

# Effectiveness of the Enterovirus A71 Vaccine in Guangdong Province, China

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## Research article

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# Abstract

**Background:** Guangdong Province started the enterovirus A71 (EV71) vaccination campaign officially in August 2016. This study aimed to evaluate the effectiveness of the EV71 vaccine against EV71, CA16, CA6, or other enterovirus infection through post-marketing surveillance in Guangdong Province in 2017.

**Methods:** A test-negative case-control design nested in a population-based cohort was used in this study. There were 3020 laboratory-confirmed child cases of hand, foot, and mouth disease (HFMD), including 409 children infected with EV71 and 2611 with CA16, CA6, or other enteroviruses. A total of 603 test-negative controls who were clinically diagnosed with HFMD in the same hospitals were enrolled in this study.

**Results:** Of all the subjects, 383 (10.6%) had received two doses of the EV71 vaccine. Only one (0.2%) out of the 409 cases with EV71 was vaccinated. In addition, 137 (5.2%) out of 2611 cases with CA16, CA6, or other enteroviruses and 21 (3.5%) out of 603 negative controls were also vaccinated. The adjusted estimate of the effectiveness was 93.6% (95% CI: 51.0%, 99.2%;  $p < 0.001$ ) for cases with EV71. The protective effect of the EV71 vaccine for cases with CA16, CA6, or other enteroviruses did not reach significance (95% CI: -130.4%, 10.6%;  $p = 0.134$ ).

**Conclusion:** The EV71 vaccine demonstrated very high protection against EV71 infection conferred by two doses of the EV71 inactivated vaccine used in Guangdong Province during 2017. No protection was found against CA16, CA6, or other enterovirus infections. Further development of combined vaccine options for HFMD control is needed.

## Background

Enterovirus A71 (EV71) is one of the major agents causing outbreaks of hand, foot, and mouth disease (HFMD) around the world[1]. In recent decades, epidemics of HFMD have mainly occurred in Southeast Asia, and EV71 has become a major public health problem for this region[2–5]. HFMD caused by CA16, CA6 and other serotypes is generally thought to be mild and self-limiting. Studies have shown that EV71 can cause serious complications in the central nervous system (CNS) and have the chance of genetic recombination, which may be responsible for the large HFMD outbreak[4]. The largest Asian epidemic of HFMD caused by EV71 occurred in China in 2008, when approximately 490,000 infections and 126 deaths in infants and young children were reported[6]. Following this epidemic, in 2008, HFMD was listed as a category C notifiable disease in China.

Guangdong Province, located in southern China, is a semitropical region in Southeast Asia with a population of 100 million. During the period from 2008 to 2017, Guangdong Province reported more than 2 million children with HFMD[7]. Among all the cases, 5238 severe cases and 276 deaths were reported with a case-fatality rate of 0.01%, and 77% of these were associated with the EV71 serotype.

In 2015, the China Food and Drug Administration (CFDA) approved the first inactivated Enterovirus A71 (EV71) vaccine for preventing severe hand, foot and mouth disease (HFMD). As one of the few preventive vaccines for children's infectious diseases generated in recent years, an EV71 vaccine is a positive measure to protect children's health in China and worldwide[8]. In China, this vaccine is manufactured by three institutes: 1. Institute of Medical Biology, Chinese Academy of Medical Sciences; 2. Sinovac Biotechnology Company; 3. Wuhan Biological Products Research Institute.

Although the EV71 inactivated vaccine has been shown to induce immune responses to EV71 successfully in infants and young children in clinical trials[9], post-marketing surveillance studies of the effectiveness of this newly developed vaccine against EV71 infection are lacking. As children remain at risk of infection from other enterovirus serotypes (other than EV71), the cross-protectiveness of the EV71 vaccine against other enterovirus serotypes, such as CA16, CA6 or other enterovirus infections is also of interest.

