Author's response to reviews

Title: Clinical Features and Phylogenetic Analysis of Coxsackievirus A9 in Northern Taiwan in 2011

Authors:

Yi-Chuan Huang (bb0479yc@gmail.com)
Ying-Hsia Chu (b95401099@ntu.edu.tw)
Ting-Yun Yen (ytingyu@gmail.com)
Wen-Chan Huang (summerdebbie@gmail.com)
Li-Min Huang (lmhuang@ntu.edu.tw)
Chang Chang (lychang@ntu.edu.tw)

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Author's response to reviews: see over
Dear Editor

We address the comments in the revised manuscript and provide this cover letter giving a point-by-point response to the concerns.

We also include a competing interest section, and an authors' contributions section in the revised manuscript.

Thank you very much for your consideration.

Sincerely yours,

Luan-Yin Chang, Department of Pediatrics, National Taiwan University Hospital, College of Medicine, National Taiwan University, 8, Chung-Shan South Road, Taipei, Taiwan 100.
Tel: 886-2-23123456 ext 71528
Fax: 886-2-23147450
Response to the comments of reviewers

Reviewer's report

Title: Clinical Features and Phylogenetic Tree of Coxsackievirus A9 in Northern Taiwan in 2011

Version: 2 Date: 13 September 2012

Reviewer: Beatrix Kapusinszky

Reviewer's report:

The paper Clinical Features and Phylogenetic Tree of Coxsackievirus A9 in Northern Taiwan in 2011 written by Yi-Chuan Huang and co-authors describes the clinical presentation of Coxsackie A9 (CV-A9) infections in order to upgrade the differential diagnosis of those infection from other diseases caused by non-polio enteroviruses and provides an inside into molecular epidemiology of CV-A9.

However the paper needs to be improved with some major and couple minor modification to meet the journal criteria before being accepted for publication.

In general the paper should be shortened and submitted as a brief report, including new findings about CV-A9 in both clinical and epidemiological aspects.

Response: According to the reviewers’ comments or suggestions, we have revised the manuscript with some major and couple minor modification to improve the paper. The manuscript cannot be shortened as a brief report, so we hope that it can be kept as an original article.

- Major Compulsory Revisions
  1. In paper title it was mentioned that infections occurred in Taiwan in 2011, however the MATERIAL AND METHODS section was describing 100 culture proven CV-A9 infections occurring in Northern Taiwan between August 2010-August 2011 and the same results were depicted in Figures and Tables. Disagreement should be corrected.

Response: We revised it for abstract (P3, L9-10), the material and methods (P7, L3-4), and results (P10, L3) to make it consistent. The 100 culture-proven was from 2011 epidemic and we did not include the sporadic cases in 2010, so we kept the original title
and revised the materials and methods and results.

2. Authors needs to clarify which infections are sporadic and how many of them are epidemic, if possible provide information in what type community (day-care, etc.) such outbreaks occurred?

Response: The 100 cases were from 2011 epidemic not from the sporadic cases. We did not have the overall community epidemiological data, so we are sorry that we could not provide the information in what type community (day-care, etc.) such outbreaks occurred.

3. In INTRODUCTION authors said that Taiwan CDC reported 3,308 non-polio HEV in 2011, and only 15% (499) was due to CV-A9, but in DISCUSSION authors described that CV-A9 was the second most common serotype in Taiwan (or Northern Taiwan?). What serotype was a first one? And what is the basis for that statement since it with disagreement with the first sentence.

Response: CA10 was the most common serotype (1303, 39%) and it caused a very common disorder of herpangina but the 2\textsuperscript{nd} most common, CA9, caused characteristic generalized febrile exanthema rather than herpangina or hand, foot, and mouth disease. That was the reason we would like to report it.

4. In METHODS section please indicate the size of VP1 amplicon based on its position in the reference genome, and also indicate the size of VP1 aminoacid sequence was used for phylogenetic analysis.

