias was assessed by Begg's funnel plot and Egger's linear regression test.^[10,11] In sensitivity analysis, a single study was omitted item by item to evaluate whether a certain study had a significant impact on the stability of the results. All statistical analysis was performed using STATA version 12.0 software (STATA Corporation, College Station, TX). A P value less than .05 was considered statistically significant.

3. Results

3.1. Basic information of the included literature

A total of 243 related literatures including 76 in English and 167 in Chinese were obtained through preliminary retrieval. After screening according to inclusion and exclusion criteria, we finally included 29 articles in this meta-analysis.^[12-39] The literature selection process and results are shown in Figure 1. Among the 29 articles, 4 were in English and 24 were in Chinese, covering more than 20 counties and cities in 11 provinces of China, and a total of 11,921 subjects were studied. AHRQ quality score in the

included literature was 6 on average, 8 on the highest score, and 5 on the lowest score. The detailed information of the included literature on the incidence of HFMD enterovirus recessive infection is shown in Table 1.

3.2. Meta-analysis

A total of 11,921 cases were reported in 29 literatures, among which 1756 cases were positive for enterovirus common nucleic acid. The heterogeneity test showed that there was significant heterogeneity in each analysis group, and the results of the random effect model were selected. Meta-analysis showed that the recessive infection rate of HEVs was 17.5% (14.9–20.1%) in healthy Chinese people, among which the recessive infection rate of EV-A71 was 3.3% (2.2–4.4%), the recessive infection rate of CV-A16 was 1.7% (1.0–2.5%), and the recessive infection rate of other enterovirus was 15.1% (11.1–17.1%), as shown in Figure 2.

3.3. Subgroup analysis

We conducted a subgroup analysis based on gender and age. The results of meta-analysis showed that the rate of HEVs recessive infection was 16.7% (12.9–20.4%) in males and 14.4% (10.8–18.0%) in females. The rate of HEVs recessive infection was 24.4% (20.4–28.5%) at 0 to 5 years old, and 9.4% (6.5–12.2%) at more than 5 years old.

