Author's response to reviews

Title: Incidence and etiology of hemolytic-uremic syndrome in children in Norway, 1999-2008 - a retrospective study of hospital records to assess the sensitivity of surveillance

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Author's response to reviews: see over
Oslo, January 24, 2014

Dear Editor,

Thank you for considering our manuscript for publication. Please find attached our revised manuscript, entitled:

**Incidence and etiology of hemolytic-uremic syndrome in children in Norway, 1999-2008 – a retrospective study of hospital records to assess the sensitivity of surveillance**

By Jenssen GR, Hovland E, Nygard K, Bjerre A, Bangstad HJ and Vold L

All the comments from the reviewers have now been addressed and are listed below, with point-by-point responses as requested, listed chronologically after concerned paragraphs and sections (dots are referee comments, our responses are clearly shown with an underlined header (“Response:”). We took the liberty of placing references made in some of the referee comments with our references at the end of this letter, as well as highlighting concerned paragraphs and sections in the referee comments for navigational purposes. Besides the references and highlighting, the referee comments are shown as originally displayed in the feedback produced by you. We hope that our responses are to your satisfaction.

**Referee 1:**

- Although there are some potential subtle differences between the patients with HUS in Norway and those in other countries (20% vs 10% exposed to EHEC develop HUS, 20% vs 40% of atypical HUS is pneumococcal) most of the numbers are fairly small and fairly comparable.

**Response:**

Although not seemingly a comment requiring a response, we would like to point out one thing; while the 23% exposed to EHEC/develop HUS-ratio would differ from other countries, this is addressed in the manuscript as a result of probable underestimation/underreporting of EHEC cases, rather than a “true rate”. In the discussion it is pointed out that, while it is possible that this rate is higher in Norway, the true rate would likely match that of other countries, had the EHEC surveillance/reporting been optimal.

- I have no specific criticism other than the lack of potential wide-spread interest of this manuscript outside of Norway.

**Response:**

Thank you for a positive response. We do believe that the paper can be used when comparing data between different countries.

**Referee 2:**

- 1. The authors might want to discuss the literature dealing with “incomplete” cases of HUS that are somewhat analogous to their cases without laboratory confirmation.
This is an interesting suggestion. In literature, “incomplete HUS” has been defined as presence of two out of the three clinical criteria (acute renal failure, hemolytic anemia, thrombocytopenia) [1], or even acute renal failure with presence of one of the two latter [2]. Our cases without laboratory confirmation are generally not analogous to these cases, as they fit all three clinical criteria. There are three cases in which the renal function did not surpass the preset 80 µmol/l serum creatinine (1-15 year olds). They were, however, clearly elevated compared to normal function. In addition, the age of the patients was 1-2 years old. Our inclusion criteria set a strict limit between the serum creatinine level of <1 and >1 year olds, something that expectedly would lead to such cases. In these three, we consulted our pediatric nephrologist, who confirmed that inclusion as a complete HUS was indicated (taking into account the dynamic process that is renal failure and the patient age and renal parameters). We chose not to include patients who did not fit all criteria, and thus decided from the start to avoid “incomplete” forms of HUS.

2. The authors might want to comment on the lower incidence of STEC HUS in Norway.

Thank you for the suggestion. We do feel that this is covered in the 4th paragraph of the discussion, where the incidence of HUS in Norway is compared to other relevant European countries/studies and explained as a likely result of the low D+HUS incidence (and thus also STEC-HUS). The reason for this presentation is that in the studies we found for comparison, a national incidence was most often calculated for all HUS. It was thus natural to compare these numbers, as the distribution of D+/D- HUS is similar. In the paragraph a possible explanation to the low incidence is also discussed. **However, we do agree that the conclusion part should contain a paragraph about the compared incidence, as it has done in previous internal revisions. This has been added to the conclusion. Should there be any need to elaborate further on this topic, we would appreciate an indication on what specific aspects we should comment (see Discussions, 4th paragraph, 2nd sentence and Conclusions, 1st paragraph, sentence 1-2).**

