Reviewer's report

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

**Version:** 1  **Date:** 9 November 2011

**Reviewer:** Tansy Peters

**Reviewer's report:**

**Major compulsory revisions**

This manuscript describes a 3 year prospective study of Salmonella enterica serovar Typhimurium using multiple-locus variable-number tandem-repeat analysis (MLVA) as the method of sub-typing. It is generally well written with a good standard of English and MLVA is a widely recognised technique that is becoming increasingly utilised due to its potential advantages over existing typing schemes. The authors have gathered a large volume of extremely useful data which is invaluable in improving our understanding of human S. Typhimurium infections.

Whilst the study clearly demonstrates the levels of diversity of S. Typhimurium from human infections I do not agree that the authors have shown prospective typing by MLVA enabled more targeted and timely public health investigations. The authors themselves state that MLVA typing data has not averted recurrences of community outbreaks of S. Typhimurium and their conclusion that further outbreaks were significantly delayed is unsound.

What do the authors mean by “the re-emergence of epidemiologically confirmed outbreaks”? Are they saying that the re-appearance of a particular MLVA profile indicates that the same food source/eating establishment/farm is harbouring S. Typhimurium and reintroducing it back into the population? They say that clusters of the same MLVA type following initial public health interventions “relapsed” – what do they mean by this? How can a cluster relapse? Also, where is their evidence that MLVA-based surveillance was a significant contributor to delays in recurrences of Typhimurium clusters that were epidemiologically investigated? It is not correct to say that an outbreak has “relapsed/recurred” based on MLVA clustering.

For MLVA in many other countries data are still being collected but it is possible that some profiles may be more common than others and therefore may be found in epidemiologically unlinked strains. A recent study by Prendergast et al (Food Microbiology Volume 28, Aug 2011:1087-1094) has shown that MLVA permitted the identification of identical profiles in isolates from the same source at different points in time but also from different sources at different points in time and that identical MLVA profiles from different sources were frequently identified.
Where apparent case clusters are detected these should be verified by other typing methods including epidemiological data looking for common risk factors. The authors have acknowledged that restriction of their cluster definition to isolates with identical MLVA patterns may be a limitation of the study. Previous work has shown that within known outbreaks and in families, isolates have been shown to vary at one locus by any number of tandem repeats although more than one dominant profile may be characteristic of an outbreak. Strains with profiles that vary at two or more loci should also be viewed carefully in relation to the strength of their epidemiological association to the outbreak as they may be from outlying cases.

Until more is known about the underlying MLVA profiles that circulate within different populations it would be unsafe to use MLVA typing in isolation without considering all the epidemiological data together with other typing methods.

Why do the authors use the Lindstedt nomenclature method of binned fragment sizes being assigned an allele number and then use the sequence length for the final locus? Larsson et al have now developed a scientifically based, rational way of assigning names to Typhimurium MLVA profiles (Eurosurveillance, Volume 14, Issue 15, 16 April 2009). Although fragment analysis is not fully comparable when using different sequencers, fluorescent labels etc this standardised nomenclature allows for normalisation of data and enables a direct comparison of data between laboratories which would be useful.

Minor essential revisions
Pg 3: Reference 10 is incorrect, it does not describe the Typhimurium Phage typing scheme
Pg 4: multiple locus VNTR analysis NOT multiple variable-number tandem-repeat locus analysis otherwise the abbreviation looks odd
Pg 4: reference for MLVA7 used in the USA
Pg 5: would the authors care to comment about the incidence of co-infection with different Typhimurium genotypes or provide a reference
Pg 5: check reference 24 – do the authors really mean this was the previously used method or should this refer to ref 25?
Pg 8: If there was no significant increase in the number of clusters or their average size but the overall Typhimurium numbers have increased was this due to the appearance of sporadic Typhimurium cases with new MLVA profiles?
Pg 9: how does MLVA typing reduce the complexity of the epidemiology of salmonellosis? If MLVA sub-divides Typhimurium Phage types surely that’s an increase in complexity?
Pg 11: When the authors say “a Salmonella infection cluster being solved” do they mean a source of infection was confirmed?
Pg 11: if the overall number of Typhimurium cases increased over the study period how could MLVA surveillance have been a significant contributor in
decreasing the burden of disease?
Pg 12: why did the authors exclude isolates differing by one repeat in three of the five loci, why not apply this to all five loci?

Discretionary revisions
Pg 2: Please do not start sentences with numerals e.g. 667 different MLVA types…
Pg 3: pulsed-field NOT pulse field
Pg 5: I don’t mind if the authors use STM or S. Typhimurium but please be consistent and decide on a single abbreviation

Figures: Explain the colour codes on the maps denoting the cluster size
If you use Google maps please remove the flags/pointers or do they have any significance?

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

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

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