Author's response to reviews

Title: Seroepidemiology of human enterovirus71 and coxsackievirusA16 among children in Guangdong province, China

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Author's response to reviews: see over
Jun 25, 2013

Dear Editors,

Thank you very much for your email of May 27, 2013, with regard to my manuscript (entitled “Seroepidemiology of human enterovirus71 and coxsackievirusA16 among children in Guangdong province, China”, Ms: 7347791494661317) together with the comments from the reviewers. According to the comments, we have revised the relevant part in the original manuscript (changes in the manuscript are highlighted in red). We also responded point by point to each reviewer comment as listed below. We hope that the revised manuscript is acceptable for publication.

Sincerely Yours,

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Responses to the reviewers’ commitments

Reviewer #1 (Jeffery Cutter):

Discretionary Revisions:

The discussion section could be strengthened by discussing the implications of the fairly high seroprevalence against EV71 and CA16 found in terms of asympomatic and unrecognized infections, public health importance and control strategies.
Response: Thank you very much for your comments. We have added discussions on this issue in the revised manuscript as “The relative high seropositive rates in health children indicated many clinically silent EV71/CA16 infections in these years. These seropositive children that had asymptomatic or unrecognized EV71/CA16 infection may serve as a reservoir for continued viral spread in the population, and should be taken into account when the government develops and implements public health interventions” in Discussion section.

Quality of written English: Needs some language corrections before being published

Response: Thanks very much for your comments. We have carefully edited our manuscript and corrected the grammatical errors and typos in our revised manuscript.

Reviewer #2 (Pengiran Hishamuddin):

Major compulsory revision

1. The authors should elaborate on the sampling methodology in this study.

Response: We are sorry that we had not described sampling methodology clearly in our original manuscript. We have rewritten this part in our revised manuscript as “The material used in this study is stored serum samples collected from the health children ≤9 years of age who had participated in seasonal immune status surveillance at Guangdong Provincial Centre for Disease Control and Prevention, China, from 2007 to 2009. Survey questionnaire was completed by trained interviewers and included information on the subject’s age, gender, vaccination history (over the past year) and presence/absence of illnesses (over the past year). All children had no sign of disease at the time of sample collection. The serum samples were stored at −80°C until testing. For the use of these serum samples, written informed consents from all participants (their parents or legal guardians) involved in survey were obtained. Serum samples from children who reported fever and vesicular exanthema on their hands, feet, mouths, or buttocks (distinct clinical presentation of HFMD) over the past year were excluded in this study and were classified into seven age groups (1, 2, 3, 4, 5, 6-7 and 8-9 years). Each group has 35-40 samples, except age group 1, 2 years in 2007(3 in 1 year group, 24 in 2 year group) and 8-9 years group in 2008 and
2009 (24 and 23 samples respectively). The sex ratios of boys to girls were 1.36:1, 1.43:1, and 1.25:1 respectively (Table 1). The study was approved by the ethics committee of the Guangdong Provincial Center for Disease Control and Prevention, and was in compliance with the Helsinki Declaration”. Besides, we also added a table (Table 1) to illustrate the demographic profile of the children enrolled in this study.

Minor essential revision

1. **The dominant virus that caused the outbreak in 2008 should be mentioned if known as this will have an impact on the results for 2008 and 2009.**

**Response:** Thanks for your nice comment. The dominant viruses that caused the outbreak in 2008 have been added and we have also discussed its impact on the results in our revised manuscript as “EV71 and CA16 were the most common causes of HFMD diseases in recent epidemics in China”; “In 2008, among 936 laboratory-confirmed cases, EV71 was found in 59% and CA16 in 26%. Considering the protective effects of neutralizing antibody in viral infection, the epidemiology surveillance data in 2008, to some degree, had verified our results. The seroprevalence of EV71 and CA16 in this year could serve as HFMD trends predictors for next year” in Discussion section.

2. **The results for the overall seroprevalence of EV71 and CA16 should be presented as a table.**

**Response:** Thanks for your nice comment. We have listed the overall seroprevalence of EV71 and CA16 in table format (Table 2) in our revised manuscript.

Quality of written English: Needs some language corrections before being published

**Response:** Thanks very much for your comments. We have carefully edited our manuscript and corrected the grammatical errors and typos in our revised manuscript.

Reviewer #3 (Li Wei Ang):

*Revisions are sought in the following sections:*
a) Materials and Methods

• It is not clear how the “cohort” study was conducted. There are no details provided on where the samples came from (childcare centres, households?), how they were collected, the sampling design and selection criteria (if any), etc.