3. It might be worth discussing the role of routine microbiological surveillance for STEC.

It would definitely be worth discussing. In discussions (paragraph 3), we have addressed the reasons for probable underestimation of STEC. These are also some of the main challenges in STEC surveillance. Because of this, countries such as France have previously used surveillance of HUS instead of surveillance of EHEC cases in their country in order to follow trends, detect outbreaks etc. [3]. This is because it is generally assumed that most (roughly 90%) of HUS cases are caused by EHEC [4-7] and that around 5-10 % infected with EHEC develops HUS [8]. HUS-diagnosis is not dependent of lab analysis, so that if you follow HUS cases you will get an overview of at least the most aggressive forms of EHEC (that causes HUS) in the area under surveillance regardless of your laboratory capacity to detect and notify EHEC cases. **We agree that this should be addressed more specifically, and have added some lines on the subject in the revised manuscript (see Introduction, 3rd paragraph, sentence 4-6).**

4. For clarity, it may be advisable to remove the material about atypical HUS which is generally not related to STEC but rather to abnormalities in the alternative pathway of complement.

This is an understandable suggestion. However, in our study, as well as assessing HUS surveillance, we wanted to assess the total burden of all clinical types of HUS. This article will be followed by an article describing the clinical aspects of both typical and atypical forms of HUS. It is thus necessary to present the epidemiologic differences of both forms here. We feel that we have separated the assessment of both in this article, while we remain open to the idea of removing it if necessary.
Referee 3:

Thank you for a thorough response – it was very useful and helped us greatly in correcting and clarifying certain issues.

Title:

• Sensitivity (also called the true positive rate) measures the proportion of actual positives which are correctly identified as such (e.g. the percentage of sick people who are correctly identified as having the condition). In this manuscript it is not possible to find out “the sensitivity of surveillance”. Because of this the title should be revised.

Response:

This must be a misunderstanding. We are here referring to the sensitivity of the surveillance system, not the sensitivity of diagnosing HUS in the hospitals. We therefore wanted to go back and check all hospital records for HUS cases (method 1) and compare to what was already in our surveillance data from our surveillance system (method 2). Although different by design and methods, the expression “sensitivity of surveillance” is used in a similar context as in what was done with EHEC surveillance in Germany in this study [9].

Please see CDC’s well known manual for evaluation of surveillance systems to check on sensitivity in the context of a surveillance system [10], quote: “From a practical standpoint, the primary emphasis in assessing sensitivity--assuming that most reported cases are correctly classified--is to estimate the proportion of the total number of cases in the community being detected by the system.” This is in our study done by comparing the number of patients who had been reported (the number of patients in MSIS) to the number of cases found with HUS when we went through the hospital journals. Because HUS was a rare disease in Norway in the beginning of the studied period, we also went through AKI’s to check for misclassifications.

Abstract:

Abstract - Background:

• 2nd sentence: “As EHEC surveillance is complex due to diagnostic challenges in detecting non-O157 infections, surveillance of HUS can be used as an indicator of the burden of EHEC infection.” This sentence could not be used as an expected scientific knowledge in the abstract. The aim in recent years is to prove the presence or absence of STEC in HUS patients [11]. Even in aHUS diagnosis, search of STEC is the first step [12].

Response:

We are not sure what the issue is here, as your comments underline our intent. What we are trying to say is that surveillance of HUS could be a way to indirectly do surveillance of EHEC: Countries such as France have previously used surveillance of HUS instead of surveillance of EHEC cases in their country in order to follow trends, detect outbreaks etc. [3]. This is because it is generally assumed that most (roughly 90%) of HUS cases are caused by EHEC [4-7] and that around 5-10 % infected with EHEC develops HUS [8]. HUS-diagnosis is not dependent of lab analysis, so that if you follow HUS cases you will get an overview of at least the most aggressive forms of EHEC (that causes HUS) in the area under surveillance regardless of your laboratory capacity to detect and notify EHEC cases. We have changed the sentence in question to better reflect this notion (see Abstract, Background, 2nd sentence).

As for using this phrase as an expected scientific knowledge, we feel that this is acceptable; even though identification of non-O157 EHEC has improved with new techniques, there are still challenges in making quick non-O157 identification easy accessible. In Norway, many microbiological
laboratories still prioritize the identification of O157 and O103 (historically considered to be the serogroups most likely to cause outbreaks) when presence of shiga toxin is found.