Response: We included the size of VP1 amplicon based on its position in the reference genome (P8, L11-14), and also indicated the size of VP1 nucleotide sequence used for phylogenetic analysis in the Methods (P9, L7).

5. Provide in RESULTS section the paragraph on Phylogenetic analysis of CV-A9 isolates.

Response: We include the Phylogenetic analysis of CV-A9 isolates in Results section (P13, L1-3)
6. How many isolates from current study was used to build the phylogenetic tree? Indicate which one is from 2010 and 2011.

Response: The isolates from the current study were all from 2011, not from 2010.

7. Also provide the GenBank accession numbers for each sequences used for phylogenetic tree construction.

Response: The GenBank accession numbers for each sequence used for phylogenetic tree construction were shown in both the methods (P9, L3-6) and the tree (Figure3).

8. Indicate on phylogenetic tree isolates from sporadic cases and those belonged to the same outbreak.

Response: They all belonged to the same outbreak of 2011.

9. Phylogenetic tree should include an outgroup, bootstrap values below 50% might be omitted.

Response: Phylogenetic tree was revised to include an outgroup (CA16 was used as the outgroup), and bootstrap values below 50% were omitted according to your suggestion (Figure 3).

10. RESULTS section. Include only results from 100 patients, additional results from other patients (n=125) with missing data are confusing.

Response: We corrected it as “a total of 100 CA9 cases with available medical records were included in our study” (P10, L2). Actually only 25 patients (rather than 125) from 2010 sporadic outbreak were not included in the analysis.

11. GenBank Accession numbers should be provided for the stain detected near the border of Mainland China and Myanmar with the reference for indicated strain in the text.

Response: We provide GenBank Accession numbers for the stain detected near the
border of Mainland China and Myanmar in the text (P13, L2).

12. Where is no clear conclusion is there any genetic difference between current CV-A9 stains and those deposited in GenBank that might influence the severity and clinical outcome of the disease.
   **Response:** We agree with the reviewer that no clear conclusion for the genetic difference between current CV-A9 stains and those deposited in GenBank that might influence the severity and clinical outcome of the disease.

13. Is there are any evidence on importation of CV-A9 from Mainland China and Myanmar?
   **Response:** We do not have any evidence on importation of CV-A9 from Mainland China and Myanmar and we address this issue in Discussion (P17, L15-17).

14. The Figures 1, 2 might be omitted from the text because their description was clearly provided in the text.
   **Response:** They were omitted as your suggestions.

15. Figure 5 needs to be reconstructed with comments described above.
   **Response:** We reconstruct the figure 5 (now Figure 3) according to your above comments.

Minor Essential Revisions:
1. The language of ABSTRACT section needs a revision by native English speaker.
   **Response:** Thank you for your suggestion. The manuscript had been edited by a native English speaker before we submitted it.

2. The title should be modified with using a scientific term: … and Phylogenetnic Analysis of Coxsackie A9”
   **Response:** We modified the title according to your suggestion.
3. In INTRODUCTION section correct the enterovirus classification was based on nucleotide sequence of VP1 region and it was accepted by ICTV in the mid of year 2000.

Response: It was corrected as your suggestion (P5, L6-7)

4. In METHODS section for case definition would be more correct to say: “In this study we collected data from 100 patients with laboratory confirmed CV-A9 infections…”

Response: It was corrected as your suggestion (P7, L3-4).

5. In METHODS section double check the method: Real-Time RT-PCR or just RT-PCR as it was described in the reference.

Response: it’s a mistake and “real time” was removed.

6. RESULTS section. Include only results from 100 patients, additional results from other patients (n=125) with missing data are confusing.

Response: It was corrected as “a total of 100 CA9 cases with available medical records were included in our study” (P10, L3-4).