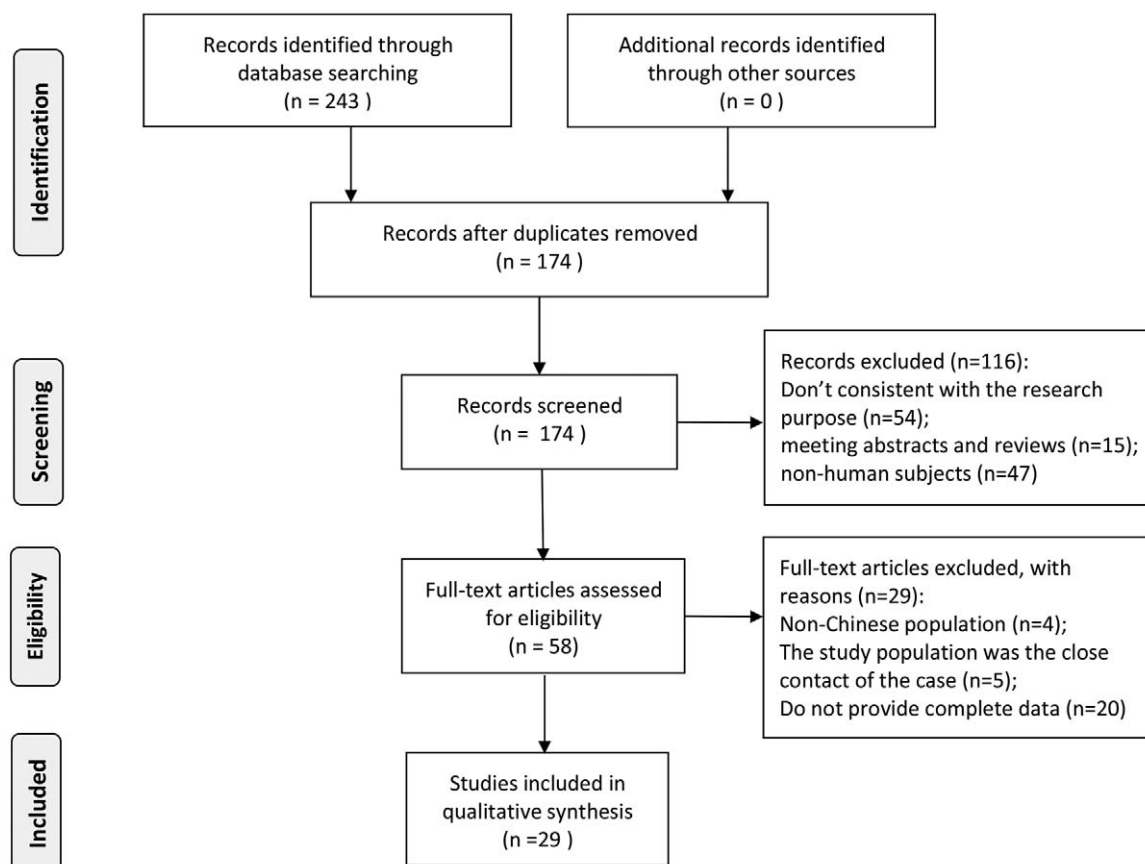


Figure 1. Selection of studies on the recessive infection of pathogens of HFMD in healthy people in China.

Table 1**Basic information of the included literature on the incidence of HFMD enterovirus repressive infection.**

Refs.	First author	Publication time	Province	HEVs (+)	Sample size	Latent infection (%)	Specimen type	Sampling time	Sampling area	Population	AHRQ score
[12]	Yang HK	2010	Guangdong	21	180	11.67	Feces	Epidemic	Rural	Children and adults	8
[13]	Kang N	2010	Guangxi	17	50	34.00	Feces	Epidemic	Urban	Children	6
[14]	Yin FQ	2011	Shandong	111	846	13.12	Feces	Epidemic	Urban and rural	Children	6
[15]	Liu CH	2011	Shandong	68	386	17.62	Feces	Epidemic	Urban and rural	Children and adults	5
[16]	Deng HL	2011	Shaanxi	13	207	6.28	Anal swab	Epidemic	Urban and rural	Children	7
[17]	Jiang WG	2011	Shandong	177	1235	14.33	Feces	Epidemic and non-epidemic	Urban and rural	Children and adults	5
[18]	Mo LF	2012	Guangdong	20	310	6.45	Feces	Non-epidemic	Urban	Children and adults	8
[19]	Ren Y	2012	Guangdong	13	150	8.67	Feces	Epidemic and non-epidemic	Urban	Children and adults	6
[20]	Ceng HR	2012	Guangdong	60	136	44.12	Feces	Epidemic	Rural	Children	5
[21]	Liu L	2012	Hebei	44	161	27.33	Feces	Epidemic	Rural	Children	6
[22]	Wang DL	2012	Guangdong	78	1305	5.98	Anal swab	Epidemic	Urban	Children	8
[23]	He Y	2012	Guangdong	18	392	4.59	Anal swab	Epidemic and non-epidemic	Urban	Children	7
[24]	Niu WD	2012	Henan	49	200	24.50	Feces	Epidemic	Rural	Children and adults	6
[25]	Yi QH	2013	Jiangsu	67	309	21.68	Anal swab	Epidemic	Urban and rural	Children	6
[26]	Chen FY	2013	Hebei	40	180	22.22	Anal swab	Epidemic	Urban and rural	Children	5
[27]	Zhang	2013	Shandong	59	254	23.23	Feces	Epidemic	Rural	Children	8
[28]	Wu	2013	Guangdong	34	320	10.63	Feces	Epidemic and non-epidemic	Urban	Children	7
[29]	Li Y	2013	Henan	71	200	35.50	Feces	Epidemic	Rural	Children and adults	5
[30]	Cai MS	2013	Guangdong	40	240	16.67	Feces	Epidemic	Urban	Children and adults	5
[31]	Liu FR	2014	Guangdong	28	118	23.73	Anal swab	Epidemic and non-epidemic	Urban	Children	6
[32]	Sun BC	2014	Zhejiang	51	395	12.91	Anal swab	Epidemic and non-epidemic	Urban	Children	8
[33]	Hou ZY	2015	Henan	26	106	24.53	Anal swab	Epidemic and non-epidemic	Urban	Children	5
[34]	Feng X	2015	Jiangxi	18	100	18.00	Anal swab	Epidemic	Urban and rural	Children	5
[35]	Zhang L	2015	Shandong	123	1275	9.65	Feces	Epidemic and non-epidemic	Urban and rural	Children	6
[36]	Gao W	2016	Hebei	11	130	8.46	Anal swab	Epidemic and non-epidemic	Urban	Children	6
[37]	Wang HQ	2016	Chongqing	211	1276	16.54	Anal swab	Epidemic and non-epidemic	Urban and rural	Children	8
[38]	Wu	2017	Yunnan	90	667	13.49	Feces	Epidemic	Rural	Children and adults	7
[39]	Yuan W	2018	Sichuan	75	193	38.86	Feces	Epidemic	Urban and rural	Children	5
[40]	Xie Y	2019	Shaanxi	123	600	20.50	Anal swab	Epidemic and non-epidemic	Urban and rural	Children	5