Abstract-Methods:

- 1st sentence: “In order to assess the sensitivity of EHEC and HUS surveillance and describe the incidence and etiology of HUS in children in Norway, we conducted a nationwide retrospective study collecting data from medical records from pediatric departments for the period 1999-2008 and compared them with data from MSIS.”

The authors do not have any “two methods” to apply to all the patients to find out the sensitivity of the method for diagnosing EHEC and/or HUS. It is not possible to calculate sensitivity in this cohort and I cannot see any information through the manuscript regarding this calculation.

Response:

We did have two methods; using medical records and using reported data to MSIS, which are two different approaches. And as previously mentioned, we are here referring to the sensitivity of the surveillance system, not the sensitivity of diagnostic methods on the patients.

Introduction:

- Is it necessary to write such a long introduction?

Response:

We do believe that the extensive length of the introduction is relevant for the better understanding of the complexity of the project.

- 2nd paragraph, 2nd sentence: “Shiga toxins are produced and released by EHEC bacteria and are an important part of EHEC-HUS pathogenesis.”

Stx is not an important part of the EHEC-HUS pathogenesis. Stx is the main cause of STEC HUS [13]. Recently instead of D+ HUS or EHEC HUS, STEC HUS is preferred. It is meaningful for description of etiology, pathophysiology and classification of HUS. I recommend the usage of “STEC HUS” throughout the manuscript.

Response:

The part about pathogenesis has been changed accordingly.

Thank you for the suggestion. We wanted to use EHEC, as this is the term used in surveillance of such infection in Norway. However, we do see the advantage of using STEC. Therefore “STEC-HUS” will be used instead of “EHEC-HUS”, and “probable STEC-HUS” for the 15 cases with typical clinical presentation and no verified STEC. However, we would like to keep D+HUS in the terminology. We feel this group has clear, defined inclusion criteria, and as it consists of both verified and probable STEC HUS cases, we feel that this best describes the group as a whole. An important point here is that we are comparing identified D+HUS cases to MSIS cases, especially when considering that the new criteria for reporting HUS in Norway are the presence of EHEC and/or a typical clinical presentation with diarrhea (or in essence: D+HUS), thus making the D+HUS category highly relevant. In addition, while it may be that trends are changing, D+HUS is still used in recent works [14-16], and more importantly in several of the articles available for comparison to our study period [7,17,18]. At the same time, we are aware of the developments in HUS terminology, and will acknowledge this in the revision (see Introduction, 1st paragraph, 4th sentence).

- 5th paragraph (last paragraph of introduction): The sensitivity issue (as told in the title and abstract-methods sections) should be re-evaluated.
Response:
Please see previous comments regarding this issue.

Methods:

Design and data collection:

• 1st paragraph 1st sentence: Why did the authors select the time interval as 1st Jan 1999-31st Dec 2008? 2006 is a special year, after that year the notification rules for HUS was changed. So it would be wise to choose a longer interval after 2006, to compare the results. Authors must explain the causes for the chosen time interval. If there is no special impediment, time interval should be arranged to increase the value of the manuscript.

Response:
It was decided to use a 10 year period for the study. The 2006 outbreak was the catalyst prompting the need for this study, as it was of interest to see what changes had been throughout this period. The data collecting part was done in 2009, when it was practically possible, due to the method used. As data was collected by travelling to all the different hospitals, it is at this moment not possible to change the data.

Case definition:

• 2nd paragraph: “A diarrhea-associated HUS (D+HUS) case was defined as a HUS case with either:
  - a clinical presentation of prodromal diarrhea, without verifiable causative etiology or
  - EHEC-HUS, defined as a HUS case with laboratory-verified EHEC-infection”
An infectious event, mainly upper respiratory tract infection or diarrhea/gastroenteritis, triggers onset of aHUS in at least half of patients [19], up to 80% in pediatric cohorts [20,21]. Interestingly, diarrhea preceded aHUS in 23% and 28% of patients in the French pediatric [20] and the Italian adult and pediatric [19] cohorts respectively, showing that the classification of HUS as (D+) or (D-) may be misleading and that post-diarrheal onset does not eliminate the diagnosis of aHUS. And also presence of prodromal diarrhea is not enough to diagnose STEC HUS. Because of this, evaluation of the medical records of the D+HUS patients after the first attack - to look for the recurrence of HUS - could be valuable to eliminate misdiagnosed aHUS patients triggered with diarrhea.