7. In DISCUSSION hand foot and moth disease= HFMD should be abbreviated the same all other the text.

Response: They were all abbreviated as “HFMD”.
Reviewer's report

**Title:** Clinical Features and Phylogenetic Tree of Coxsackievirus A9 in Northern Taiwan in 2011

**Version:** 2  **Date:** 4 September 2012

**Reviewer:** Teruo Yamashita

**Reviewer's report:**

Major Compulsory Revisions:

1. Page 8 (Viral identification): Authors use RNA from throat swabs for RT-PCR. However, Oberste et al. use cell culture supernatant in reference 9. I guess the method is not suitable for amplifying the virus nucleotides from throat swabs of patients.

**Response:** We can use RNA from throat swabs for RT-PCR and we have used it for about 8 years in our lab. This method can be used for amplifying the virus nucleotides from throat swabs of patients. Our recent works with this method were published as the following: Lee MH, Huang LM, Wong WW, Wu TZ, Chiu TF, **Chang LY** (Correspondence). Molecular Diagnosis and Clinical Presentations of Enteroviral Infections in Taipei during 2008 Epidemic. J Microbiol Immunol Infect 2011 Jun;44(3):178-83, and Huang WC, Huang LM, Kao CL, Lu CY, Shao PL, Cheng AL, Fan TY, Chi H, **Chang LY** (Correspondence). Seroprevalence of enterovirus 71 and no evidence of crossprotection of enterovirus 71 antibody against the other enteroviruses in kindergarten children in Taipei city. J Microbiol Immunol Infect. 2012;45(2):96-101

Page 8, last sentence: Accession nos. are need for 19 formerly reported CV-A9 sequences and isolates in this study.

**Response:** Accession nos. are listed in the text (P9, L3-6) and the Phylogenetic Tree according to your suggestion.

2. Page 10 (Results), line 7: Patients no. of age <1 and 1 are more than age 2. Age 9 and 10 are more than age 8. Why aged 2 and 8 years old?

**Response:** The age distribution was not even. The possible reasons why Patients no. of age <1 and 1 are more than age 2: (1). children younger than 2 years may be more...
susceptible, (2). Since NTUH is a medical center, younger children infected with enteroviruses tend to develop severe diseases and more younger children were referred to this hospital. The patient no. of age 8, 9, 10 were 2, 4, 3, respectively. We did not think the no. was significantly different.

3. Page 10 (Results), line 3 from bottom: What are influenza-like viral illnesses in table 1?
   **Response:** To prevent confusion, we revised it as “CA9-infected patients sometimes experienced fever and symptoms of upper respiratory tract infection, defined as rhinorrhea, cough, and/or sore throat (Table 1)” (P10, the last paragraph).

Minor Essential Revisions:
1. Page 5(Introduction), line 5: “VP2” is incorrect. VP1 region is used for typing.
   **Response:** VP2 had been corrected as VP1 (P5, L5).

2. Page 7(Methods), 6: “real-time” should be removed.
   **Response:** “real-time” was removed.

3. Page 11, line 4, Page 14, line 3 from bottom: The most commonly seen rash pattern?
   **Response:** The sentence had been corrected as “The most common rash pattern (56%) was generalized maculopapular rash (size from 1 to 3 mm)” in both pages.

4. Page 14, line 5 from bottom: 61 should be Sixty one.
   **Response:** “61” had been corrected as “Sixty one”.

5. Page 15, line 8: 96 should be Ninety six.
   **Response:** “96” had been corrected as “Ninety six”.

6. Page 16, line 5: usually is ?
   **Response:** The sentence had been revised as “the clinical course is benign and self-limited” (“usually” was deleted).
7. In references: There are no titles or bold characters in reference 2, 5, 10.
   
   **Response:** reference 2, 5, 10 had been corrected.

8. Table 1: Age (year) --- 4.6(3.4) Need asterisk mark. Male/female (ratio) ---- 65/35(1.9)* Remove asterisk mark.

   **Response:** Table 1 was corrected as your suggestions.