Author's response to reviews

Title: Symptoms and sources of Yersinia enterocolitica -infection: a case-control study

Authors:

Elisa Huovinen (elisa.huovinen@thl.fi)
Leila M Sihvonen (leila.sihvonen@thl.fi)
Mikko J Virtanen (mikko.virtanen@thl.fi)
Kaisa Haukka (kaisa.haukka@thl.fi)
Anja Siitonen (anja.siitonen@thl.fi)
Markku Kuusi (markku.kuusi@thl.fi)

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Author's response to reviews: see over
Dear Dr Koutsos,

We thank the reviewers for their invaluable comments and insights on our paper. After the careful consideration of the comments, we agreed with most of their criticism and suggestions and undertook the revision of the manuscript according to their comments. However, the task was not easy since some of the comments of different reviewers were contradictory. In the context of the sources of infection (Table 3) two reviewers (JV and KM) had conflicting opinions. We agreed more with reviewer KM comments and based on his, as well as reviewer IA´s, recommendations we have softened our claims on the potential pathogenicity of YE BT 1A strains and refocused the whole paper. In addition, we excluded the discussion on the frequencies of YE and low-lactose diet since it was criticized by three reviewers. We hope that the manuscript in its revised form merits publication.

Yours sincerely,
Leila Sihvonen

List of the changes made and responses to the reviewers' comments:

**Reviewer: Gianluca Matteoli**

**Discretionary Revisions:**

1) In order to increase the overall clarity of the work, I would advise the authors represent the data (number of strains and/or symptoms) with graphics such as histograms or pie charts.

- We have made a histogram of symptoms from table 2 and present the onset of symptoms to sample taking in a table, since we found that data was not easy to illustrate with graphics. We also tested the data of table 1 as a graphical presentation, but since the differences in numbers are considerable, we think data suits better in a table format.

2) Moreover, the definition of an integrated analysis (e.g. regression analysis) associating the occurrence of symptoms to the infections would be beneficial to the comprehensive meaning of this case-control study

- Source analyses were done using univariate conditional logistic regressions, i.e. between cases and controls, which are rather a standard procedure in case-control studies (Clayton D, Hills M. Statistical models in epidemiology, Oxford University Press, 1993).

**Minor Essential Revisions:**

1) The authors should carefully review their manuscript and ensure that the English they used is correct. (es. line 16 page 5, line 7 page 6, line 21 page 8)

- The sentences mentioned have been checked.

2) It would be interesting to display as supplementary material the questionnaires submitted to the patients and to their controls, in order to assess the effectiveness of the survey and to exclude possible biases in the questions.

- We agree, however, the questionnaire is only in Finnish language and at the moment we do not have resources to translate it.

3) In the discussion section, the authors state that “The higher frequency of
vomiting among patients with YE BT 1A might be due to enterotoxin YstB known to be produced by some BT 1A strains”. It would be of extreme interest to analyse the frequency of the Y.e. BT 1A strains expressing the enterotoxin YstB in their isolates.

- We have started to analyze strains with PCR to detect ystB genes. We hope to publish the results in the near future.

**Major Compulsory Revisions:**

1) The authors report more cases of continuous diarrhoea in the BT 1A-infected patients compared with the pathogenic Y.e.-infected patients. Considering that the infection with the pathogenic Y.e. is generally correlated with a worse clinical outcome, it would be worth mentioning the therapeutic actions undertaken by these patients, in order to exclude influence of pharmacological treatments on the course of the infection.

- You are right in saying that therapeutic actions might affect the course of infection and therefore we have changed the MS. We have removed the section “Symptoms continue at the time of questionnaire’’ from Table 2 and a corresponding sentence from the symptoms paragraph: ”Symptoms also continued at the time of filling the questionnaire more often among the cases with YE BT 1A.”

2) The association of joint symptoms with the pathogenic Y.e. infection may be ambiguous and a more accurate analysis with a larger number of patients would be required to support this conclusion and implications of the different Y.e. strains. The fact that a so high percentage of individuals form the control group reported joint symptoms may lead to inappropriate conclusions.

- The association of joint symptoms with a Yersinia infection is difficult to measure with a questionnaire study like this. Anyhow, we think that our results are quite interesting. The high percentage of joint symptoms among controls brings out the variety and frequency of joint symptoms. We have tried to highlight this now in the discussion section. We defined a probable reactive arthritis in methods as swelling with gleam/redness in a joint and same time being able to report the accurate onset of joint symptoms. The cases that met these criteria were 10 % of pathogenic YE patients and 3 % of YE BT 1A patients and 0.3 % of the controls. We think these numbers are not very biased, since reactive arthritis is quite common due the high prevalence of HLA-B27 in Finnish population.

3) The authors need to clarify and to support with more details their statements about the correlation between low-lactose diet and infection with the BT 1A strains.