Response:
This is an important point. While we do acknowledge that this classification may be considered misleading, we would like to point out that the articles mentioned here show a percentage of aHUS cases triggered by diarrhea as 28 and 13 %, respectively. As aHUS is considered accountable for around (5-)10 % of all HUS cases [4], the total percentage of post-diarrheal aHUS would be quite low (here 2,8 and 1,3 % of all cases, respectively). From this one would consider the probability of finding one post-diarrheal aHUS in 15 patients with D+HUS (and no proven etiology) as quite low. In our study, all of the 15 cases were identified before 2007. The data on all of them were gathered in the summer of 2009 or later. Therefore, at least 1.5 years have passed (in most cases more) from time of diagnosis to the point of data extraction. Thus we had access to their long-term follow-up, which in neither showed recurrence of or clinical suspicion of atypical HUS. From this we consider it likely that they fit the D+HUS category. This has been clarified in the revised manuscript (see Results, Incidence and etiology of all types of HUS in children 1999-2008, 4th paragraph, 10th sentence).

As for the D+HUS terminology issue, please see previous response (2nd point in “Introduction” section).
Results:

Sensitivity of the HUS surveillance:

- **1st paragraph:** “In the period 1999 to 2008 27 HUS cases among children <16 years of age were notified to MSIS. One case from 2007 was identified and excluded from this study as it was initially admitted to a hospital abroad. In the same period, 102 cases of EHEC infection were notified in the same age group (Fig 1). Accordingly, 23% of EHEC- cases notified to MSIS in the period were cases with HUS.”

There are some conflictions which I could not solve in this paragraph.

1- Cases notified to MSIS: 27 HUS cases- 1 case excluded = 26 cases. In table 1 there are 25 cases? In table 2 there are 23 EHEC identified HUS cases. Then 2 cases after 2006 in MSIS must be EHEC-? EHEC status of 5 patients after 2006 should be clarified. Where is the 26th case?

Before 2007 all reported cases should be EHEC+ and the number is 20. However when I count figure 1, I found 21 EHEC+ HUS patients before 2007, and 26 EHEC+ HUS patients in total. Total D+? HUS: 25, 26, 27? and The number before 2007? 20, 21?

Response:
Thank you for the observation made regarding the missing MSIS case. You are indeed correct. In light of this, we went through the data thoroughly, and found it necessary to change and specify the following. First of all, there are in fact 28 cases of HUS reported to MSIS in the study period. Two cases were excluded from the MSIS numbers, as they were both originally admitted at a hospital abroad. In addition, the 28th case is a “double listing”, or more specifically; one of the two cases excluded was transferred between and reported from two major hospitals, and there are no traceable records of other patients with HUS at these hospitals in the year in question (2003).

This is also the missing 26th case. The reason for it being “missing” is that this was identified as a part of the MSIS cases in a second review, so the earlier revisions operated with only one excluded case. However, the later revisions have been correct, so I actually do not know how this was changed in the process, but it probably occurred in one of the final revisions were changes were made to the order of the results section. We have apparently missed this when submitting our article to you. This also answers your question regarding the “extra” case in Figure 1, as the extra case before 2007 is in fact one of the “double listed” admittances related to the “missing” case. Table 2, however, is correct concerning number of cases. To conclude, you are correct, it was missing – **it has now been corrected in the manuscript.** We also decided that, to avoid confusion, the 29th reported case, although not being an actual case, should be mentioned and explained (see Figure 1, figure, title and legend, and Results, Sensitivity of the HUS surveillance, 1st paragraph, sentence 1-2).