Guangdong Province officially started the EV71 vaccination campaign in August 2016. A test-negative case-control design nested in a population-based cohort was used in this study to evaluate the effectiveness of the EV71 vaccine against EV71, CA16, CA6, or other enterovirus infections in 2017 to prevent laboratory-confirmed hand, foot, and mouth diseases (HFMDs) in children in Guangdong Province.

## Methods

### Study population and data collection

The inactivated EV71 vaccine was recommended for children in the age range of 6 months to 5 years via the primary health care system. As per the immunization protocol[10], two separate doses of the vaccine are required with a one-month interval as part of existing routine immunization schedules[10]. The EV71 vaccine was not included in the Chinese free vaccine programme; therefore, parents were expected to pay for the vaccine. The vaccine supply was adequate in Guangdong Province. There were no specific targeted populations for receiving the vaccine, and parents or guardians chose to give the vaccine to their children voluntarily.

As the number of children immunized with the EV71 inactivated vaccine was very low in 2016, we carried out the study for all of 2017. The study covered the period from week 1 to week 52 of 2017. The HFMD case data were obtained from the National Disease Report System for the period from 1 January to 30 December 2017. Meanwhile, the immunization data of these cases were collected through the Guangdong Provincial Immunization Program Information Management System. Subjects were considered to be protected 28 days after vaccine administration. Written informed consent was obtained from the parents or guardians whose children were given the vaccine. According to the Diagnostic Criteria for HFMD, which was promulgated by The National Health and Family Planning Commission in 2009, a clinically diagnosed HFMD patient was defined as someone who had rashes on the hands, feet, mouth, or buttocks and ulcers or vesicles in the mouth with or without fever[11]. A laboratory-confirmed patient was

defined as a probable patient with laboratory evidence of infection with EV71, CA16, CA6, or other enteroviruses[11]. The diagnostic tests used for enterovirus detection were reverse transcription PCR and real-time reverse transcription PCR[12]. All the clinically diagnosed and laboratory-confirmed cases of hand, foot, and mouth disease were made statutorily notifiable to the National Disease Reporting Information System in China from May 2, 2008.

Twenty-two sentinel surveillance hospitals in twenty-one cities of Guangdong Province were selected for HFMD case surveillance. The physicians and paediatricians in the sentinel hospitals were asked to take stool specimens from 3 to 8 patients who were clinically diagnosed with HFMD every week of 2017 per hospital. The fixed sampling date and sampling size per week were arranged in every hospital, and the patients were chosen at random, which helped minimize the selection bias. Written informed consent are obtained from parents or guardians and verbal assent are obtained from children before study enrolment. Verbal informed consent had to be obtained from the parents or guardians before sampling. The stool specimens were processed by reverse transcription PCR and real-time reverse transcription PCR in the laboratories in the prefecture-level centres for disease control and prevention (CDC), and positive samples were characterized for EV71, CA16, CA6 and other enteroviruses.

### **Study design and statistical analysis**

We carried out a nested case–control study based on the HFMD sentinel surveillance system in Guangdong Province. The study population included all persons who had submitted stool specimens from physicians in the sentinel network during the period from week 1 to week 52 of 2017. The study population included 3623 children aged from 3 days to 14 years.

From the Guangdong Provincial Infectious Disease Information Monitoring System, we obtained the following baseline characteristics: sex, age, district of residence, onset date and swabbing date.

Two different comparisons were conducted to assess vaccine effectiveness and to estimate the ORs. For the first comparison, all laboratory-confirmed patients with laboratory evidence of infection with EV71 in sentinel hospitals were considered as cases, while clinically diagnosed HFMD patients who were negative for EV71, CA16, CA6, or other enteroviruses in the same sentinel hospitals acted as controls. For the second comparison, laboratory-confirmed patients who were negative for EV71 but positive for CA16, CA6, or other enteroviruses were considered cases, while those with a negative test acted as controls. The purpose of the first analysis was to provide an estimate of the effectiveness of the EV71 vaccine in preventing EV71 infection. The second analysis aimed to estimate whether the EV71 vaccine has the ability to prevent the occurrence of CA16, CA6, or other enterovirus infections.