HEVs = human enteroviruses, HFMD = hand, foot, and mouth disease.

3.4. Meta-regression analysis

In order to further explore the source of heterogeneity, a meta-regression analysis was conducted with the Chinese population HEVs repressive infection rate as the dependent variable and the sample type, sampling period, sampling area, and study population as the covariables. The results showed that the size of the heterogeneity explained by the covariates included in the model was $R^2 = 34.86\%$, and the joint test of all covariates in the model was $P = .002$. The factors affecting the HEVs repressive infection rate in the Chinese population were the sampling period ($t = 2.56$, $P = .016$), the sampling area ($t = 2.16$, $P = .039$), and the study population ($t = 3.03$, $P = .005$), as shown in Table 2.

3.5. Sensitivity analysis

Meta re-analysis was conducted by removing single studies one by one to observe the influence of a single study on the results. The sensitivity analysis results showed no significant difference in the associated repressive infection rate, indicating that the meta-analysis results were relatively stable and that a single study would not have a significant impact on the meta-analysis results, as shown in Figure 3.

3.6. Publication bias analysis

The Begg's funnel plot and Egger's linear regression were used to evaluate publication bias, which showed that there was a significant publication bias, as shown in Table 3 and Figure 4.

4. Discussion

The results of the present meta-analysis showed that the repressive infection rate of HEVs was high among healthy people in China, mainly with other enteric viruses. The repressive infection rates of EV-A71 and CV-A16 were also high, at 3.3% (2.2–4.4%) and 1.7% (1.0–2.5%), respectively. Subgroup analysis showed that male HEVs repressive infection rate was slightly higher than female. The repressive infection rate of HEVs was significantly higher in the population aged 0 to 5 than in the population aged more than 5 years old. The gender and age distributions of HEVs repressive infection rate in healthy Chinese population are consistent with the distribution characteristics of HFMD cases in China.^[41] It is suggested that the prevention and control measures focus on young children should be taken during the epidemic of HFMD.

Meta-regression analysis showed that the factors affecting the HEVs repressive infection rate in Chinese population included sampling period (epidemic and non-epidemic), sampling area (city and rural), and study population (children and adults), which were consistent with the epidemiological characteristics reported in China such as the epidemic period of HFMD, higher incidence in rural areas than in urban areas, and more cases concentrated in children under 5 years old. In the future, health education on HFMD prevention in nurseries and primary schools in rural areas should be strengthened, and good hygiene practices should be promoted, especially during epidemics of HFMD.