As for your second observation, thank you for highlighting this. We agree that the MSIS cases should be clarified as to identification of EHEC. In fact, all of the cases reported after 2006 are verified EHEC cases. There are three cases reported prior to the change in notification criteria, who are in fact not EHEC verified. Two of these were admitted to hospital just before the occurrence of an outbreak was acknowledged, the last one in the months following. All three had a clinical profile similar to those involved in the outbreak, so although EHEC was not verified, it is likely that they were reported as possible outbreak cases.

According to the numbers, this leaves one EHEC verified case prior to 2007. As we are able to retrace all of the above mentioned cases according to source of reporting listed in MSIS, we are also able to pin-point the remaining case. It is indeed a verified EHEC case from 2005. The reason as to why it was not reported is unknown, but according to the medical record EHEC verification was made in a late analysis of material, thus one could assume that it was simply overlooked. So in essence (especially related to several of the following issues raised; there are indeed 23 EHEC-HUS cases and 15 remaining D+HUS cases).

We have clarified the above mentioned in the revision (see Results, Sensitivity of the HUS surveillance, both paragraphs).
2. **Figure 1**: EHEC and HUS cases? Are these EHEC+HUS cases? Or D+HUS? Counts are confusing: 23 # 26?

**Response:**
This figure is supposed to show the distribution of EHEC and HUS cases reported to MSIS, the idea being that HUS cases reported to MSIS necessarily are also EHEC cases. However, in light of the previous question, this is slightly undermined. What we want to illustrate is the proportion of all EHEC cases resulting in HUS (as one has to report both separately according to Norwegian law). Therefore, we have now changed the figure to show the proportion of EHEC-HUS cases identified in total (and not reported to MSIS) in relation to all EHEC-cases reported to MSIS. This will reflect what we want to illustrate – that there probably are too few EHEC cases reported (thus the numbers we use in this figure are now the same as those we use to calculate and show that the proportion of EHEC-HUS cases compared to the common EHEC/HUS-ratio) (see Figure 1, title, legend and figure).

3- If the total is 26 (actually I included the 2 - which I suppose clinically diagnosed, EHEC- after 2007) than 26/102 is not 23%, it is 25.4%. If the authors did not use the 2 EHEC- HUS( is my finding correct?) than 23/102 is 22.5%.

**Response:**
Actually, your finding is not in itself correct, but your final answer is. This is our fault. The number used to calculate 23% (or 22.5%, as you show), is indeed 23 – but based on the 23 EHEC-HUS cases we have identified in our medical record search (and not the MSIS cases alone). This is the true amount of EHEC-HUS cases in the study period. Please see the previous answer(s) for a closer explanation, where we have explained the changes we have had to make (as three of the cases reported before 2007 are in fact not verified EHEC cases, but all of the reported after 2006 are).

4- In this part, as far as I understand authors are reporting D+ HUS. Then the title must be “Sensitivity of the D+HUS surveillance”. I don’t discuss sensitivity subject again. Definition of sensitivity is presented in the title chapter.

**Response:**
Thank you for pointing this out. Our thought was that one generally does not survey atypical HUS, thus making this evident, but it is a valid point to avoid misunderstandings. This has been changed accordingly (see Results, Sensitivity of the D+HUS surveillance). For our reply to the sensitivity issue, please see mentioned title chapter answer.

2nd paragraph: “Twenty of the HUS cases in MSIS were notified from the start of the study period up to and including 2006, and five after 2006. The corresponding numbers from the medical records search were 33 and five for the period 1999-2006 and 2007-2008, respectively (Table 1).”

1- 13 patients were found from the medical records: Were these 13 patients all diagnosed as D+HUS in the medical records? Were these 13 patients all diagnosed as D+HUS in the medical records or were there any patient found from the AKI group? How many criteria were found for each patient, how many were diagnosed with a pediatric nephrology consultation? A table would be helpful for the convincing of reader.