- We have excluded text concerning low-lactose diet from the MS.

**Reviewer: Kare Molbak**

**Major revision:**

Overall, I find that this is an interesting study. The study has two aims: one is to determine the clinical picture by different types of Yersinia enterocolitica (YE) and the other to identify risk factors for YE according to bio/serotype. The main conclusion (taken from the abstract) is that some strains of YE biotype 1A, which in general is considered nonviralent, may cause an illness but the symptoms differ from yersiniosis caused by the classic pathogenic YE bio/serotypes. I would be more cautious on the interpretation.
- We agree and have rewritten the parts of the MS concerning potential pathogenicity of BT 1A.

_Cases were identified among patients who submitted stool samples for faecal analysis, and it is thus inevitable that patients with YE biotype 1A (and any other finding for that matter) suffer from some sort of gastrointestinal complaint. On this basis, it is difficult to draw this conclusion. The 263 patients with YE biotype 1A were identified from a sample base of 41,848 stool specimens (representing an unknown number of patients). It would be interesting to learn whether the prevalence of YE biotype 1A among these patients were different from the prevalence of YE biotype 1A among healthy controls/the general population._

- We actually studied 200 samples of healthy people as a part of the project and found one healthy person without any symptoms that had YE BT 1A. However, since this result is not statistical significant we wish not report this in the study. We think a larger group of healthy persons should be examined before any conclusions.

_Unless these data become available, I propose to focus the paper more on risk factors and sources (where there are clear differences between the classic pathogenic YE bio/serotypes and YE biotype 1A), and speculate less on the pathogenic potential of YE biotype 1A._

- We have refocused the MS and reformulated parts concerning potential pathogenicity of BT 1A

**Minor revisions:**

_Background. To clarify, please mention that YE-like species are defined as selected non-enterocolitica species within the genus Yersinia. Otherwise this remains unclear until the reader reach page 7 where the YE-like species are mentioned._

- Species are now listed in Background section

**Methods. How many patients do the 41,848 stool specimens represent?**

- Unfortunately, we cannot answer this. Sample we kindly provided from the Finnish clinical microbiology laboratories but we have patient information only from those samples that contained yersinia (n=462). The number 41,484 contains also multiple samples of some patients.

**Did the authors analyse the semi-quantitative data by exposures and clinical picture?**

- Yes we did, but we did not detect any correlation. It may be that the variation of time of the stool sample taking (from the onset of the symptoms) or some other factors affected the results.

**There were 27 isolates (how many patients?) with another gastrointestinal pathogen. What were the types of Yersinia identified on those polymicrobial patients?**

- Following table demonstrates the polymicrobial patients.

<table>
<thead>
<tr>
<th>Yersinia species</th>
<th>additional isolate</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. enterocolitica 4/O:3</td>
<td>Campylobacter</td>
<td>2</td>
</tr>
<tr>
<td>Y. enterocolitica 4/O:3</td>
<td>Salmonella</td>
<td>2</td>
</tr>
<tr>
<td>Y. enterocolitica 4/O:3</td>
<td>Y. enterocolitica 1A</td>
<td>3</td>
</tr>
<tr>
<td>Y. enterocolitica 1A</td>
<td>Y. enterocolitica 1A</td>
<td>1</td>
</tr>
<tr>
<td>Y. enterocolitica 1A</td>
<td>Campylobacter</td>
<td>6</td>
</tr>
<tr>
<td>Y. enterocolitica 1A</td>
<td>Salmonella</td>
<td>4</td>
</tr>
<tr>
<td>Y. enterocolitica 1A</td>
<td>Shigella</td>
<td>1</td>
</tr>
</tbody>
</table>
Y. enterocolitica 1A  Cryptosporidium  1
Y. enterocolitica 1A  Small round virus  1
Y. enterocolitica 1A  Norovirus  3
Y. enterocolitica like sp.  Salmonella  1
Y. enterocolitica like sp.  Campylobacter jejuni  2

**tot. 27**

A two week exposure period seems long; the incubation period < 10 days, usually 2-3 days. Definition of reactive arthritis: Was it defined how long after YE infection the signs of reactive arthritis might occur? What was the case definition of “probable reactive arthritis” as used in the results section.
- We admit that two weeks exposure period is a quite long. According to literature, the incubation time typically is 4 to 6 days, varying from 1-14 days (Red book: 2006 Report of the Committee on Infectious Diseases. American Academy of Pediatrics; 2006, p. 732–4). Therefore we asked exposures for two weeks period.
- Reactive arthritis might have occurred after a patient had answered the questionnaire. This has been mentioned in the discussion. The definition of probable arthritis has been explained in the methods: “A probable reactive arthritis in a case or control was defined as swelling with gleam/redness in a joint and the onset of joint symptoms reported with the accuracy of 1-3 days.”