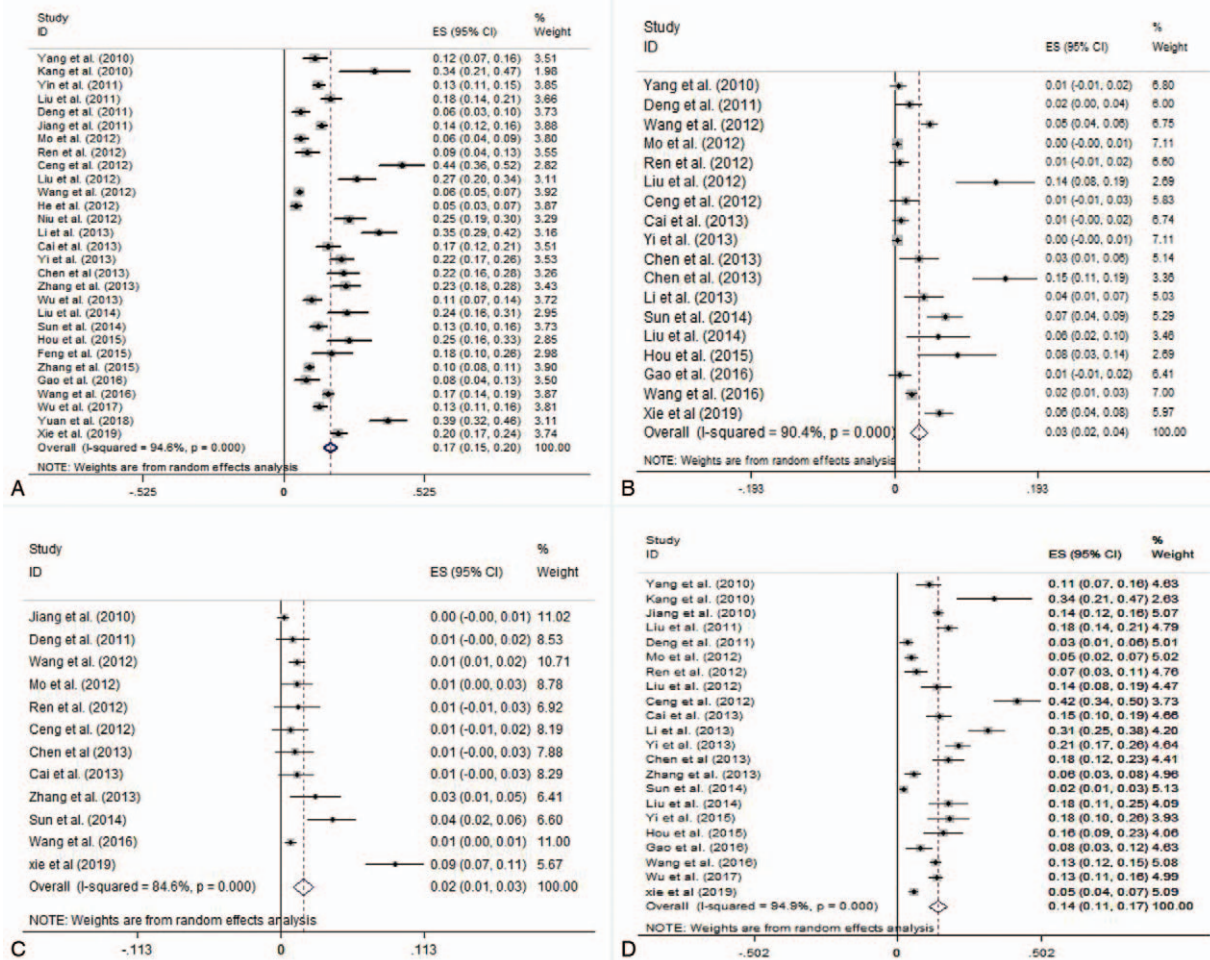


Figure 2. Forest plots for the pooled recessive infection of pathogens of HFMD in healthy people in China (a. HEVs, b. EV-A71, c. CV-A16, and d. other HEVs).

Kindergartens, primary schools, and other key units should strengthen morning screening to identify cases as soon as possible. If a suspicious case is found, the one should be encouraged to return to the hospital for medical treatment and placed in home isolation. Schools should pay close attention to

the physical condition of close contacts of the case, and classes may be suspended if necessary.

There was a large heterogeneity in this meta-analysis, so subgroup analysis and meta-regression analysis were used to explore the source of heterogeneity. Gender and age were selected

Table 2
Meta-regression analysis results of the latent infection rate of HFMD pathogens in healthy Chinese population.

