Author's response to reviews

Title: Outbreak of Cryptosporidium hominis following river flooding in the city of Halle (Saale), Germany, August 2013

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Author's response to reviews: see over
Covering letter – revised version of manuscript

Dear Madam, dear Sir,

we would like to thank the editors and reviewers for their helpful comments. We are happy to submit the revised version of the manuscript entitled “Outbreak of Cryptosporidium hominis following river flooding in the city of Halle (Saale), Germany, August 2013” by Gertler and coauthors, initially submitted in August 2014.

We addressed all the remarks of the reviewers. Please see the point-by-point description in the annexes (reviewer 1 and 2) below.

Corresponding author remains Dr. Maximilian Gertler, Robert Koch Institute, Department for Infectious Disease Epidemiology, Seestr. 10, 13353 Berlin, Germany,

Please change my corresponding email-address to mgertler@gmx.de

We hope, that our modification meet the reviewers’ and editors’ requests.

Best regards,

Maximilian Gertler
Annex 1: answer to the editors

-- Requesting Ethics statement

Research involving human subjects (including human material or human data) 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/en/30publications/10policies/b3/index.html). 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.

**Answer:** With regards to a desired ethical review process and informed consent of study participants, we have to state that: In accordance with Article 25 paragraph 1 of the “German Infection Protection Act” a formal ethical review process and approval was not required for these outbreak investigations. This is now included in the methods section of the manuscript.

-- Requesting consent statement:

Please state in the Methods section whether written informed consent for participation in the study was obtained from participants or, where participants are children, a parent or guardian.

**Answer:** Study participation was voluntary and based on informed consent. This is now included in the methods section of the manuscript.

Annex 2: answers to reviewer 1

This manuscript describes the response and analysis for a Cryptosporidium hominid outbreak following a flood event in an urban landscape. The work implicates contamination of human use areas persisting after flood waters withdrew, which is an important finding for public health officials in determining the safety of public land use in the wake of such events. The methods are sound and the report is generally straightforward. Some English usage could be corrected.

**Answer:** We improved the language.

A lack of emphasis on the source of the problem should be addressed. Was effluent from the treatment plant analysed before and after sterilization?

**Answer:** No, we focussed on the river water, we did not question the effectivity of the treatment plant. The treatment plant is downstream of the city of Halle and therefore not the primary source of the problem.
Species determination in environmental samples was ‘not successful’ and molecular typing showed 32/106 fecal samples were C. hominis. From these data the conclusions extrapolate to C. hominis from environmental sources were the cause of the outbreak? I think that conclusion should be softened/qualified a bit more than the paper presents.

Answer: We already include this limitation in the discussion “However, evidence of the environmental findings is limited as species identification based on pcr-amplification in samples from the river water was not successful.” - We now include the sentence: “Thus, no definitive source tracking to the river was obtained.”

Specific comments:
P2 L6 perhaps: "the river Saale overflowed its banks, flooded the part of the city centre in the food plain, and resulted in failure of the central sewerage system."
Answer: - changed accordingly.

P2 L16 Italicize Cryptosporidium (here and throughout)
Answer: - changed accordingly.

P4 L2-3 "The protozoa in the genus Cryptosporidium are important parasitic pathogens causing water and food contamination leading to diarrheal disease."
Answer: - changed accordingly.

P4 L4-5 "...recreational water exposure as well as drinking water consumption.
Answer: - changed accordingly.

P4 L6 replace 'or' with 'and'
Answer: - changed accordingly.

P4 Ln 12 "...species in the genus have been identified. Disease in..."
Answer: - changed accordingly.

P4 L13 delete '(C.)'
Answer: - changed accordingly.

P4 Ln17-18 "...chlorine disinfection as commonly applied in drinking water and sewage effluent treatments. Transmission takes place by oral ingestion. The incubation...."
Answer: - changed accordingly. We left out the word commonly out as chlorine disinfection was not applied in Halle and is often not applied in Germany.

P4 Ln 20-21 "...to notify the local public health department of patients with stool tests positive for Cryptosporidium by Federal Law."
Answer: - changed accordingly.

P4 Ln 22-23 "...annually between 2008 and 2012 in Germany. Outbreaks resulting in notifications were restricted …"
Answer: - changed accordingly.