Specific analyses were performed under different situations, including only patients aged less than 5 years because they were high-risk individuals for EV71 infection, considering only swabs taken in the first 3 days after symptom onset, and including only the epidemic period of weeks 14 to 45. All relevant covariates, such as age, sex, residence, research time, sampling time and immunization information, were included in the models for each specific analysis. Percentages were compared by  $\chi^2$ . Vaccine

effectiveness was estimated as  $(1-OR) \times 100$ . Logistic regression techniques were used to calculate crude odds ratios (ORs), and ORs were adjusted for the mentioned variables. When necessary, exact logistic regression was used.

## Results

### Description of study subjects

From 1 January to 30 December 2017, 3623 HFMD patients, with a median age of 4 years (range 1 month-9 years), were enrolled and swabbed. Most, 93.5% (3386) of patients were less than five years old. The male to female ratio was 1.68:1. Approximately, 75.0% (2719) of patients were swabbed within 3 days of symptom onset. The weekly number of swabbed patients peaked between weeks 14 and 45, which was largely consistent with the pattern of HFMD incidence in the population (Fig. 1). Across the entire sampling period, most 83.3% (3020) were confirmed for enterovirus: 13.4% (409) for EV71, 3.0% (92) for CA16, 69.1% (2087) for CA6 and 14.3% (432) for other enteroviruses. The predominant strain between weeks 14 and 20 was EV71 (the constituent ratio of EV71 varied from 35–63%), changing to CA6 between weeks 21 and 45 (the constituent ratio of CA6 varied from 36–100%). Among 3623 patients enrolled in the study, 44 (1.2%) subjects (all were the other enterovirus cases) had been vaccinated with only one dose of the EV71 vaccine, and 383 (10.6%) were vaccinated with two doses of the EV71 vaccine prior to illness onset by the immunization protocol for optimal effectiveness. As we had too few subjects who received one dose of the EV71 vaccine, the efficacy of the EV71 inactivated vaccine with one dose was not analysed in this study.

There were small differences between cases and controls. Compared with confirmed cases of EV71, the test negative controls had a higher proportion of children who were less than one year old and who were diagnosed between weeks 14 and 45 of 2017. A smaller proportion of EV71 cases than controls had been vaccinated with two doses of the EV71 vaccine. Compared with other enterovirus cases, the test negative controls had a smaller proportion of children who were less than five years old, who were male and who were diagnosed between weeks 14 and 45 of 2017. A higher proportion of enterovirus cases than controls had been vaccinated with two doses of the EV71 vaccine, were located in the Pearl River Delta Region and had been swabbed within 3 days of symptom onset (Table 1).

### Effectiveness of the EV71 inactivated vaccine with two doses against confirmed EV71 cases

Out of 409 cases confirmed for EV71, only one (0.2%) had been previously vaccinated with two doses of the EV71 vaccine. This case was a 3-year-old girl who had completed the vaccination protocol (2 doses a month apart?) on May 10 and started symptoms on June 12. Out of 603 negative controls, 21 (3.5%) had been vaccinated. Compared with the test-negative controls, a smaller proportion of confirmed EV71 cases had received the EV71 vaccine (OR: 0.064; 95% CI: 0.008; 0.49;  $p = 0.009$ ). In the analysis adjusted for age, sex, district of residence, swabbing date, and week period of diagnosis, the effectiveness of the EV71 vaccine was 93.6% (95% CI: 51.0%, 99.2%;  $p < 0.001$ ). In the analysis restricted to subjects aged less than 5 years, the effectiveness was 93.6% (95% CI: 50.9%, 99.2%;  $p < 0.001$ ). Estimates of vaccine

effectiveness were similar for patients swabbed within 3 days of symptom onset (93.0%; 95% CI: 46.0%, 99.1%;  $p = 0.011$ ) and those diagnosed between weeks 14 and 45 of 2017 (93.8%; 95% CI: 53.3%, 99.2%;  $p < 0.001$ ) (Table 2).