**Response:**
As explained in previous questions, there are 15 patients (D+HUS without EHEC verified; now “probable STEC”) found from the medical records. Three patients were found in the AKI group, two of which were included as D+HUS as they were both misdiagnosed (given the wrong ICD-10 code). One of these was also reported as an EHEC-HUS to MSIS (in 2007) and thus included as an EHEC-HUS (now “STEC-HUS”). The last patient found in the AKI group was an SP-HUS; also given the wrong diagnose code. All three matched the clinical criteria and were confirmed as HUS by our pediatric nephrologist. This has now been specified in the revision (see Results, Incidence and etiology of all types of HUS in children, 1999-2008, 1st paragraph, 3-5).
• 2- First sentence of this paragraph should be changed as “20 of the D+HUS cases…” They are not D- or aHUS? Throughout the manuscript there is confusion because of terminology. “Possible STEC HUS” should be used generally instead of D+; EHEC+ should be used specially where required.

Response:
As for the actual sentence; we understand the point being made, as – true – there are no atypical HUS cases there. However, the idea here is that, prior to actually finding the cases, one could not know for sure that there weren’t any atypical HUS cases misdiagnosed as D+HUS there, and thus “wrongly” reported, and thus we felt that using only HUS here is indicated. This is done on purpose to separate the MSIS cases from the medical record identified ones.

As for the terminology issue, “probable STEC-HUS” would be misleading as a replacement for the entire D+HUS group, as it contains both verified and probable EHEC-HUS cases. We have changed “EHEC-HUS” to “STEC-HUS” and the D+HUS cases without verified EHEC to “probable STEC-HUS”. This has also led to a number of changes, as the Norwegian notification criteria specifically state EHEC identification. In general, and especially after following your advice to change the terminology of these groups, we feel that these different groups are well defined in the study and that the group in question is specified accordingly. See previous answer to this for elaboration on the subject.

• 3- Figure 1 show 21, table 1 and this part shows 20? cases. It is very confusing and like a puzzle. I will propose a table like this. Then authors also will see the missing or excess cases 1999-2006 2007-2008
Total MISIS cases (text number 26, table 25) 20-21(EHEC+HUS) 5(There must be 2 possible STEC HUS) 25-26?
EHEC+HUS cases 20-21? (figure1: 21) 3?,5? (Text 3?,figure1: 5?) 23-24? (figure 1: 26, table 2: 23?, table 3: 23?)
Medical records D+HUS cases 33(there must be 13 possible STEC HUS) 5(There must be 2 possible STEC HUS) 38
Medical records D-HUS cases 9-x X=0? 9

NB: AUTHORS COMMENT – this is the formatting I was able to extract from the document provided – it originally shows a table with numbers according to lines listed above.

Response:
Please see previous answers for explanation to the differences in Figure 1 and Table 1. We want to emphasize the difference in what Table 1 and Table 3 illustrates. Table 1 compares the MSIS numbers with the medical record identified numbers; further rows here would downgrade the importance of this. Table 3, on the other hand, illustrates the distribution of cases (into EHEC-verified, D+, D- and total HUS cases). As the MSIS cases are not relevant to several of the aspects shown in this table, we consider a merging of these two unnecessary.

For the record – again, thank you, the table and your comments did help us greatly in finding the missing case.

Incidence and etiology of all types of HUS in children 1999-2008:

• In the 1st paragraph 1st sentence total case number is 47, in table 2 total case number is 48??

Response:
Thank you for pointing this out; you are correct, this is wrong. This error has occurred as we, at a point, excluded a patient and the content within the table was changed, but we apparently forgot the overall (as you will notice, it is not an automated table). We have changed the number to 47 (see Table 2).

- **1st paragraph 3rd sentence**: “Three cases, all D+HUS, were identified as HUS through the diagnostic code for acute kidney injury (AKI); N17.”
  What did the authors find to change the diagnosis for each patient?

Response:
All three patients had been given the wrong diagnose code, as they clearly fit the inclusion criteria and were acknowledged as HUS in their respective medical records. We were unsure whether this issue was supposed to be answered in the actual revision, but we clarified it to be sure. However, it is incorrect that all three of them were D+HUS – one was an SP-HUS. This has been changed (see Results, Incidence and etiology of all types of HUS, 1st paragraph, 3rd sentence).