Response: We are sorry that we had not described sampling methodology clearly in our original manuscript. We have rewritten this part in our revised manuscript as “The material used in this study is stored serum samples collected from the health children ≤9 years of age who had participated in seasonal immune status surveillance at Guangdong Provincial Centre for Disease Control and Prevention, China, from 2007 to 2009. Survey questionnaire was completed by trained interviewers and included information on the subject’s age, gender, vaccination history (over the past year) and presence/absence of illnesses (over the past year). All children had no sign of disease at the time of sample collection. The serum samples were stored at −80°C until testing. For the use of these serum samples, written informed consents from all participants (their parents or legal guardians) involved in survey were obtained. Serum samples from children who reported fever and vesicular exanthema on their hands, feet, mouths, or buttocks (distinct clinical presentation of HFMD) over the past year were excluded in this study and were classified into seven age groups (1, 2, 3, 4, 5, 6-7 and 8-9 years). Each group has 35-40 samples, except age group 1, 2 years in 2007(3 in 1 year group, 24 in 2 year group) and 8-9 years group in 2008 and 2009 (24 and 23 samples respectively). The sex ratios of boys to girls were 1.36:1, 1.43:1, and 1.25:1 respectively (Table 1). The study was approved by the ethics committee of the Guangdong Provincial Center for Disease Control and Prevention, and was in compliance with the Helsinki Declaration”.

• Was there a sample size determined for this study?

Response: Thanks very much for your constructive comments. As a retrospective analysis, all of the serum samples used in this study was stored samples collected for other study (seasonal immune status surveillance in 2007-2009). The relative small sample size in our study has its
limitation. To compensate this, we have tried our best to balance the three years samples in respects such as age, gender and used the proper statistic methods to analyze the data.

• Among the “healthy” children, was there information gathered on whether they had been infected with HFMD previously?

Response: As mentioned in our revised manuscript, survey questionnaire was completed by trained interviewers and included information on the subject’s age, gender, vaccination history (over the past year) and presence/absence of illnesses (over the past year). All children had no sign of disease at the time of sample collection. Besides, serum samples from children who reported fever and vesicular exanthema on their hands, feet, mouths, or buttocks (distinct clinical presentation of HFMD) over the past year were excluded in this study.

• Instead of computing GMT of all samples (there is no justification to assigning a value of 4 to the seronegative samples in the analysis), it would be better to confine the analysis on GMT to seropositive samples with antibody titers > 8.

Response: Thanks very much for your constructive comments. We have re-computed GMT only in seropositive samples. And based on these re-analyzed data, we also have rewritten the Result and Discussion sections. All of these changes in our revised manuscript are highlighted in red. For example, in Result section, we have rewritten as “To analyze the immunity level, the geometric mean titer (GMT) of EV71 and CA16 neutralizing antibodies in seropositive individuals was tested. The overall EV71 NA showed a moderate level, with GMT values of 51.6 (95% CI: 40.6-64.5), 88.1 (95% CI: 71.3-108.9) and 56.6 (95% CI: 41.6-76.8) in 2007, 2008 and 2009 respectively (Fig.2). For CA16, significant higher values were observed than those for EV71 in each year tested (p<0.05) (Fig.2). The highest CA16 antibody level was identified in 2007, with a value of 175.5. Then the GMT values gradually declined to 143.4 and 135.8 in 2008 and 2009 respectively (Fig.2)”, and as “GMT values of EV71 NA were slightly higher in 1-5 years group than in those 5 years older. The highest GMT of EV71 in 2007 was in children aged 2 years group (GMT 114.0). Then this value deduced across age groups, fell to approximately
40.0 among those 5 years or older (Fig.3A). Compared to 2007, the GMT in 2008 was relatively high in most of the age groups. Children in 1-5 years groups had values no less than 87.7. Then the immunity level decreased to 39.8 and 60.9 in children aged 6-7 and 8-9. In 2009, the highest GMT was identified in 1 year old children (GMT 128.0). Contrary to 2007, the 2 years group had a lowest value of 8 in 2009. Then this value gently increased with age, rising from 39.0 to 101.6 in 3-5 year groups. Again, for those aged more than 5 years, the GMT in seropositive individuals was relatively low. No one was found to be more than 50.0. For CA16 (Fig.3B), the GMT values were approximately equally distributed in 2007 and 2008. Significant higher values were only observed in 2-3 years groups in 2007 and in 3 year group in 2008. While in 2009, declining trend across age was identified, with a sudden dip at age of 2 years”

• What was the statistical test used for comparison of the GMT by year and age group?.