P5 Ln 8 "...along the river and on the..."
Answer: - changed accordingly.

P5 Ln 16 insert comma after 'Bathing'
Answer: - changed accordingly.

P6 Ln 3 "...Halle. By comparison, the …"
Answer: - changed accordingly.

P6 Ln 18 delete 'initially'
Answer: - changed accordingly.

P6 Ln 21 "...test procedures, laboratory testing frequency, and ...."
Answer: - changed accordingly.

P7 Ln 3 Italicize Cryptosporidium
Answer: - changed accordingly.

P7 Ln 5 "...7 days of the 2 to 14 day incubation period..."
Answer: - changed accordingly.

P7 Ln 7 "...household, kindergarten, or school..."
Answer: - changed accordingly.
P7 Ln 10. I do not see any hypotheses stated in this section. Either remove the heading, or clearly state the hypotheses being tested by this study.

**Answer:** - This part explains our methods leading to the generation of hypothesis described in the results section. We would like to keep the heading.

P7 Ln 21. main hypothesized sources/transmission modes are not clearly stated in the above section.

**Answer:** - after revision, these are now stated in the results section, we preferred to have them here as they were based on RESULTS of the explorative interviews.

P8 Ln4 Kindergartens in Halle were informed

**Answer:** - changed accordingly.

p8 Ln 8 The informational material provided to the parents of control subjects contained

**Answer:** - changed accordingly.

p8 Ln 9 delete 'by the parents of controls'

**Answer:** - changed accordingly.

p8 Ln 16 replace 'comprise' with 'utilized'

**Answer:** - changed accordingly.

p9 Ln 10-12 ...August 2013), 13 from pools of public baths, 12 samples from 10 different sites int he river Saale including lateral branches and 5 samples from other bodies of water in Halle..."

**Answer:** - changed accordingly.

p9 Ln 20 parasite (no 's')

**Answer:** - changed accordingly.

p10 Ln 11 one 'L' in initial, ",... in Halle, 7 of 11 (64%) reported..."

**Answer:** - changed accordingly.

P10 Ln 12-15. This does not make sense to me, please rewrite and clarify

**Answer:** new phrasing: Initial interviews with laboratories reveal that the different institutes do not perform stool testing based on the same eligibility criteria and 3 out of 4 major laboratories active in Halle perform tests even only on specific request or in children or in
children <2 years (2/4 laboratories).

P11 ln 1-7 replace ; with , patients were male, 60/167
Answer: - changed accordingly.

P11 ln 3 ...and 21/60 (35%) of the secondary cases were from kindergartens.
Answer: - changed accordingly.

p11 ln "...4 of 16 diarrheal cases in children confirmed as Cryptosporidium by laboratory analysis was detected in …"
Answer: new phrasing: A cluster of 16 diarrhoeal cases in children confirmed as 
*Cryptosporidium* by laboratory analysis was detected in two kindergartens sharing the same premises. In the rural district Saalekreis which is encircling the city

p11 ln 5-7 "In the rural district of Saalekris which encircles the city of Halle, 23 cases were reported during weeks 27 through 47 compared to the annual average for 2008 to 2012 of 5 cases/yr."
Answer: - changed accordingly.

p11 ln 9 replace 'of' with 'with'
Answer: - changed accordingly.

p11 ln 11 "...pools that tested positive..."
Answer: - changed accordingly.

p11 ln 15 delete 'background'
Answer: - changed accordingly.

p11 ln 16 space after 'holidays.'
Answer: - changed accordingly.

p13 ln 4 'sampling'
Answer: - changed accordingly.

p13 ln 6 'Saale were confirmed'
Answer: - changed accordingly.
p13 ln 7 if there is nothing to report, do not make it a separate section, just a sentence int he methods would suffice.

**Answer:** - changed: – no separate section but methods described

p13 ln 17-19 "....later, after considering the findings front he exposure interviews and with the first tap water samples testing negative for oocysts. Two public swimming pools were positive and were closed."

**Answer:** - changed accordingly.

p17 ln 22" ...and advised against bathing..." add to results- some description of municipal agency operation of the waste water treatment facility and any Crypto testing done there. Any pre and post effluent sterilization tests available? Tracking to source seems by inference, rather than direct tracking to source. Samples form upriver of the treatment facility?

