Author's response to reviews

Title: Molecular and Epidemiologic Analysis of a County-Wide Outbreak Caused by Salmonella enterica subsp. enterica serovar Enteritidis Traced to a Bakery

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PDF covering letter

Dear Editor,

We highly appreciate the reviewers' comments and revised the munuscript '**Molecular and Epidemiologic Analysis of a County-Wide Outbreak Caused by *Salmonella enterica* subsp. *enterica* serovar Enteritidis Traced to a Bakery**' according to their suggestions.

For Reviewer Meirion Evans' comments

1. Abstract

1.1 Should reflect revisions made to the text as detailed below.

1.2 Background should be clarified and epidemiological study design should be explained.

1.3Conclusion should emphasise not just importance of alertness in the emergency room but role of the microbiology laboratory in surveillance and the importance of prompt and through investigation to trace the source of outbreaks and to institute appropriate control measures.

1.4 The implicated bread is the vehicle of infection, but not necessarily the source of the outbreak. The source may have been a contaminated ingredient, or the cause othe outbreak may have been cross-contamination in the kitchen, or contamination by an ill food handler.

The abstract is revised according to the above comments.

2. Introduction

2.1 Final two sentences should be moved to Discussion

The final two sentences is already moved to the Discussion section.

3. Methods

3.1 Page 5, line 2. Were these patients attendances at or admissions from the emergency room? Elsewhere (P.8, line 10) 31/34 patients are said to be hospitalised.

They are attendees but not admitted cases. The sentence is revised.

3.2 No case definition is given

Case definition is presented on the last sentence of page 5 of the revised manuscript.

3.3 Details of the case searching exercise undertaken are vague

We found cases from ER record by ER personnel and infection control nurses. It is presented in the 2nd sentence of Page 5 of the revised manuscript.

3.4 Was an analytic study (e.g. case-control study) carried out? This is is unclear. If it was, it should be described including details of cases included/excluded from the study and selection of controls.

When comparing the relationship of *Salmonella* and the implicated food, we use Chi-Square test to analyze. Otherwise, we report a cohort of cases.

3.5 How were data on cases (and controls) collected? There is mention of chart review of all patients(P.5, line 12) and review of demographic features and food histories by the infection control team(P.5, line 4) on a sub-sample (?) of patients. How were these two reviews linked? Did the review of food histories include interviews with patients? Was a standard questionnaire used (for both cases and controls) and what kind of questions were asked, especially about food items eaten? Page 7, sentence 1 should be included in this description.

Interview cases without gastroenteritis at ER was not underwent. Only cases with gastroenteritis are interviewed with a case record form that was filled by hospital personnel but not using a questionnaire to be replied by patients.

3.6 There is virtually no description of the environmental investigation. How was the traceback undertaken? Was the implicated bakery inspected? Were food handlers interviewed and food hygiene standards reviewed? Were food and/or environmental samples obtained from the bakery? If so, what and how many? Who carried out the inspection?

The environmental investigation was rewritten in page 7 and page 11.

3.7 Food samples were obtained from patient's homes (P.5, line 10) but insufficient detail is provided about this. Were all patients/cases visited and by whom? On what basis was a food sample 'suspected as a possible cause of gastroenteritis'? (These details should ideally be brought together in a section on Enviromental investigation along with the information described in 3.6 above.)

The basis of a suspected food sample was shown in page 7, environmental investigation, the first sentence.

3.8 Data analysis (P.7, line 3-4) should include calculation of odds ratios with 95% confidence intervals if a case-control study was done.

Odds ratio and 95% confidence intervals were also calculated when Chi-Square test was used.

4. Results

4.1 This section describes 28/34 cases consuming the implicated food and 6/131 cases not consuming the food as being positive for Salmonella (P.8, line 4-5). Where do these numbers come from? The previous paragraph refers to 162 patients during the week of the outbreak and 506 in the 6 weeks beforehand.

The case number 131 is incorrectly written. Actually, it is 128.