Response: As mentioned in our revised manuscript, Kruskal–Wallis test was used for comparison of the GMT by year and age group.

b) Results

• A table on the demographic profile of the children enrolled in the study by year could be provided.

Response: Thank you very much for your comments. We have added a table (Table 1) on the demographic profile of the children enrolled in the study by year.

c) Discussion

• There is a need to discuss the prevalence trends together with the epidemiological trends of HFMD cases reported in Guangdong province.

Response: Thank you very much for your nice comments. In our revised manuscript, we have added discussions about the prevalence trends together with the epidemiological trends of HFMD cases reported in Guangdong province. For example:
In 2007, nearly 40,000 HFMD cases were reported in Shandong and over 10,000 cases were
recorded in large cities such as Beijing and Shanghai. However, little reports have described the
HFMD situations in Guangdong in 2007. The high CA16 seroprevalence in 2007 indicated that
frequent asymptomatic and/or unrecognized CA16 infections have occurred before and in this
year.

According to the Guangdong HFMD web-based surveillance system, a total of 47,660 HFMD
cases were reported in 2008. And this number almost doubled in 2009 (92,998 reported cases),
was five-fold in 2010 (230,978 reported cases). To correlate the level of immune protection to
the incidence rate of HFMD cases, seroprevalence of EV71 and CA16 NA in and after the 2008
were also investigated. We didn’t find significant higher positive rate of EV71 and CA16 in and
after 2008. Previous study suggested that HFMD presents a seasonal pattern every 2 - 3 years.
Together with the increased incident rates of HFMD cases in 2009 -2010, our results suggested
that the peak of recent HFMD epidemic cycle in Guangdong was not in year 2008 but in the
years 2009 and 2010. Consistent with these, Yu, et al. identified that children in Lu’an had
significant higher seropositive rate in 2010 when compared to that before 2008. The ensuing
HFMD epidemics from 2008 to 2010 largely increased the exposed chance of viral infection and
thus the seropositive rate of viral NA in children.

- More information on the HFMD outbreaks including the age profile and the associated main
  predominant circulating enterovirus type(s) based on laboratory surveillance is warranted to
  associate with the findings of the study.

Response: Thank you very much for your constructive comments. We have added more
information on the HFMD outbreaks including the age profile, the main predominant circulating
enterovirus type(s) based on laboratory surveillance in our revised manuscript. We also have
correlated these characteristics with our findings in the Discussion section. For example:

As a common febrile illness of early childhood, HFMD occurred mainly among children \( \leq 5 \)
years old. In Guangdong, a total of 47,660 and 92,998 cases have been reported to the provincial
surveillance system in 2008 and 2009 respectively. The number of children \( \leq 5 \) years old
accounted for the largest proportion (from 87.5% to 93.3%). These were highly consistent with
the age related seroprevalence trends in our study. In our results, both EV71 and CA16 NA rise with age among children less than 5 years and reach a plateau thereafter. We also observed significant reduction at 2 years group, the most susceptible population that had the highest incidence rates of HFMD in 2008 and 2009 epidemic year in Guangdong.

The high CA16 seroprevalence in 2007 indicated that frequent asymptomatic and/or unrecognized CA16 infections have occurred before and in this year. In 2008, among 936 laboratory-confirmed cases, EV71 was found in 59% and CA16 in 26%. Considering the protective effects of neutralizing antibody in viral infection, the epidemiology surveillance data in 2008, to some degree, had verified our results. The seroprevalence of EV71 and CA16 in this year could serve as HFMD trends predictors for next year.

• How does the issue of cross reactivity of EV71 and CA16 affect the interpretation and use of the results?

Response: Thank you very much for your constructive comments. To illustrate this issue, we added some discussions in our revised manuscript as “EV71 and CA16 were highly diverse in the nucleotide sequences of structure proteins which serve as major antigen in host immune response. Li et al. used VP1s and VP4s as antigens to detect of serum antibodies against EV71 and CA16. Their results suggested immunological reaction to VP1 and VP4 of both EV71 and CA16 was different. The study from the clinical patient also indicated that individuals with or without prior EV71neutralizing antibody showed a similar incidence of non-71 Enterovirus infection. EV71 and CA16 were the most common causes of HFMD diseases in recent epidemics in China. To evaluate the immune protection level against HFMD, we also calculated the proportion of individuals that were positive for both the EV71 and CA16”.

Quality of written English: Needs some language corrections before being published

Response: Thank you very much for your comments. We have carefully edited our manuscript and corrected the grammatical errors and typos in our revised manuscript.