Covariates	Coefficient	S.E.	t value	P	95%CI
Sample type					
Feces	0.056	0.053	1.08	.291	-0.051 to 0.164
Anal swab	Reference				
Sampling period					
Epidemic	0.094	0.036	2.56	.016	0.019-0.168
Non-epidemic	Reference				
Sampling area					
Rural	0.053	0.024	2.16	.039	0.003-0.104
Urban	Reference				
Study population					
Children	0.105	0.035	3.03	.005	0.034-0.075
Adults	Reference				
Constant	0.167	0.071	2.37	.025	0.023-0.312

HFMD=hand, foot, and mouth disease.

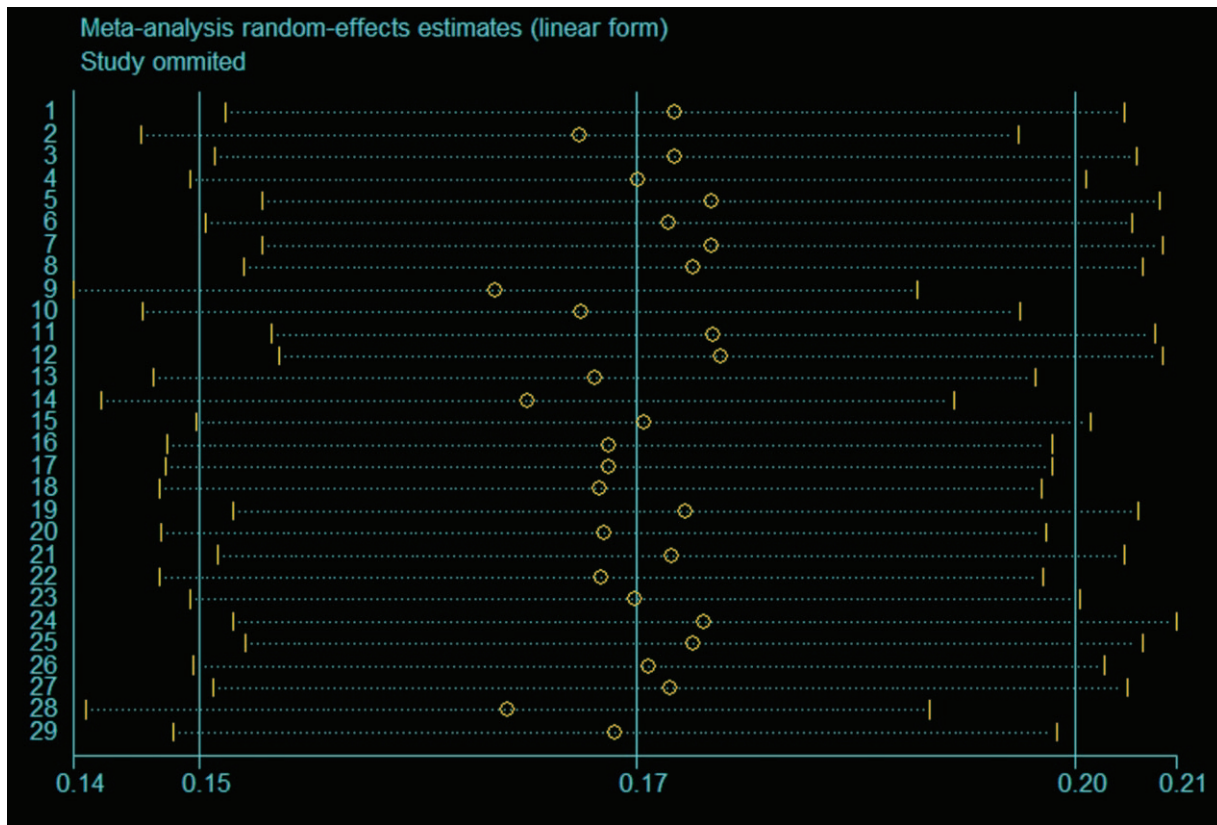


Figure 3. Sensitive analysis for the recessive infection of HEVs in healthy people in China by removing single studies one by one.