### **Effectiveness of the EV71 inactivated vaccine with two doses against CA16, CA6, or other enterovirus infections**

Out of 2611 cases confirmed for CA16, CA6, or other enterovirus infections, 137 cases (5.2%) had been vaccinated with two doses of the EV71 vaccine. Compared with the test-negative controls, the crude OR was 1.535 (95% CI 0.961 to 2.451;  $p = 0.073$ ) for the vaccine. In the analysis adjusted for age, sex, district of residence, swabbing date, and week period of diagnosis, the protective effect of the EV71 vaccine did not reach significance (95% CI: -130.4%, 10.6%;  $p = 0.134$ ). No significant relationship was observed when the analysis was restricted to three specific groups (subjects aged less than 5 years; patients swabbed within 3 days of symptom onset; those diagnosed between weeks 14 and 45 of 2017) (Table 3).

## **Discussion**

As a newly developed vaccine, the EV71 inactivated vaccine generated concern from public health professionals and parents, as it provided a new prevention tool for protecting children from EV71 infection. We conducted a nested case–control survey in 2017 to test the effectiveness of the vaccine. The findings provide useful evidence towards a comprehensive evaluation of the effectiveness of the vaccine for the post-marketing stage in which little research had previously been done.

Our study comprised only laboratory-confirmed cases that were compared with test-negative controls treated in the same hospitals, which would provide better comparability and reduce selection bias. This nested case–control design has been used in many studies that have evaluated vaccine effectiveness, such as influenza and hepatitis B[13–15]. This kind of research method was first used to evaluate the effectiveness of the EV71 vaccine to prevent unmeasured confounding[16].

Using the HFMD sentinel surveillance system in Guangdong Province for case finding, we detected excellent protection against EV71 infection conferred by two doses of the EV71 vaccine in 2017. Specifically, 28 days or more after vaccination, we estimated a vaccine effectiveness of 94% (95% confidence interval 51–99%) against medically attended, laboratory-confirmed EV71 cases in 2017. Our estimates of protection by vaccine are consistent with immunogenicity studies indicating a very high antibody response to the EV71 vaccine in children[9, 17].

A longer time between symptom onset and swabbing can lead to a lower positive rate of EV71 virus detection, which could underestimate vaccine effectiveness[18, 19]. This effect was considered in the design phase, and 75% of the specimens were collected during the first 3 days after symptom onset. In subsequent analysis, this variable was also controlled, and the analysis was carried out repeatedly after eliminating the cases swabbed after the first 3 days. No changes were discovered in the estimate of vaccine effectiveness.

HFMD cases occur every month, and an obvious seasonal pattern is evident in Guangdong Province, which generally had a large peak from April to June and a second smaller peak from September to October[11]. In 2017, 89.7% of the HFMD cases and 95.1% of the EV71 cases occurred from weeks 14 to 45 (April to October). The analysis was limited to this period for maintaining the effectiveness of the vaccine, although we finally found that the effectiveness remained stable.

In China, the EV71 vaccine has been indicated for children aged 6 to 59 months who have the highest risk of the disease[20]. Restricting the analysis to this population group, the EV71 vaccine was also effective, similar to children aged over 59 months.

In our study, the EV71 vaccine demonstrated very high protection against EV71 infection in each specific group of study subjects.

Although some other clinical studies have shown a weak cross-protective phenomenon in EV71 and CA16 infection[21, 22], others have reported no cross neutralization or interference between EV71 and other enteroviruses, such as CA16 and polioviruses[23–25]. This finding is consistent with other findings indicating that the EV71 vaccine is not protective against CA16, CA6, or other enterovirus infections[17]. To maintain confidence in this user-pay vaccination for HFMD, parents and guardians should be informed of the remaining risk of contracting HFMD, even after EV71 vaccination. Further, to reduce the additional burden associated with other serotypes, we need to continue to develop multivalent vaccines (e.g., EV71 combined with other prevalent circulating serotypes, CVA16 and CVA6) to prevent HFMD among infants and young children in the future.