4.2 How are these cases defined (P.8, line 4-5)? According to the Abstract, a person had to be culture positive for Salmonella to meet the definition of a 'case'.

Case definition is presented on the last sentence of page 5 of the revised manuscript.

4.3 The association between food and illness is incorrectly described (Page8, line 4-7)implying that the 'disease' is eating the bun and the 'exposure' is having salmonella infection. It is the proportion of cases (ill) who ate the bun compared with controls (not ill) who ate the bun that is relevant.

4.4 There should be a Table describing associations between illness and a range of relevant items. The associations should be described as odds ratios with 95% confidence intervals both in the tableand in the text(if this was a case-control study).

The relationship of *Salmonella* and the implicated food among cases with gastroenteritis are examined. The relationship of gastroenteritis and the implicated food was not statistically tested for not interviewing the ER cases without gastroenteritis.

4.5 Three months follow-up is mentioned (P.8, line 16) though there is no reference to this in the methods.

4.6 Control measures (P.8, line 17-18) should be included in a separate paragraph together with further results of the Environmental Investigation.

4.7 The date the bakery was closed should be indicated by an arrow on Figure 1.

4.8 Page 9, second paragraph should be moved to the Discussion section.

The above comments are revised.

5. Discussion

5.1 This should be introduced by a paragraph briefly summarising the key findings including for example the final part of the Introduction (see 2.1) and the penultimate paragraph of the Results (see 4.8)

5.2 There should be more discussion on how the bread may have been contaminated (and any public health implications arising from this.

5.3 P.11, line 12-13. What other food samples were examined from the bakery? - this should be described in the Results.

5.4 P.12, line 3. Why was there no investigation of bakery staff or bread ingredients? This is after all a large outbreak of a type uncommon in Taiwan according to the next paragraph.

5.5 P.12, line 17. If this was an egg glaze on the bread it is difficult to see how it might onnly be lightly cooked.

5.6 P.13, line 6. Could there be another explanation for the apparent short incubation history e.g. another food vehicle. Only 21% of cases are explained by the bread according to P.8, line 1 – how were the others infected?
5.7 Final paragraph should also emphasis importance of laboratory surveillance for identifying outbreaks. This should allow an excess of Salmonella cases to be identified even if they attend several different ERs.
The discussion part is rewritten according the above comments. For point 5.6, the possibility of another vehicle was presented in the last paragraph, Page 14. The added Figure 2 and the related statistics showing the increased cases on Saturday and Sunday may contributed to the gastroenteritis cases not consuming the implicated food.

6. References
6.1 Mention eacg reference only once - there is multiple duplication
7. Figures
7.1 It is difficult to distinguish different grey shades in this figure. Aslo indicated date of bakery closure by an arrow (see 4.7).
The above are revised.

For Reviewer Anja Siitonen,

1. The authors report that the cause of the outbreak was Salmonella Enteritidis of a certain PFGE genotype and that the egg-covered bread, contaminated with this particular Salmonella type, was the source of this outbreak. The outbreak was traced to a bakery producing that bread. However, most of the patients felling ill with gastroenteritis during 29th and 30th of July had not consumed bread produced the bakery (Fig 1). The finding that indistinguishable Salmonella strains were present in stool samples of the patients consumed bread and in a bun obtained from a patient, is not the evidence that the source was bread. Unfortunately, no stool samples of the patients who did not consume bread were cultured for Salmonella. Actually, it seems more likely that also some other source(s) or vehicle(s) existed. The authors should critically discuss about these issues.

The possibility of another vehicle was presented in the last paragraph, Page 14. The added Figure 2 and the related statistics showing the phenomenon of increased cases on Saturday and Sunday at that ER may contributed to the increased gastroenteritis cases not consuming the implicated food on July 28 and 29 (Saturday and Sunday). Critical discussion was performed at the discussion section.

2. In addition, no food items were collected from the bakery for culturing of Salmonella. Since S. Enteritidis was isolated from only one of the buns obtained from the patient, the contamination of the bun at home of the patient cannot be excluded. This should also take into account in the discussion of the results.

