Reviewer's report

Title: Enterovirus Genotypes Causing Hand Foot and Mouth Disease in Shanghai, China: A Molecular Epidemiological Analysis

Version: 2 Date: 18 February 2013

Reviewer: Audrey Mirand

Reviewer's report:

The authors report the genotyping results of enteroviruses associated with hand, foot and mouth (HFMD) in children hospitalized at children’s hospital of Fudan university in Shanghai between May 2010 and April 2011.

Enterovirus genome was detected from stool specimens and genotyping directly performed with the cDNA used for diagnostic. Genotyping was based on the sequencing of the partial VP1 coding sequence.

This work provides valuable information on the epidemiology of enteroviruses in Shanghai because it is based on direct genotyping from clinical samples. In fact, most of the studies rely on genotyping results obtained from culture supernatants, which involves subsequent biases. Moreover, the typing of EV-positive samples has been obtained with a great success rate (269/277, 97.1 %).

Major compulsory revisions

The aim of the authors was to “identify any associations between enterovirus types and clinical manifestations”. I’m not sure that stool specimens are the most appropriate specimens for investigating enteroviruses associated with HFMD. Enteroviruses may be shed in the faeces 3-5 weeks after infection. Stool samples may thus contain a virus that has nothing to do with the symptoms. Therefore, the clinical samples used in this study are not adequate to fulfil the objectives claimed by the authors. Moreover, the clinical symptoms are not fully described in the methods or the results sections. In my opinion, the report should focus on the genotyping results. The phylogenetic analyses should be reinforced and improved as the analysis of the figures does not fit the data reported in the manuscript.

Methods

- Page 5 – Sample collection:

The way by which the authors have collected clinical information for the patients included in the study should be indicated. Was the information collected prospectively or retrospectively?

-Clinical complications should be more precisely described. For example, neurological complications following HFMD and herpangina have been clearly

- Page 7 – Enterovirus genotyping: the approximate length of the amplified products for the partial VP1 sequence should be indicated.

It would be interesting for the scientific community if the sequences obtained in this study could be deposited in international databases.

- Page 7 – Phylogenetic analysis: the mathematic model used to estimate the genetic distances should be indicated.

Results
- Page 8, lines 158-160: The detection rates of each enterovirus types should be calculated with the number of positive clinical specimens, i.e 277: EV71 (66.7 %, 185/277) for example.

- Page 8, lines 163-164: The phylogenetic analysis of partial VP1 sequences of enterovirus 71 strains was performed with 165 sequences. The authors reported in the precedent section that 185 strains of EV71 were detected. Please explain why.

I have the same remark for the other serotypes: CV-A16 (15 instead of 20), CV-A6 (23 instead of 24), CV-A10 (23 instead of 26).

- Page 9, lines 175-178: the CV-A16 sequences are distributed in two clusters B1a and B1b by the authors. The authors should indicate on which criteria the clusters B1a and B1b have been defined, as bootstrap values do no support the differentiation of the two clusters.

- Page 9, lines 179-183: same remark for the phylogenetic analysis of the CV-A6 sequences. Bootstrap values don’t allow distinguishing “two genetically distinct branches”.

- Page 10, lines 193-194:

Supplemental material Table 1: It is not clear why the babinski’s and the brudzinski’s signs have been combined in the same line in the table, as they do not correspond to the same clinical information.

Considering the aim announced by the authors to “identify any associations between enterovirus types and clinical manifestations”, it is not clear why the table is included as supplemental material.

Discussion
- Page 11, lines 212-221: this paragraph could be shortened. It does not really correspond to a discussion of the obtained results.

- The authors should point out the weakness of not using more appropriate specimens (vesicles, throat or buccal swabs) for the diagnostic of enterovirus
infections associated with HFMD and discuss how it could have influenced the results.

- It has been shown than EV-71 and CV-A16 infections were predominantly associated with HFMD while the other serotypes were more frequently responsible for herpangina. Was this the case in the study?

- What was the seasonality of enterovirus infections?

- The improvement of the phylogenetic analyses could allow a better analysis of genetic relationships between strains isolated in Shanghai with other Chinese strains and with strains isolated in other countries.

Legends of figure and figures
For each figure corresponding to phylogenetic analyses, the length of the VP1 sequences should be indicated, as well as the number of sequences of reference strains used in the analyses.

For clarity, only the bootstrap values of over 70 % can be indicated on the dendograms.

The country in which the reference strains used were collected should be indicated.

Minor essential revisions
- Page 6, line 110: “Human enterovirus was identified with highly conserved 5'UTR primers”. The 5'UTR region is used for the diagnostic of enterovirus infection. The term “identify” is not appropriate here.

- page 5, line 81 : “severe compliations”. Please correct.

- Reference section : lines 320 and 323 : “Oberate” should be replaced by Oberste.


- Line 442 : “Clin Microbiol Infect’ : Clin Microbiol Infect

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.