Despite the many study design strengths, several limitations of this study are noted. First, compared with outpatients, we did not observe the effectiveness of the EV71 vaccine against hospitalized cases whose illness might be more severe and who have a lower response to the vaccine. Second, the total vaccination rate with two doses in our sample was low at approximately 10.6%. Although we found that only one out of 409 cases with EV71 had been vaccinated previously, this may be partly due to the low vaccination rate. Finally, there were systematic influences of bias (selection, information) in the sentinel system for monitoring vaccine effectiveness, which have been described previously[26–28]. Our study utilized a public health surveillance approach, making it very difficult for it to be as rigorous in controlling modifying factors as is possible in controlled clinical trials.

## Conclusions

This study demonstrated very high protection against EV71 infection conferred by two doses of the EV71 inactivated vaccine used in Guangdong Province during 2017, with particular reference to children less than five years old. No protection was found against CA16, CA6, or other enterovirus infections, consistent with other research. Our results provide additional evidence of the risk reduction and subsequent public health benefits of HFMD prevention in children provided by this user-pays vaccine and may help to inform the further development of a combined vaccine option for HFMD control.

# Abbreviations

EV71: Enterovirus A71

# Declarations

## Ethics approval and consent to participate

This study protocol was approved by the ethics committee of the Guangdong Centers for Disease Control and Prevention. Our study population included 3623 children aged from 3 days to 14 years. Written informed consent are obtained from parents or guardians and verbal assent are obtained from children before study enrolment.

Written informed consent is obtained from parents and verbal assent is obtained from children before study enrolment.

## Consent for publication

Not applicable.

## Availability of data and material

The datasets generated and/or analysed during the current study are not publicly available as per ethical approval from the ethics committee of the Guangdong Centers for Disease Control and Prevention, which stipulates that the data will not accessed or analyzed by others.

## Competing interests

The authors have no competing interests to declare.

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## Authors' contributions

FY, HZZ, MZ, VC, YFL PH and LMS contributed to the conception and design of the study. YF drafted the manuscript. YF, SR and TL revised the manuscript and all authors approved the final manuscript. RER is responsible for project oversight.

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## Tables

Table 1  
Characteristics of cases and controls

	<b>EV71 cases n(%)</b>	<b>Other enterovirus cases n(%)</b>	<b>Test negative controls n(%)</b>	<b>p-value (EV71 v.s control)</b>	<b>p-value (other v.s control)</b>
Total	409(100)	2611(100)	603(100)		
Age groups (years)				< 0.001	< 0.001
< 1	38(9.3)	494(18.9)	120(19.9)		
1–2	114(27.9)	1050(40.2)	197(32.7)		
2–3	91(22.2)	496(19)	107(17.7)		
3–4	70(17.1)	301(11.5)	69(11.4)		
4–5	44(10.8)	151(5.8)	44(7.3)		
≥ 5	52(12.7)	119(4.6)	66(10.9)		
Sex				0.611	0.025
Male	248(60.6)	1669(63.9)	356(59)		
Female	161(39.4)	942(36.1)	247(41)		
Residence				0.001	< 0.001
The Pearl River Delta Region	222(54.3)	1498(57.4)	264(43.8)		
The Other Region	187(45.7)	1113(42.6)	339(56.2)		
Period				< 0.001	< 0.001
Week 1 to 13	17(4.2)	54(2.1)	23(3.8)		
Week 14 to 28	325(79.5)	837(32.1)	232(38.5)		
Week 29 to 45	64(15.6)	1562(59.8)	228(37.8)		
Week 46 to 52	3(0.7)	158(6.1)	120(19.9)		
Delay between symptom onset and swabbing				0.079	< 0.001
≤ 3 days	303(74.1)	2000(76.6)	416(69)		
> 3 days	106(25.9)	611(23.4)	187(31)		
Dose of EV71 vaccine received				< 0.001	< 0.001

