Author's response to reviews

Title: Sentinel surveillance for human enterovirus 71 in Sarawak, Malaysia: Lessons from the first 7 years.

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Dear Editors

We are resubmitting our revised manuscript and we have attempted to address all the comments and suggestions of the 3 reviewers. Our responses to the comments follow this, and we hope that we shall hear from you soon regarding your decision about publication.

Thank you.

Yours sincerely,

Jane Cardosa

Response to Reviewers’ Comments

Reviewer: Steve Oberste

Serotype identification based on VP4 has been well validated for HEV71 strains but not for other serotypes: In this work we are only concerned with HEV71, CVA16, CVA10 and a few minor species A enteroviruses which are associated with HFMD, and not the full range of enteroviruses in species B and species C. We thus feed that because of the volume of isolates we are handling, the simple VP4 methodology is adequate. We have in fact verified the reliability of VP4 based identification for these viruses and retesting using VP1 based methods has not produced any conflicting identifications. This information is included in the manuscript in the methods section.

Genogroup C in endemic circulation in Sarawak: We agree that subgenogroup C1 viruses show a background sporadic endemic picture. However, we cannot extend this conclusion to all genogroup C viruses as we have never isolated any other subgenogroups (no C1, C3 or C4 viruses isolated during the 7 years, not even during the presurveillance outbreak of 1997).

Genogroup B viruses arising from an unknown/undetected reservoir in Sarawak or repeatedly introduced from outside. Clearly we are unable to prove conclusively that the genogroup B viruses arise from an internal reservoir, but we know that temporally, HEV71 outbreaks in Singapore and in peninsular Malaysia occur later than in Sarawak. Furthermore, in temperate Asia (eg Japan, Korea) HEV71 outbreaks happen during the summer while in Sarawak these outbreaks begin in February. This clarification is included in the text.

Why does genogroup B cause such big outbreaks? This is true for Sarawak, where the outbreak in 1997 was due to subgenogroup B3 but in Taiwan in 1998 the outbreak was predominantly due to subgenogroup C2. This information is in the manuscript in the introduction.
Virus isolation data – why is overall experience different from first 18 months: We have modified the text in the results section to clarify this. Basically we are not getting the same isolation rates when there is an HEV71 outbreak and when there is a CVA16 outbreak. HEV71 is harder to isolate than CVA16, thus because the first 18 months saw only CVA16 and some non-HEV71 enteroviruses, the isolation rate was similar to that obtained in 2002 when CVA16 and CVA10 caused an outbreak of HFMD. We have corrected the text in the results section to reflect this.

Significant digits in age: we have made the corrections of the number of decimal places to one decimal place.

Figure 5: Figure refers to HFMD cases. In the revised manuscript this is now Figure 4.

Annual birth cohort: We have now included this information in the discussion – 48,000 to 49,000.

Reviewer: Katsumi Mizuta.

Suggestion to focus on the point that HEV71 outbreaks occur every 3 years and that the viruses are genetically distinct from each other: We agree entirely and have revised the conclusion to reflect this.

Compulsory revisions:
Point 1. genogroup B evolving within Borneo. Dr Mizuta argues that this is difficult to accept because (a) the distribution of subgenogroup B4 does not support this idea and (b) need to explain why genogroup C viruses do not evolve within Borneo. Dr Mizuta argues that Borneo may be one of the epicentres of HEV71. We agree with this latter idea. The fact that of the genogroup C viruses, we only find C1 viruses in Borneo, and these viruses occur only sporadically and do not vary much from year to year, suggests that in Borneo the C1 viruses are not highly transmissible. The genogroup B viruses on the other hand are highly transmissible and each outbreak throws up a new distinct subgenogroup. The fact that in 2000 subgenogroup B4 viruses were found throughout the region does not negate this idea because the HEV71 outbreaks in Borneo begin in early February while the rest of the region sees summer outbreaks, occurring after the Sarawak outbreaks have swept through the state. The point of the temporal sequence of HEV71 outbreaks in the region is made in the revised manuscript.

Point 2. The use of RD cells. We have explained this more thoroughly in the section on methods. We are aware that RD cells are most sensitive to species A enteroviruses. These were our target pathogens, and we did not use any other cell lines. However, although we did isolate 2 coxsackie B viruses in RD, we do not imply that RD cells are efficient for isolation for CVB viruses. We explain in the methods that for this surveillance exercise we were only concerned with HEV71 and the other species A enteroviruses.

Point 3. Neutralization as the gold standard. We understand this argument, but we have not access to the LBM pools or to other typing sera as we only began to get
interested in enteroviruses when we had our first outbreak in 1997. Nevertheless, the literature is replete with publications about why molecular serotyping is becoming necessary and we have used these methods carefully. The issue of misidentification of CVA16 as HEV71 is no longer an issue as we have HEV71 specific primers designed based on recent regional isolates.

Discretionary revisions:
1. We maintain that it is important to present both our VP4 and our VP1 data as there are still groups who use VP4 as a rapid method for identifying species A enteroviruses in their HFMD surveillance programmes. We would like to enable all groups to compare their data with ours.

2. Figure 4 is not essential as suggested. We have deleted it from the main manuscript but have offered it as a supplementary figure because the X axis is in weeks and not months and offers a more detailed snapshot view of what is happening in the early weeks of this outbreak. Furthermore, the data includes hospital specimens as well.
3. Conclusions to be included in discussion: We agree, and we have incorporated both conclusions (d) and (e) in the discussion and deleted (d), (e) and (f) from the conclusion.

Reviewer: Mark Pallansch.

Compulsory Revisions:
Relevance of Table 4 if CVA10 children are younger. It is true that in Table 3, there is a trend showing that children with CVA10 were younger than those with CVA16 and HEV71. However, we had written in the results section that there was no statistical significant difference in age of children with CVA10, CVA16 and HEV71. Hence the difference in presentation with fever shown in Table 4 is relevant and not trivial. This difference is statistically significant and the p values for all this is clearly stated in the text of the results section.

Discretionary revisions:

Change in the ratio of mild to severe disease over time: Ours is a sentinel surveillance programme and we are only tabulating cases notified by primary care doctors. Hence these are all mild cases, and only serve to tell us what is happening in the community.

Single cell culture line: As we were only really concerned with HEV71 and CVA16, we do not think that any serious observational bias has been introduced. Furthermore, the main objective of this exercise was to determine the epidemiology of HEV71 and to discover if we could work out an early warning system.

Ages of children positive for virus and negative for virus: We have provided the overall mean age of the children in the results section. This includes both children with virus isolated and children who were negative. The mean age did not significantly differ between these groups.
Did specimen testing change during the study? We tested the majority of specimens (89%) from a majority of cases (95%). This oversight has been now included in the revised manuscript.

Change of system and effect on results: This is difficult to determine because there was not an annual occurrence of the same viruses throughout the period of study. We have attempted to address this question by looking at the isolation rates of the first 18 months (44%) compared to that of the species A outbreak in 2002 (40%). We have commented on this in the revised manuscript.