**Results. Consider to move the two first sentences to Methods.**
- We wish to have this data in results section since it refers results in Table 1.

There were cases and controls that were lost because the matching in the analysis was maintained. In particular, only 758 controls of the 1002 who returned their questionnaire were used for the analysis. To preserve statistical power, did the authors consider to break the matching (and adjust for the matching variables in the analysis)? If yes, did it affect the results?
- This was never considered. It is a valuable suggestion. However, the matching criteria were relatively tight. For instance, adjusting for spatial effects implied in matching by the commune of residence, would unnecessarily complicate the analyses.

**Symptoms. Almost all cases…. Better to provide the number.**
- added numerical values (240/248) in the text
**At page 10, second line, a % is missing**
- added missing %

**Discussion.**
As mentioned above, the limitations of the study need to be addressed, and the authors should be more cautious when they address the potential pathogenicity of YE biotype 1A. There seems to be some indications that the patients with YE biotype 1A had more unspecific complaints and may suffer from a more ill defined gastrointestinal illness. Less severe symptoms may also result in a delayed contact with health care, which may explain some of the observations.
- We have soften the claims of pathogenicity of BT 1A in the text.

In addition, vomiting was common among patients with biotype 1A which the authors ascribe to either enterotoxin YstB or to undiagnosed viral gastroenteritis. I assume that it is not possible to confirm the latter hypothesis by testing of preserved specimens.
Yes, it is unfortunately impossible.

- Did the patients fulfill the Kaplan criteria for suspected norovirus infection? This may give some clues.
- At page 13, the authors discuss different frequencies of low-lactose diet. I assume that they refer to soybean and soybean products that were a common exposures among YE biotype 1A cases. Please elaborate.
- Reviewer: Ingo Autenrieth
  1. abstract: the last sentence is overstated as other causes for diseases in patients with type 1A isolates could not be excluded. thus it is just speculation that the "apathogenic" biotype 1A may cause any disease and symptoms.
  - We agree and have changed the conclusion of abstract as follows:
    “The symptoms of the patients with YE BT 1A differed from yersiniosis caused by the classic pathogenic YE bio/serotypes. In addition, the patients with YE BT 1A had more protracted gastrointestinal disorders and unspecific complaints. Small children were overrepresented in classic pathogenic bio/serotypes while in BT 1A or YE–like species were not found among children younger than two years. This suggests the lacking virulence of the BT 1A strains. We can not, however, rule out the possibility that some strains of genetically heterogeneous group of BT 1A may cause an illness.”
  2. in agreement with this concern is the fact that young children were not overrepresented in this group (usually these are the patients for the actual cases with pathogenic yersiniae because they are highly susceptible to enteric infections). therefore, it might well be that this biotype 1A group just reflects other infections, or even other totally different causes of bowel irritation, vomiting etc, even psychosomatic reasons.
  - We agree with the reviewer and have added a sentence in a paragraph concerning children & infection in discussion “Since children are known to be more susceptible to enteric infections due to immature immune system (Cohen MB: J Ped 1991, 118(4):34-39.), this observation suggests that YE BT 1A does not cause a disease in otherwise healthy persons.”
  
- Reviewer: Jugsharan Virdi
  Reviewer's report:
  In Table 1, the analysis pertaining to percentage of men is not very relevant to epidemiology of Yersinia enterocolitica and should be excluded from this table.
  - The earlier statistics (National Infectious Diseases Register) in Finland have stated that women have YE infections more often than men. This has been an artifact due not separating YE biotypes. We think it is also interesting that proportion of men is
significantly higher in classical pathogenic bio/serotypes compared with BT 1A cases. Therefore, we would wish to have this information in this table.

*Data given in Table 1 and Table 2 reiterate some of the earlier observations reported from other parts of the world. However this reiteration is worth reporting. Thus the whole MS needs to be reframed from the data presented in these two tables. The data related to the sources of infection (Table 3) is very rudimentary and there are not enough patients in each source to arrive at any significant or unequivocal results. In view of this, the authors need to analyse a larger sample of patients or else this data should be deleted.*

- We agree with the reviewer that the table 3 is very rudimentary. However, since reviewer KM wanted to focus the paper more on risk factors and sources we wish to keep the table in the paper.

*Minor Essential Revisions*

The abbreviation NBT for non-typeable strains is not appropriate and should be avoided.

- We have deleted NBT from the paper and use the term non-biotypeable instead of it.

The reference related to low lactose-diet and its relationship to *Y.enterococitica* is not substantiated by the presented data and it should be deleted from the text.

- We have excluded text concerning low-lactose diet from the MS.

In the bibliography, citation no. 23 needs to be written properly.

- Citation 23 has been corrected