Reviewer's report

**Title:** A snapshot from ongoing enhanced surveillance of invasive listeriosis in Lombardy, Italy, 2006-2010

**Version:** 2  **Date:** 20 November 2012

**Reviewer:** Stephen Knabel

**Reviewer's report:**

**Major Compulsory Revisions:**

1. The title needs to be more specific to indicate the conclusions reached, not just the type of study conducted. For example, the title might better read “Enhanced surveillance of invasive listeriosis in the Lombardy region of Italy from 2006-2010 reveals major clones and an increase in serotype 1/2a”

2. “Enhanced surveillance” needs to be more clearly defined in the introduction. Does this include conventional epidemiology or just Laboratory-based molecular epidemiology? Throughout the manuscript the authors state that no listeriosis outbreaks were notified in the Lombardy Region during the period in question (2006-2010), and they repeatedly emphasize the potential for “hidden” outbreaks to go undetected. The authors mention listeriosis outbreaks were detected in Europe in the past, were they ever detected in the Lombardy Region of Italy? If so, when and what was/were the food vehicles responsible? More discussion is needed about how conventional epidemiology was conducted in the Lombardy Region during the period in question. For example, in the U.S. epidemiologists often use shopper cards, which allows CDC to electronically track specific food items and brands purchased by ill and control customers, which allows them to perform more accurate case-control studies to identify the specific food vehicle responsible for a listeriosis outbreak. For example, the US CDC successfully detected the recent 2012 Italian Ricotta Cheese outbreak using a combination of conventional and molecular epidemiology – this two-pronged approach needs more emphasis in the discussion in this manuscript. Was this type of conventional epidemiology investigation being done routinely in the Lombardy Region of Italy during the period in question? If not, why not? Were case-control studies routinely being performed when these time and geographically-linked clusters were repeatedly detected by molecular epidemiology? Again, if not, why not. It is painfully obvious in this study that without good conventional epidemiology it is impossible to know the epidemiologic relevance of data from different subtyping methods. Therefore, specific recommendations concerning “enhanced” conventional epidemiology strategies need to be also included in the conclusions.

3. Pulse-field gel electrophoresis of Lm isolates was conducted using Ascl and Apal; however, in the legend to Fig. 2 it says Xbal was used. This needs to be corrected.
4. Exactly how were the PFGE clusters defined? This needs to be inserted at the end of the PFGE section in the Materials and Methods on page 7.

5. The authors need to review ALL STs to make sure they were assigned to the correct clonal complexes. For example, the authors claim ST38 is in clonal complex 38, when in fact ST38 is in clonal complex 101 (see Ragon et al., 2008 and Chenal Francisque, 2011). In Fig. 2 one ECII isolate (code 233) has the same ST/CC (2) as ECIV isolates, but should be in CC6 (See Ragon et al., 2008). This is probably due to either a MLST or MVLST sequencing error or an error in assigning this isolate to CC2. This same concern occurs with ECIII isolate code 118 being assigned to CC14, which should be in CC11, like isolate code 226. Also, isolate code 191 is not listed as ECIII, but it has the same ST/CC (11) as ECIII (Ragon et al., 2008). The authors need to recheck their results and/or rerun their experiments to make sure that data in all Tables and Figures agree with one another and with the literature. If they disagree with the literature after rerunning MLST and/or MVLST, the possible reasons for these discrepancies need to be discussed in the Discussion section.

6. The authors report finding ST8 and ST120 in CC8, which are most likely ECV. ECV-specific primers and the MVLST sequences for ECV have been published (Knabel et al., 2012; Gilmour et al., 2010). Therefore, these analyses need to be performed and this data entered into Table 2. If all 11 ST8 isolates listed in Fig. 3 are ECV, then this would increase the number of epidemic clones detected (from 3 to 4) and the total percentage of isolates that are epidemic clones (from 32% to 40%). This information needs to be included in the Materials and Methods, Results and Discussion.

7. An outbreak due to ECIV associated with corn occurred in the Piedmont Region of northern Italy, which is next to the Lombardy Region (Lomonaco, 2012. EPIDEMIC CLONES OF LISTERIA MONOCYTOGENES: DETECTION, TRANSMISSION AND VIRULENCE. In, Listeria Infections: Epidemiology, Pathogenesis and Treatment). This needs to be discussed in the context of the results presented and the reference for this outbreak (Aureli et al., 2000) needs to be also included in the manuscript.

8. The subheaders in Table 2 should read “Number of cases with underlying condition” and “Isolate/Subtype data”. Plus explain below this Table why PFGE clusters 1 and 10 were not included.

9. On Figure 1 indicate “percent of isolates by serotype” and “total number of isolates” on the left and right x-axis, respectively.

10. On Figure 2, put PFGE Cluster on far left, followed by code, year, serotype, ST (CC) and epidemic clone. This will make the order consistent with that in Table 2. Also, indicate if ECV is present. Also delete title at bottom.

11. On Figure 3, change 38 to 101 and recheck all other STs to make sure they are the correct clonal complex.
12. Rather than using the term “molecular subtype clusters”, the authors need to be specific and call them PFGE clusters throughout ms. Also, if ST, CC or EC are used then different clusters result, as mentioned above this needs to be discussed – how and why are these clusterings (using different molecular markers) different? What do they mean? Which markers are more relevant/accurate/useful for epidemiologic investigations? This needs to be included in the discussion section.

Minor Essential Revisions
1. The manuscript needs a thorough review by someone fluent in correct English grammar to clarify meaning in many, many places

2. In many cases the authors say there is a difference, but then say it is not significant (see bottom of page 9). Either delete these statements or say there was no significant difference.

3. Need references to support statements like, “Lombardy an Italian region accounting for 16% of the nation’s total population, is notifying 55% of the listeriosis cases” Where did the data come from to allow calculation of these values? What time span are the authors referring to? How many outbreaks in total were reported in Italy during that time span?

4. Need to discuss why PFGE Clusters, STs, CCs and epidemic clones don’t always agree (see Table 2).

Discretionary Revisions
1. Paragraphs following the first paragraph need to be indented

2. Need to clarify the time period (span) in question in many places

3. It would help to know what national and regional Italian Health Agencies were responsible for investigating listeriosis cases and detecting outbreaks in the Lombardy Region of Italy and what their different roles were during the period in question

4. Poor word choice in many places, need to choose the right word

5. How many total cases of listeriosis were there by year throughout the period in question?

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

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