Author's response to reviews

Title: Improving public health surveillance for human Salmonella enterica serovar Typhimurium infection: 3 years of prospective multiple-locus variable-number tandem-repeat analysis (MLVA)

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Author's response to reviews: see over
Dear Editor,

RE: MS-1840003316607872 - Improving resolution of public health surveillance for human Salmonella enterica serovar Typhimurium infection: 3 years of prospective multiple-locus variable-number tandem-repeat analysis (MLVA)

We would like to thank you and your reviewers for your constructive criticism and helpful comments. Please find attached a new version of the manuscript revised according to the reviewers’ suggestions. All significant changes are highlighted in the text of the marked-up copy as requested. We would also like to address specific comments raised by you and the reviewers:

Associate Editor

1. The manuscript is rather long

The revised version has been shortened from 3,904 words to 3,600. We have tried to provide sufficient additional detail requested by reviewers and to remove statements that perceived to be controversial.

2. Determination of repeat numbers should be clarified and the associate editor suggested plotting the observed sizes of repeats against the number of repeats for each locus.

We have taken up the suggestion and added the plots of observed sizes and repeats as a supplementary figure. Additional information has also been provided about the MLVA method and its reproducibility.

3. The need to correlate MLVA results with epidemiological information

We have carefully revised the manuscript in accordance with the feedback. However, we are passionate about the key concept that rapid molecular typing techniques have shifted the power of laboratory investigations which has perhaps not been fully appreciated by the public health community. In the past, laboratories were expected to confirm epidemiological hypotheses and had the luxury of plentiful time to apply multiple methods to provide the most accurate conclusion. Such investigations have usually been associated with large-size outbreaks with single-source exposures leading to food recalls and court proceedings. In contrast, the PCR-based typing offers a novel opportunity to be ahead of the curve by detecting small clusters early and to generate hypotheses for epidemiological investigations. Our work aims at translating STM MLVA typing into pre-emptive public health actions and, we hope, provides some evidence to the foundation of this new approach.
4. Figures 5 and 6 are difficult to read and may not be essential for the narrative

Figures 5 and 6 have been omitted from the revised version.

**Reviewer 1**

5. Disagreed that authors have shown that prospective typing by MLVA enabled more targeted and timely public health interventions.

The range of existing opinions on the maturity of Salmonella Typhimurium (STM) MLVA technique and its translational potential must be acknowledged. Additional details have been provided on the turn-around-time and impact of prospective MLVA typing to address this criticism (pages 4 and 6). With due respect we would argue that the facts that (a) MLVA had a higher resolution than conventional phage typing as well as (b) the number of clusters that were investigated and epidemiologically confirmed as the result of prospective MLVA typing, potentially averting further cases of STM disease, both strongly support our statements about potential benefits of such approach. Two of co-authors are field public health practitioners who have used the information provided by our state reference laboratory for three years and witnessed the growing acceptance of MLVA results by the public health community in Australia.

6. The conclusion that further outbreaks were significantly delayed is unsound.

The conclusions have been modified to address this concern.

7. The meanings of “re-emergence” and “relapse” have to be properly defined.

The results and discussion sections have been modified to clarify the ambiguity of “clusters” and “outbreaks” and the wording of “re-emergence” was changed to “re-occurrence” throughout the text.

8. It is possible that some MLVA profiles may be more prevalent than others and may be found in epidemiologically unlinked cases.

This is a valid point which we have added to the discussion with the reference offered by the reviewer (pages 10-11).

9. The limitation of the MLVA cluster definition to STM isolates with identical MLVA patterns should be acknowledged.

The acknowledgement of our cluster definition has been duly added to the manuscript (pages 11-12).

10. Where apparent case clusters are detected these should be verified by other typing methods including epidemiological data looking for common risk factors.
We share the reviewer’s view that each potential cluster suggested by a molecular typing method must be carefully evaluated. In fact, we have used Table 3 to show details of fifteen clusters suggested by our laboratory surveillance based on STM MLVA that have been confirmed by full-scale epidemiological investigations looking for common risk factors. Furthermore, we occasionally investigate additional STM variable loci to assess the discrimination power but strongly feel that there is a body of literature assembled already and cited in the manuscript to support prospective use of STM MLVA when the turn-around-time of laboratory reporting is of paramount importance.

11. Until more is known about the circulating STM MLVA profiles it would be unsafe to use MLVA typing in isolation.

To avoid the argument with an opinion expressed by the reviewer relevant statements have been added to the Discussion (pages 11-12).

12. Why do the authors use the Lindstedt nomenclature method? Fully standardized nomenclature would be more useful.

This is an excellent point. We are aware of recent harmonization efforts by the European community and participate in the ECDC STM MLVA QAP Program. We initially opted for reporting our findings in the Lindstedt nomenclature adopted by several reference laboratories in our country. However, we have added an appropriate statement to the Discussion section (page 11) and one additional reference [36] to raise the point about MLVA harmonisation.

13. Reference 10 is incorrect.

This reference has been replaced with more appropriate one.

14. Odd abbreviation on page 4 has been corrected.

15. Reference for MLVA-7 used in the USA.

Unfortunately, apart from personal communication with the CDC, we cannot offer peer-reviewed reference for the CDC method so we decided not to mention this variation of the method.

16. Comment on the incidence of co-infection with different STM genotypes.

Co-infection with multiple salmonellas in many tropical countries is well-recognized. However, there is not much literature on STM genotypes co-circulating in those populations. We have added the reference to support our statement.

17. References have been checked and re-assigned to 25.

18. Ambiguities in some statements on pages 8, 9 and 11 have been clarified.
19. Why does the exclusion of isolates differing by one repeat not apply to all five loci?

   We have applied this exclusion criterion to all five loci.

20. All discretionary revisions suggested by the reviewers have been gratefully accepted.

21. Figures that required further explanation and editing have been deleted due to the space constraints.

   **Reviewer 2**

22. It is relevant to describe how the correct assignment of repeats was ensured.

   The section detailing this methodology has been expanded to provide more detail in this respect.

23. Further detail is required on the distribution of the MLVA profiles in clusters, especially about one dominant MLVA type within the PT170.

   Additional information has been added to the Results section as requested.

24. It is not clear how cluster definitions were used in the active surveillance.

   While the cluster definition was prospectively applied to generate suspected clusters for further epidemiological investigations, the ratio between novel and persistent MLVA types was not relevant in this respect. Two explanatory sentences have been added (page 6).

25. Background reference 10 should be corrected.

   This reference has been replaced and the definitive phage types of STM have been used.

26. The references to figures should be checked in the text.

   Figure numbers have been checked and references to Figures 1 and 2 corrected where necessary.

27. Figures 5 and 6 are difficult to read.

   Both figures have been removed from the revised version of the manuscript.

28. All discretionary revisions suggested by the reviewers have been gratefully accepted. The only exception was the answer to the question about the inclusion of monospecific variants of STM into the MLVA testing scheme. We did include them into the typing scheme but felt that this addition of this point is not warranted due to a very low frequency of such variants in our STM populations.
Regards,

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