**Answer:** - The treatment plant is downriver of the city of Halle and as therefore was not regarded as the primary source of the problem in the city.

P14 ln 13 human fecal samples

**Answer:** - changed accordingly.

P14 ln 14 PCR confirmation was obtained for all but 8 (7%) if 114 samples recorded as positive for visual fluorescent antibody testing. The ability of PCR to determine the presence of Cryptosporidium in samples negative for visual analysis was not determined.

**Answer:** - changed accordingly.

P14 ln 18-20 ".evidence for exposure from human activities in the areas affected by the flood waters, including numerous playgrounds and the river beach, as a primary risk factor for the infection of children.

**Answer:** - changed accordingly.

P14 ln 22 capitalize Cryptosporidium here and throughout.

**Answer:** - changed accordingly.

p15 ln 21 which should be which
Answer: - changed accordingly.

p15 bottom- thus no definitive source tracking to the river was obtained, and the conclusion that contamination of the floodplain by the river flood is made by inference.

**Answer:** We now rephrased this paragraph. “However, evidence of the environmental findings is limited as species identification based on pcr-amplification in samples from the river water was not successful. Thus, source tracking to the river was limited to the findings of Cryptosporidium oocysts in the water.”

P16 ln 19 'weather'

**Answer:** - changed accordingly.

p17 ln 3 "such that activities can both can be" does not make sense, rewrite.

**Answer:** New phrasing: The Halle zoo was not overflowed by the flood. However, one of its exits leads directly to recreational areas in the floodplains, so that visits to the zoo and to the floodplain could easily be combined.

P 17 ln 17 main conclusion should be persistent infectious disease contamination of the floodplain after flooding

**Answer:** - yes, please compare new first phrase of conclusions

Figure 2 is too small, and legend type too small

**Answer:** both enlarged – please see new file
Annex 3: answers to reviewer 2

In the abstract mention that the River Saale is in Germany

Answer: – done

Background paragraph1 - Mention average and range of how long symptoms can last for

Answer: – done

Background para 2 - Cool environments are suited for survival as well. Could say a bit more about evidence and survival in river systems. For example rivers are demonstrated in many cases to act as the transfer medium from livestock to public water supplies. Cryto can also survive quite well in river sediments.

Answer: – done

Background para 2 - You may not be able to quantitify but would be worth highlighting that reported cases are only a small fraction of community cases

Answer: - possibility discussed in discussion para 1

Background last paragraph - It should read "outbreak and recommend"

Answer: - changed accordingly

Methods, environmental samples - Table 1 does not list the details of the environmental samples.

Answer: - table 1 only presents incidence in city districts, environmental samples is presented in table 3

Methods, species determination - Why did you only send 3 samples to the uk?

Answer: - when we decided to do so, we did not have left more than these 3 samples
(information added to manuscript)

Figure 1 - Would benefit from adding in the date of the flooding

Answer: - now added

Table 2 - Exposure descriptions need to be clearer to the reader

Answer: -- info added

Discussion para 6 crypto has a long survival in river water and sediments so the continual detection
may not represent new crypto being added to the river.  
**Answer:** -- *new phrasing:* As *Cryptosporidium* oocysts were shown to being able to survive long periods in river water sediments, our findings cannot explain if the high concentrations of *Cryptosporidium* oocysts detected up to five months after the flooding, were exclusively caused by the preceding flooding or if ongoing sewage seepage or spills in a city with ongoing *Cryptosporidium* transmission contributed to this.

Discussion para 9. Given the survival of crypto ingestion of soil, e.g. muddy hands, could also be a source of crypto. This could be made clearer.  
**Answer:** - *changed accordingly*

Discussion - In terms of limiations need to discuss that you did not ask in detail what the children were doing on the floodplain. Also none of your samples were soil samples. so whether this is some kind of bathing of soil ingestion remains unclear.  
**Answer:** -- *added:* We need to consider that the participants were not asked what precisely they did in the floodplain (e.g. bathing, eating, playing) and our findings do not provide information on what the exact mechanism of transmission actually is.

Conclusions para 2 - need a reference as to why flooding may become more common.  
**Answer:** -- *added: due to climate change and stream straightening*

Conclusion - Need to highlight the uncertainty as to what the exact mechanism of transmission actually is.  
**Answer:** -- *added in discussion as mentioned above*