Author's response to reviews

Title: Characteristics and risk factors for symptomatic Giardia lamblia infections in Germany

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Author's response to reviews: see over
response to the editor:

1) Ethics –

Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

➢ This epidemiological study has been performed in compliance with the Helsinki Declaration. It was conducted under the provision of the German Protection against Infections Act (IfSG). No invasive procedures were performed in this study and no risks to the patients existed. Participation was voluntary. The study was performed in accordance with the standards for data protection established at the Robert Koch Institute, Germany. A statement to this effect has been added to the methods section.

2) Copy-editing –

We recommend that you copyedit the paper to improve the style of written English. Please bear in mind that as we are a free-access publisher, we cannot bear the costs of copyediting English ourselves. You may wish to use a professional copyediting service. Examples are those provided by the Manuscript Presentation Service (www.biomedes.co.uk <http://www.biomedes.co.uk>), International Science Editing (http://www.internationalscienceediting.com/) and English Manager Science Editing (http://www.sciencemanager.com/). BioMed Central has no first-hand experience of these companies and can take no responsibility for the quality of their service.

➢ The manuscript has been revised by a native English speaker.

3) journal style

Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals ). You should remove the footnote page (page 2) and include the necessary contact information on your title page. It is important that your files are correctly formatted.

➢ The style of the manuscript has been revised conforming to the Journal style.
Reviewer's report
Title: Characteristics and risk factors for Giardia lamblia infections in Germany
Version: 1 Date: 18 June 2009
Reviewer: Lucy Robertson

Reviewer's report:

This is an interesting and useful study which seeks to analyse which of the Giardia infections notified to the authorities in Germany are autochthonous, and risk factors associated with these infections. The study is certainly worthy of publication and the data are provided are of interest. However there are various points which I believe should be addressed by the authors before the manuscript is published. These revisions are of the minor essential/discretionary type, although I think that some discussion around the 2 genotypes (point 5) is very important/essential.

1) It is frequently noted in the Giardia literature that symptomatic cases of giardiasis may be acute, with the sudden onset of severe diarrhoea and other intestinal problems, or there may be a prolonged illness with intermittent bouts of diarrhoea followed by apparent recovery lasting until the next bout of diarrhoea.

The reasons for this cyclicity in symptoms are not properly understood at present, although associations with particular giardia genotype have been suggested, although probably the true picture is more complicated. This prolonged manifestation may follow a period of months or even longer.

Thus, for those patients who decide to visit their doctor after a recurrence of symptoms in a case with prolonged clinical picture may have been infected long before the 3 week exposure period, as defined in this study.

Although the authors partially address this point with the additional question on foreign travel in the 12 month period prior to symptom onset, I think that this point should be made more clearly.

Cases were asked for their first onset of symptoms and the exposure period was defined by this date. To take earlier exposure in foreign countries into account, we asked about foreign travel in the last year. In the comparison of autochthonous and travel associated cases, foreign travel in the last year was more frequent in travel associated cases. Also, in the case-control study foreign travel in the last year was not a risk factor.

To clarify this point, we have included information on foreign travel destinations to “high risk” and “low risk” countries in table 1 and table 2 (see methods, results, tables).

However, as we looked at symptomatic cases, it is possible that we identified risk factors for the expression of symptoms following asymptomatic carriage. We added this to the limitations.

2) In the case-control study more information is needed regarding the two matched controls per case. In what respects were the controls matched with the cases – age? Sex? Water supply? Socio-economic position? Level of education? Employment? All these variables may possibly impact on likely exposure/infection. However, I perhaps understand from the paragraph that the controls were only matched by age and county of residence. Is this correct? This needs to be clarified. It would perhaps have been interesting to have had controls which had a diarrhoeal infection during the same period as the case, but diagnosed as being of non-Giardia aetiology, however I can appreciate that this would not have been simple to organise.

Controls were only matched by age and county of residence. We have made this clearer now in the methods section.
3) I understand from the first paragraph of the results that over 8% of cases notified do not meet clinical case definition. This seems a very high proportion to me. What is the reason for the notification if there has been no lab diagnosis and no link to a lab-confirmed case? This requires some clarification or comment.