The possibility of contamination at house or at six markets simultaneously at the same day is low. The point is presented in page 16.

3. What might be the other reasons or causes for the increased number of gastroenteritis cases during the outbreak period (shown in Fig 1)?

The added Figure 2 and the related statistics showing the phenomenon of increased cases on Saturday and Sunday at that ER may contributed to the increased gastroenteritis cases not consuming the implicated food on July 28 and 29 (Saturday and Sunday). Critical discussion was performed at the discussion section.

4. Table 1 is not interesting, and can be deleted. Instead, the authors could briefly describe the case record form, the data enquired and the data obtained from the patients.

The discussion of Table 1 is presented in the 2nd paragraph in page15 according to reviewer Frank Rodgers's comment (point 8).

5. Page 7.

The authors have used Fisher's exact test to test if there was a significant

increase in number of patients with gastroenteritis visiting the hospital during the outbreak period compared to the number of a week or 6 weeks before the outbreak. The significant increase was found, $P < 0.005$. It would be good if an expert statistician could check that the method really is suitable for comparison between these completely independent patient populations. In addition, the authors should give exact P values (still no more than with 3 decimals) always when it is possible. It seems that the P values are $P < 0.001$ rather than $P < 0.005$. If the authors used any software for calculations, it should be mentioned.

The statistics was examined by expert at National Health Research Institute, Taiwan. Chi-Square test with calculation of odds ratio and 95% CI was suggested. Fisher's exact test was more suitable than Chi-Square test in the 2nd paragraph, page 10.

6. Page 27, Figure 3.

It seems that the profiles contain too much DNA or the exposure time has been too long resulting in excessive brightness of the bands. However, it does not matter since all the outbreak profiles can be seen and they clearly are indistinguishable from each other. However, despite of the brightness, the markers cannot be seen. Probably therefore, the authors have not been able to mark the molecular weights on the side of the gel at the right positions. This should be corrected, or the marks for different kilobases should be removed from the picture. In fact, in this kind of picture where only profiles of the outbreak and non-outbreak strains are compared, the kb markers are not very important.

The figure is adjusted according the reviewer's comments.

For reviewer Carl Schroeder

1. The first paragraph should have been broadened. For instance, have any outbreaks of salmonellosis been reported in Taiwan? What about those related to S. Enteritidis? Expansion of the Introduction would greatly aid the reader in placing the current study in context. As is, it seemed unduly abbreviated.

2. Somewhere in the manuscript the authors should have elaborated on whether the mayonnaise or pork toppings may have been vehicles of infection. Specifically, have mayonnaise and pork been implicated in infection previously? Granted, the authors state that cultures were not available for each part of the bread; nevertheless, this point deserved further consideration/discussion.

3. Regarding the antimicrobial susceptibility testing, it is essential that the authors include description of what if any quality control organisms were used. Also, a description of tested dilution ranges and breakpoints would have provided valuable information.

For point 1-3, we revised the manuscript according the comments

4. A list of quality control organisms should also have been provided for the PFGE experiments.

Repeated PFGE experiment to examine the correctness and duplicating ability of PFGE.

5. On page 8, the authors state that 6 of the 131 not consuming the implicated food were culture-positive for Salmonella. Were these cultures determined to be S. Enteritidis?

The details of the mentioned 6 isolates were presented in the 2nd paragraph, page 12.

6. On page 9 the authors state that blood samples from two patients were positive for S. Enteritidis, thus implying invasive infection. A further elaboration/discussion of this point would have been valuable. For instance, what was the treatment regime? The clinical outcome? Etc.

The details are presented in line 5-6, page 11.

7. In general, further information about patient demographics would have greatly improved the manuscript. For instance, information about patient age would have been valuable in learning more about the epidemiology of salmonellae infection.