as the grouping factors in this meta-analysis. Since most of the literature grouped these 2 factors, the analysis of these 2 subgroups could appropriately avoid the influence of other confounding factors. For other factors that may influence heterogeneity, meta-regression analysis was used to include the possible influencing factors into the covariables. However, meta regression analysis requires a certain number of covariables, usually no more than the number of included references/10, otherwise, type I error will increase significantly.^[42] In this meta-regression analysis, 34.86% of the heterogeneity was explained. Other sources of heterogeneity may include geographical location, climatic factors, sample size, sampling method, etc.

In order to evaluate the robustness of meta-analysis results, sensitivity analysis showed that the removal of a single reference would not have a significant impact on the combined results, indicating that the study results were relatively stable. The analysis of publication bias showed that there was significant publication bias in each comparison group. The publication bias may be caused by the unpublished literature with low recessive infection rate or the unsearched gray literatures.

The results of this meta-analysis need to be interpreted with caution and have some limitations. Firstly, the heterogeneity of the studies included in each research group is relatively large. Although subgroup analysis and meta-regression were used in

Table 3 Meta-analysis results of the latent infection rate of HFMD pathogens in healthy Chinese population.

Group	No. of study	Latent infection	95%CI	Cohran's Q test for heterogeneity (P value)	I ² (%)	Model	Begg's P value	Egger's P value
HEV type								
HEVs	29	0.175	0.149–0.201	<.001	94.6	Random	<.001	<.001
EV71	18	0.033	0.022–0.044	<.001	90.4	Random	<.001	<.001
COA16	12	0.017	0.010–0.025	<.001	85.8	Random	.024	.008
Other HEVs	22	0.151	0.111–0.171	<.001	94.9	Random	.010	<.001
Gender								
Male	13	0.167	0.129–0.204	<.001	86.5	Random	.009	.001
Female	13	0.144	0.108–0.180	<.001	86.1	Random	.161	<.001
Age								
0–5yr old	27	0.244	0.204–0.285	<.001	95.7	Random	.003	<.001
>5yr old	17	0.094	0.065–0.122	<.001	87.3	Random	.001	<.001

HEVs=human enteroviruses, HFMD=hand, foot, and mouth disease.

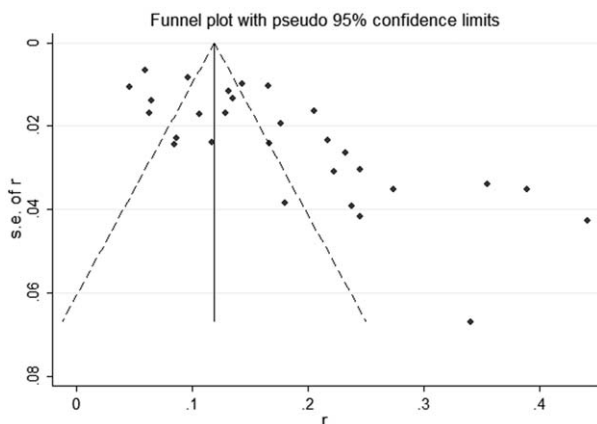


Figure 4. Begg's funnel plot for the pooled recessive infection of HEVs in healthy people in China.

this meta-analysis to explore the sources of heterogeneity, some heterogeneity could not be explained due to the lack of information in the original literature. Secondly, publication bias may lead to higher analytical results, and literature retrieval and screening may result in missing literature inspection. Finally, in the subgroup analysis, some literatures were not stratified according to the grouping factors, resulting in different numbers of literatures included in each comparison group.

In conclusion, Chinese healthy people have a high rate of HEVs recessive infection. The rate of HEVs recessive infection in males was higher than that in females. The recessive infection rate of HEVs was higher in people aged 0 to 5 than in people aged more than 5 years old. The factors influencing the HEVs recessive infection rate in Chinese population include sampling period, sampling area, and study population. Limited by the quantity and quality of the included studies, more high-quality studies are needed for further verification in the future.

Author contributions

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