⇒ All notified cases except the 6 cases with an epidemiological link had a lab confirmation. We applied the case definition used for surveillance in Germany, which specifies that *Giardia*-infections are either 1) laboratory confirmed by microscopy or antigen-test and presenting with at least one of the following clinical signs or symptoms: diarrhoea, abdominal cramps or bloating or 2) epidemiologically linked to a laboratory-confirmed case and presenting with at least one of the above mentioned clinical signs or symptoms. *Giardia*-infections not fulfilling this case definition or *Giardia*-infections without any reported information are not included in the national statistics. This has been clarified in the methods section.

Altogether, 14.4% of notified cases (86/597) did either not meet the clinical case definitions (49) or the clinical picture was unknown (37).

4) A relatively high number of cases required hospitalisation. Some further information on the reason for hospitalisation would be interesting (were these cases with other diseases, impaired immunity, or particularly elderly/young).

⇒ The median age of hospitalized cases was 38 years (range 3-92) and the male proportion was 43.5%. Four of the hospitalized cases (17.4%) were immunocompromised and five (21.7%) were on therapy with antacid medication. This information has been added to the results section (page 10, line 1-4).

5) very important/essential.

There are no comments or discussion on the two different genotypes of *Giardia* (A and B) which are infective to humans are made throughout the manuscript. Whilst I realise that information on the genotype of the majority of the infections is probably unavailable, I think that there is room for appropriate comment in the discussion.

⇒ The information about the two genotypes has been included in the background. However, there is no information on the genotype for the notified cases. Therefore, our data can not contribute to the discussions around the different assemblages.

We added a sentence to the conclusions: “To better understand the epidemiology of *Giardia*, it would be of interest to further study the role of the different genotypes of *Giardia lamblia* infective to humans.”

6) It is mentioned that lettuce may be contaminated by irrigation water contaminated by human waste. What about animal waste? Is this a plausible route? It is mentioned in the discussion that ‘assemblages in animals apparently do not cause symptoms in humans’ (with a reference) – but it should be noted that although the majority of animal infections are probably with genotypes which are not infective to humans (particularly in domestic animals and pets), this is certainly not exclusive, and genotypes of *Giardia* infective to humans have been isolated from a range of domestic and wild animals.

⇒ This point has been included in the discussion.
7) Comparison with autochthonous/travel-related case ratio from other European countries.

- The notification rates for giardiasis in European countries vary greatly (ref ECDC). and people returning from travel abroad probably import a significant proportion of cases in some countries (ref WHO). We added this information to the discussion.

8) Comparison of similar data from other similar countries to Germany would be very interesting, and more in depth discussion regarding risk factor studies from other comparable countries (I believe that there are a few from New Zealand).

- We found some information from other European countries reported by ECDC and a report from the United States and added this information on page 13, lines 6-7.

9) English language: whilst I appreciate that the first language of the authors is not English, I think that the quality of the paper would be very much improved if the text could be corrected by a native English speaker so that small grammatical errors, with which the text is peppered, could be corrected.

- The revised manuscript has been read and corrected by a native English speaker.

I enjoyed reading this manuscript and believe the data presented are interesting and though provoking and an important contribution to our understanding of giardiasis in industrialised countries. I commend the authors and look forward to seeing the final publication.

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:

I declare that I have no competing interests
Reviewer's report
Title: Characteristics and risk factors for Giardia lamblia infections in Germany
Version: 1 Date: 18 June 2009
Reviewer: Kristine Mørch
Reviewer's report:
This is an interesting and clearly written report. I have a few comments:

Discretionary revisions:
1. Abstract; Methods section.
It needs to be written more clearly that the case-control study included only autochtonous cases: I suggest to change 3. sentence to: “We conducted a case-control-study including autochtonous Giardia cases…
⇒ This has been changed in the methods section of the abstract (line 5).

2. Background section.
It is true that many Giardia cases are self-limiting, but reports for instance from the large Bergen outbreak in 2004, states that there was a high level of symptomatic infections in immunocompetent individuals, a high level of protracted infections, and a surprisingly high level of post-infectious fatigue and IBS. The last sentence in first paragraph may not be a correct description of the natural course of the infection.
⇒ A sentence on protracted infections has been included in the background section (page 3, sentence starting in the last line).