- **4th paragraph last sentence**: “The remaining 15 cases presented with diarrhea, but without verified EHEC infection or etiology.”
  The follow up information of these patients should be clarified from the medical records. As I presented in case definition part diarrhea can precede aHUS in 1/3 of aHUS patients. Medical history of presence of diarrhea may cause a mistake.

Response:
We agree; this has been clarified in the revised manuscript. Please see our previous response (in the “Case definition” section) for elaboration on the issue of misdiagnosing aHUS cases.

D-HUS / atypical HUS:

  - Infection-induced
    Shiga toxin-associated: E. coli (STEC), Shigella dysenteriae Type 1 and other bacteria
    Invasive infection with Streptococcus pneumoniae (p-HUS)
  - Complement dysregulation
    Genetic
    Acquired
    - ADAMTS13 protease deficiency
    Genetic
    Acquired (including ticlopidine)
    - Defective cobalamin (B12) metabolism
    - Quinine
    - With Disease associations
      (Adapted from Besbas et al.)
  This classification is in accordance with etiology and avoids misdiagnosis of diarrhea preceded aHUS. I would recommend figure 2 to be like this:
  HUS cases: 47
  Infection induced
  STEC (EHEC+, D+) should be included in this group with pointing the suspects (how many bloody, how many watery? A few of them could be aHUS triggered by diarrhea. Follow up information of these patients with follow up time could decrease suspect)
  Strep
  Campylobacter
Response:
While we appreciate the suggestion in light of recent trends in classification, this would mean changing our inclusion criteria in retrospect, something we would not feel comfortable doing. The criteria for inclusion in both groups are clearly defined. This would also contradict some of the purpose of this study, to compare identified D+HUS to MSIS cases, as the new criteria for reporting HUS in Norway are the presence of EHEC and/or a typical clinical presentation with diarrhea (or in essence: D+HUS). In addition, as previously mentioned, while it may be that trends are changing, D+HUS is still to some degree used in recent works [14-16], and more importantly in several of the articles available for comparison to our study period [7,17,18].

Regarding the question concerning the D+HUS cases, please see previous comment on the subject. This can also be applied to the other issue raised here; the three “unknown D-HUS”. We did review their medical record within several years after the first episode. None showed recurrences, and might thus well have been STEC-associated, but were placed in this group according to our inclusion criteria.

In general, whether one chose the infection/non-infection or the D+/D- classification, arguments could be raised against either. For example, if a case of EHEC-triggered HUS where a genetic defect was identified, but where there were no recurrences – in which category would this best be placed when considering an infection/non-infection classification? In addition, the inclusion of other infection-incurred cases of HUS, especially in Europe, would “add few to the many”, that is a few “atypical” cases to the overrepresented “typical” cases. What practical impact would this have? In our case, it was natural to use the D+/D- HUS classification to accommodate the surveillance strategies currently in use in Norway.

Discussion

- **1st paragraph 1st sentence**: 2 years and 5 patients? Are these data really enough to conclude like this?

*Response:*
We assume the sentence in question here in the 4th in this paragraph (?) and that the conclusion in question is from the first paragraph in “Conclusions”, quote “The diagnostics have improved after the outbreak in 2006.”? This is probably a misunderstanding. In the first paragraph, we do not conclude on the subject, we simply state that the amount of cases corresponded to those identified after 2006. The phrase quoted from “Conclusions” is in fact not meant as a conclusion referring to the amount of cases before/after 2006. This simply reflects the fact that before the 2006 outbreak, most laboratories focused on the identification of O157 by cultures (and to a certain degree O103). After the 2006 outbreak, measures were made to implement diagnostic procedures based on PCR screening for the presence of Stx. Ring tests were sent out to all microbiological laboratories on a regular basis after 2006, and these showed improved diagnostic capabilities. **We agree that this should be clarified, which has now been done in the introduction (see Introduction, 4th paragraph, end lines).**

- **2nd paragraph 2nd sentence**: what about aHUS triggered by diarrhea??

**Response:**
Please see previous responses regarding this issue.
• **2nd paragraph 4th sentence:** “This may reflect that the HUS cases were caused by other etiological agents causing D+HUS that we were not able to recognize, but it is more likely due to problems with diagnosing EHEC in stool samples from HUS patients.”