8. On page 11 the authors state that the bread sold in the Chinese traditional markets “might not have been” protected through sanitary regulations and that production and distribution were “unlikely” to have been subjected to routine hygiene inspections. If the authors knew who produced the bread, wouldn’t it have been possible to determine whether production was subjected to hygiene inspections, etc. Inclusion of this information would have been helpful and helped

to tighten the Discussion.

For point 7 and 8, the manuscript is revised according to the reviewer's comments.

For reviewer Frank Rodgers

1. English usage and grammar should be improved throughout the manuscript.

2. Page 2: When the authors use "incidences" here, I think they mean the "number of new cases".

3. There is no consistency in the usage of the organism name Salmonella enterica subsp. Enterica serovar Enteritidis. Capitals and italics are interchanged liberally. Furthermore, after Salmonella enterica subsp. enterica serovar Enteritidis has been defined as such, then the abbreviated form S. Enteritidis should be used thereafter throughout the manuscript.

4. Figure 2 adds no value and should be deleted.

For point 1-4, the manuscript is revised according the reviewer's suggestion.

5. My copy of figure 3 (the PFGE) was very unclear. Could a sharper figure not be used? Actually I think figure 3 should be re-thought out. A gel with 25 identical lanes comprising 22 outbreak isolates, a single bread isolate, a blood isolate and a stool isolate (all identical) seems unnecessary.

A sharper PFGE figure with more clear legend is modified as the reviewer and reviewer Anja Siitonen's suggestion.

6. Pages 5-7 and Table 1: Was phage typing or any other molecular techniques undertaken to identify these isolates further. Although the PFGE and antibiogram profiles would appear to confirm the tight clustering of these isolates, further typing procedures might assist in the characterization and sub-clustering of these pathogens. This is particularly pertinent given the data in figure 1. The vast majority of cases of disease occurred during this period in individuals who had not consumed the bread in question. How many of the isolates from these were sub-typed? Was subsequent food contamination by infected individuals within families or other groupings a factor in these cases? Indeed, it is particularly interesting to note that in the apparent absence of consumption of the contaminated vehicle, the levels of acute gastroenteritis prior to the outbreak ran at around 10 or fewer per day between Feb. 23 and the first day of the outbreak (Feb. 28), but on Feb 29, the number of cases of acute gastroenteritis without consumption of the bread rose to 38 people. Again, in the absence of consumption of the proposed vehicle these numbers remain higher than "normal" for some time after. The authors make no comment on these observations. How many isolates were made from each of the groupings across figure 1; how many of the group "cases without consumption of bread" were serotyped; how many were subjected to PFGE and antibiogram analyses. This information is not present in the M&M. Were bakery personnel and food handlers examined?

The added Figure 2 and the related statistics showing the phenomenon of increased

cases on Saturday and Sunday at that ER may contributed to the increased gastroenteritis cases not consuming the implicated food on July 28 and 29 (Saturday and Sunday). Critical discussion was performed at the discussion section.

The details of the 6 isolates from cases not consuming the implicated food were presented in the 2nd paragraph, page 12.

7. Page 5: It is not clear on how many individuals and by what methods stool and blood cultures were performed?

The matierial and method part is revised as the reviewer's commements in the first paragraph in page 6. 'Twenty-eight *Salmonella* isolates were cultured from 32 available stool specimens from 34 cases who consumed the implicated food.' The last line in page 11.

8. Table 1: these data are presented without comment. If significant then appropriate discussion should be made otherwise it should be deleted.

Discussion of table 1 is presented in page 15.

9. Discussion, page 10: There have been a number of reports, including a number from our group, which suggests that PFGE, when applied to S. Enteritidis, has failed to distinguish epidemiologically unrelated isolates. Indeed, S. Enteritidis, phage type 1 and phage type 4 often present with identical PFGE patterns.

Phage typing is not available in our institutes. The fact that PFGE, when applied to S. Enteritidis, has failed to distinguish epidemiologically unrelated isolates was presented in Discussion part.

10. This is a well circumscribed outbreak with little new to commend it as different from many other such outbreaks; therefore, if accepted it should be reduced in length to present only the essential and new information.