	<b>EV71 cases n(%)</b>	<b>Other enterovirus cases n(%)</b>	<b>Test negative controls n(%)</b>	<b>p-value (EV71 v.s control)</b>	<b>p-value (other v.s control)</b>
Two doses	12(2.9)	319(12.2)	52(8.6)		
One dose	0(0)	44(1.7)	0(0)		
No	397(97.1)	2248(86.1)	551(91.4)		
EV71 vaccine received > 28 days before onset of HFMD illness				0.001	0.071
Yes	1(0.2)	137(5.2)	21(3.5)		
No	408(99.8)	2474(94.8)	582(96.5)		

Table 2

Effectiveness of the EV71 inactivated vaccine with two doses against confirmed EV71 cases in 2017 in Guangdong, China

	<b>EV71 cases, N(%)</b>	<b>Test negative controls, N(%)</b>	<b>Crude OR (95% CI)</b>	<b>p-value</b>	<b>Adjusted vaccine effectiveness% (95% CI)</b>	<b>p-value</b>
Overall	409	603				
Vaccinated	1(0.2)	21(3.5)	0.064(0.008–0.490)	0.009	93.6(51.0 to 99.2%)	< 0.001
Unvaccinated	408(99.8)	582(96.5)				
Subjects aged less than 5 years	357	537				
Vaccinated	1(0.3)	21(3.9)	0.064(0.008–0.491)	< 0.001	93.6(50.9 to 99.2%)	< 0.001
Unvaccinated	356(99.7)	516(96.1)				
Patients swabbed within 3 days of symptom onset	303	416				
Vaccinated	1(0.3)	17(4.1)	0.07(0.009–0.540)	0.013	93.0(46.0 to 99.1%)	0.011
Unvaccinated	302(99.7)	399(95.9)				
Week 14 to 45	389	460				
Vaccinated	1(0.3)	18(3.9)	0.062(0.008–0.467)	0.007	93.8(53.3 to 99.2%)	< 0.001
Unvaccinated	388(99.7)	442(96.1)				

Table 3

Effectiveness of the EV71 inactivated vaccine with two doses against the other enterovirus cases in 2017 in Guangdong, China

	<b>Other enterovirus cases, N(%)</b>	<b>Test negative controls, N(%)</b>	<b>Crude OR (95% CI)</b>	<b>p-value</b>	<b>Adjusted vaccine effectiveness% (95% CI)</b>	<b>p-value</b>
Overall	2611	603				
Vaccinated	137(5.2)	21(3.5)	1.535(0.961–2.451)	0.073	-43.6(-130.4 to 10.6%)	0.134
Unvaccinated	2474(94.8)	582(96.5)				
Subjects aged less than 5 years	2492	537				
Vaccinated	136(5.5)	21(3.9)	1.418(0.887–2.268)	0.144	-31.6(-111.5 to 18.1%)	0.256
Unvaccinated	2356(94.5)	516(96.1)				
Patients swabbed within 3 days of symptom onset	2000	416				
Vaccinated	106(5.3)	17(4.1)	1.314(0.778–2.217)	0.307	-24.4(-111.0 to 26.7%)	0.419
Unvaccinated	1894(94.7)	399(95.9)				
Week 14 to 45	2399	460				
Vaccinated	123(5.1)	18(3.9)	1.327(0.801–2.199)	0.272	-17.0(-95.3 to 30.0%)	0.549
Unvaccinated	2276(94.9)	442(96.1)				