Bacteriologically all EHECs are not STEC, but all STECs are EHEC and the cause of the disease (HUS) is STEC. Than the suspected point should be the HUS patients with D+ but unknown STEC status is really STEC HUS (according to authors EHEC HUS)? At this point such a retrospective data could be strengthen just with review of medical records of these suspected cases for the presence of recurrence, for misdiagnosis of aHUS.

**Response:**
We assume this is a spelling mistake – the reviewer means the other way round? All STEC’s are not EHEC’s. STEC simply means an E coli that produces shigatoxins. EHEC means a STEC that can cause illness in humans. Since the capability of the bacteria to cause illness in humans is not solely based on the ability to produce Shigatoxins, but also occurrence of for instance eae and other attachment mechanisms, other virulence factors, possibly serotype etc., this is a crucial issue for the Food Authorities. This is a big problem in the food sector, as the reviewer is probably aware of – if you detect a STEC in a batch of meat – is this necessarily a threat to human health and should the batch therefore be withdrawn from the market with all political and economic issues related to this? Or should one pursue the issue and isolate the bacteria and see whether there is reason to be believe that this is actually a bacteria with potential to cause disease in humans (ie an EHEC, not solely a STEC) based on additional criteria like presence of eae and other virulence factors etc. Since this is a sensitive issue with no good and clear answers so far, a lot of research is done in this area to better classify the bacteria according to threat to human health/ability to cause disease.

We also suspect there has been a misunderstanding here; we are saying the very same thing you are – that they are probably STEC-HUS. The sentence is rhetorical, as we cannot fully exclude other etiological agents, but still strongly suspect that STEC is the causative agent. Again, we had access to the follow-up of the patients; this has been addressed in previous points above and is now clarified in the revised manuscript.

• **2nd paragraph 8th sentence:** “In our study, we found that 64% of verified EHEC cases were non-O157 when the outbreak-related EHEC-HUS cases are excluded.”

I could not find this information in the text of results also in the tables. In table 3 there is 7 non-O157; 7/14 is 50%?

**Response:**
As for your first question, this information is found in Results; Incidence and etiology of all types of HUS in children 1999-2008; 5th paragraph 3rd sentence; quote “The remaining nine (64%) sporadic cases were non-O157.”

As for your second question, in Table 3, there are 7 defined non-O157 and 2 unknown serotypes. These remaining two were examined by laboratories testing for O157 and O103 only, and were specified in the medical journal as presence of an EHEC with stx, but excluding O103/O157-origin. We chose to classify these two as “unknown”, but accept that this might be misleading. **We have changed the group name to “non-O157/O103” accordingly.**

• **2nd paragraph 9th sentence:** “However, O157 was the most frequently isolated serogroup causing sporadic HUS in Norway”

Isn’t this sentence contradicts with 2nd paragraph 8th sentence?

**Response:**
We do not see why this would be contradictory. Table 3 mentions all serogroups identified, with 5x O157, 2x O103, 2x O145, 2x O26, 1x O87 and two unknown. Granted, with 15 suspected cases without verified etiology and only 14 verified sporadic cases, this is not conclusive, but it remains “the most frequently isolated serogroup”.


• 3rd paragraph 1st sentence: 23%? Is it correct? (Detailed discussion is in results part)

Response:
Yes, please see mentioned comment in results part. It is also important to see this in relation to the rest of the paragraph, were it is discussed as an unrealistic number (which is the point).

References:

• References should be written according to the rules. Month and journal number should be erased. The authors copied the references just from pubmed summary (text).

Response:
We have not copied any references from pubmed summary. We used the style file “Biomed Central” from the “Authors instructions” page on your website, which for some reason initially leaves most references in need of retyping. As to why the references have been formatted back to their initial form before submission, we have no good answer. References have now been corrected. Please let us now if there is need of further retyping/proofreading.

Editorial Requirement

• Please update your ethics statement to include the name of the specific ethics committee that approved your study.

Response:
We have updated the ethics statement accordingly (see Ethical considerations, 1st sentence).

The final manuscript has been seen and approved by all authors.

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Best wishes,
Gaute Reier Jenssen

Reference List