3.
Chronic infection and asymptomatic carriage could also be discussed, since chronic infection from previous travel could lead to underreporting of travel-associated cases in this study.
⇒ We provided more details on foreign travel in the last year prior to onset of symptoms (see methods, tables). We added to our limitations, that as we have no information on asymptomatic carriage in cases, it is well possible, that in some cases we looked for risk factors for the expression of symptoms following asymptomatic carriage rather than for risk factors related to the transmission of the pathogen. Therefore it is well possible that some cases became infected with Giardia long before our defined exposure period.

4. Results.
273 cases were included out of 326 interviewed cases, because these cases could provide a specific date of onset of symptoms. The 53 excluded cases, could potentially have had prolonged infection, possibly acquired on travel, leading to underreporting of travel related cases. This should be discussed as a limitation.
⇒ Only cases with a specific date of onset of symptoms were included in order to be able to specify the inquiry period. The fact that chronic infection and asymptomatic carriage from previous travel could lead to underreporting of travel-associated cases has been included in the discussion (see above).

5.
As many as 38% of the autochtonous cases had travelled abroad in the 12 months before onset of disease, and 52% had travelled in the control group; Did they travel to endemic or non-endemic areas, was the travel destination different in the groups?
The information on travel destinations in the year before infection/interview was included in the results (see results and tables). Travel destinations were classified according to geographical regions: Central America, South America, Asia, Northern Africa and sub-Saharan Africa were considered as high-risk areas and others (including Europe, United States of America and Australia) as low risk travel destinations for contracting Giardia infections. World travellers (e.g., airplane staff) were also considered to have a high risk of contracting Giardia infections (this has been specified in the methods section).

6. Could there be a selection bias of the 11 out of 131 cases not included in the case-control study?

The mean age of these 11 cases was 44.5 years, the youngest was 10, the oldest 80 years. Cases from both sexes were not included: five were male and six were female.

7. Male sex was found to be a risk factor, drinking tap water was not a risk factor. As many as 67% of cases were male, while there was a predominance of women among controls. After an outbreak in Bergen in 2004, a predominance of infection in women was reported, and amount of water consumed was a risk factor, possibly because young women exercise and drink much water as a cultural phenomenon to stay healthy. In this report tap water daily was reported, but not amount of water. This could be discussed.

Thank you for this comment. Our data can not contribute to the discussion about the infectious dose of giardia in tap water, as tap water was not a risk factor in our study. Furthermore, we do not have information on the amount of water consumed.

8. Was effect modification by gender on the association between tap water drinking and giardiasis tested in the statistical analyses?

Yes. We didn’t find effect modification by gender on this association. Stratification by gender yields significant odds ratios (“protective”) for males and females.

9. Tables
It would have been of interest to include p-values in all three tables.

We prefer to give the confidence intervals as they provide the information on statistical significance but in addition also information on the magnitude and precision of the effect.

10. All abbreviations should be written out in footnotes.

This has been checked.

11. Minor essential revisions:
Typographical error: Page 11, second last sentence, two dots at the end of sentence.
Page 13, 9. sentence: Products?

We have deleted one point. We did not change "produce", as it is used to imply that the products are fresh and generally in the same state as where they were harvested.
Level of interest: An article of importance in its field
Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Declaration of competing interests:
I declare that I have no competing interests
Reviewer's report
Title: Characteristics and risk factors for Giardia lamblia infections in Germany
Version: 1 Date: 2 July 2009
Reviewer: Ryan Lennon

Reviewer's report:

1. Major Compulsive
Page 15 - Limitations
The fact that women and people living in rural areas were more willing to participate should be in
the Results section, quantified if possible. How many controls refused and were replaced by other
controls? These limitations are important enough to receive more attention than a few sentences in
the Discussion, but should not be considered a barrier to publication.
➔ We provided more details on the selection of controls in the methods section. Extra figures in
the results section show the age and sex distribution of autochthonous cases and controls.
Quantification was not possible as notes were not taken consistently by all interviewers.