## Figures

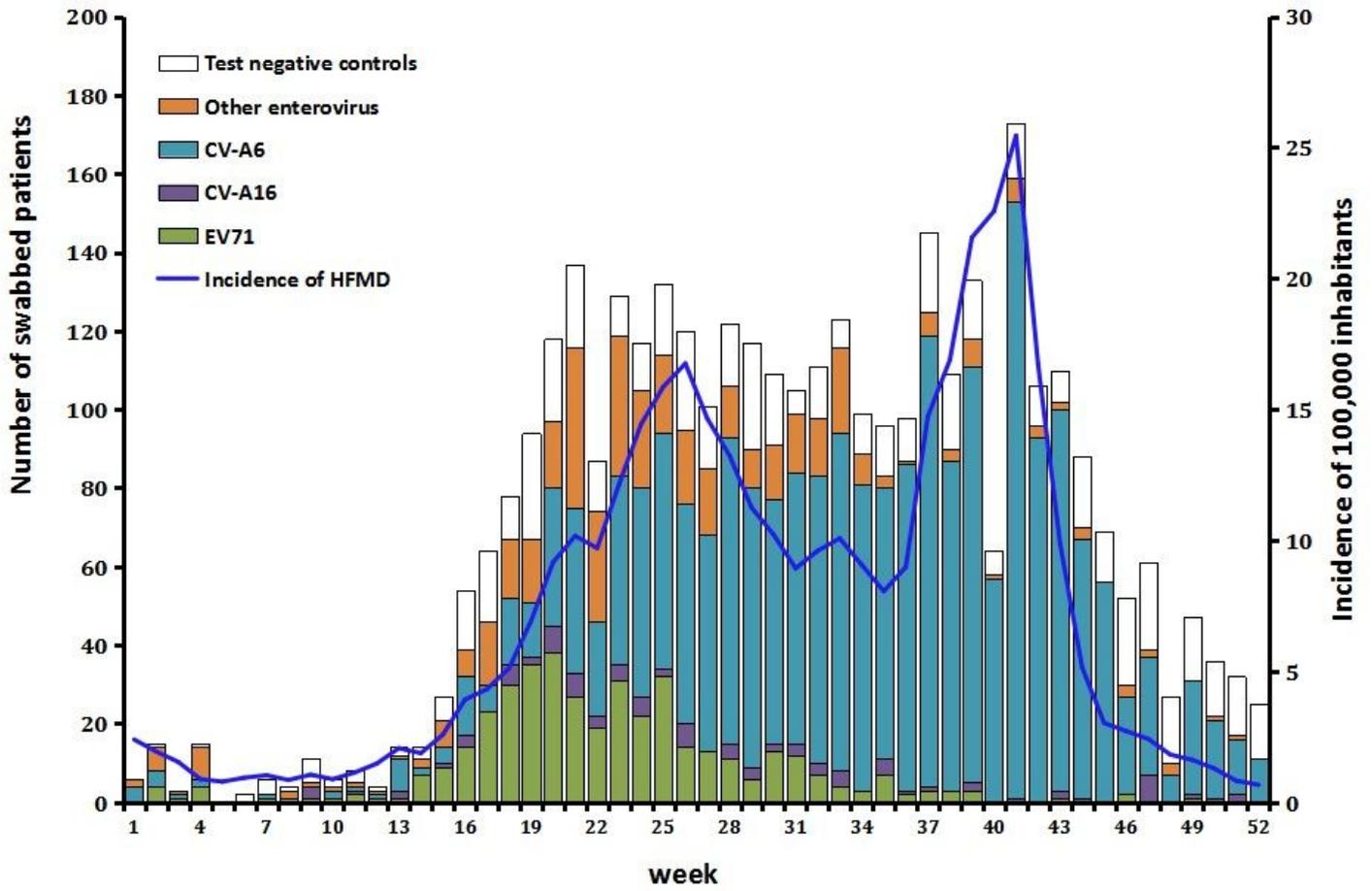


Figure 1

Number of test-negative controls and HFMD cases and incidence of HFMD cases by week in 2017 in Guangdong, China