2. Major Compulsive
It is arguable that the study should have matched the controls based on both age and sex, since they
are both non-modifiable factors. This would have precluded an analysis of the association between
sex and infection, yet oversampling of women in the controls also sheds doubt on the significant
association of gender seen in the this analysis. This should be more clear in the limitations.
➔ As surveillance data in Germany have shown repeatedly that male persons more often fulfil the
Giardia case definition which requires lab diagnosis and the typical clinical picture, we wanted to
investigate a possible association of gender and symptomatic Giardiasis. Therefore we didn’t match for sex. Since women were unfortunately oversampled in the controls
the study provides limited information about an increased Giardia risk among males. This warrants
further epidemiological studies (eg, focusing on specific risk factors among males (see
conclusions page 15).

3. Major Compulsive
Table 1 - Residency
The levels for this category are mutually exclusive, hence this represents a single ordinal variable.
A rank sum test should be used to test for the difference and produce a single p-value (instead of 4
highly correlated p-values).
➔ Thank you for this comment. We have used the rank sum test and changed the information in
table 1.

4. Major Compulsive
Table 1 - Reason for laboratory examination
Similar to "Residency" this should be a single hypothesis test due to mutually exclusive categories.
However, this is not an ordinal variable, so a Pearson chi-squared or exact test would be
appropriate.
The categories are not mutually exclusive since one person could fulfill several reasons for laboratory examination. Therefore we did not change these results in table 1.

5. Major Compulsive
Table 2 - Residency
Again, this is a single variable. The analyst should select "<5000" as the reference category and then the odds ratios for the other categories would be referenced against that level. A single p-value for this variable may be calculated using conditional logistic regression with three indicator variables for three of the four residency levels. The overall model p-value is then the p-value of interest.

Since we have matched for county of residence, the chance for a control to live in a larger of smaller city depends on the special geographic characteristics of the county; therefore we decided not to include residency as a variable in the case-control study.

6. Major Compulsive
The authors may have to re-do their multiple regression model (Table 3) depending on the single p-value for this variable.

As the p-value of the variable is larger than 0.20, we did not include the variable in the multivariable regression.

7. Minor Essential
Page 10 - "p=0.011"
Change to "p=0.011" for consistency.
^) Page 10 - "> 100,000 inhabitants"
change "." to "," for consistency.

This has been changed.

8. Discretionary
Page 8 - "Controls were matched by county of residence..." Matching for county of residence might have been too strict as a rule. It would seem that any case that lived in a city>100,000 would be very likely to have a control also in that same city, since the city population might dominate the county. (Perhaps selecting within some distance radius, e.g. 100km. As a result, the association between residence and infection might be underestimated. This is also complicated by the fact that rural controls were more likely to participate, which pushes the bias in the opposite direction.

Thank you for this comment. Since we have matched for county of residence, the chance for a control to live in a larger of smaller city depends on the special geographic characteristics of the county; therefore we didn’t include residency as a variable in the case-control study.

9. Discretionary
Page 9 - "Of all notified cases, 326 (65%)..."
Change "notified" to "confirmed" since the 505 confirmed cases must be the denominator. I took notified to mean the 597.
The case definitions in the method section have been clarified. (lab confirmed symptomatic giardiasis). On page 8 (previously page 9) notified was changed to lab confirmed.

10. Discretionary
Page 14-15 - "Finding travel to the Indian subcontinent...”
The authors imply that travel to Indian is a risk factor for Giardia-infection. However, they would have to compare their percentages to the percentage of all travels with India as its destination. If 21% of all travels are to India, then there is no evidence of additional risk in such travel.

We found a reference stating that in 2008 for Germany only 6.6% of all foreign travel was to Asia (which includes India). Thus travel to India is associated with a higher risk of acquiring symptomatic giardiasis.

11. Discretionary
Table 2 - "MOR"
What does "MOR" stand for? Matched odds ratio? Mantel-Haenszel odds ratio?
We decided to use the abbreviation OR$^{\text{MH}}$, which stands for Mantel-Haenszel odds ratio, and changed the footnotes accordingly.

**Level of interest:** An article whose findings are important to those with closely related research interests
**Quality of written English:** Acceptable
**Statistical review:** Yes, and I have assessed the statistics in my report.
**Declaration of competing interests:**
I declare that I have no competing interests.