PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Hospital-based surveillance of rotavirus gastroenteritis among children under five years of age in the Republic of Ivory Coast: A cross-sectional study
AUTHORS	AKOUA KOFFI, Chantal ; ASSE, Vincent; Jean Jacques, Yao Atteby

VERSION 1 - REVIEW

REVIEWER	Prof. George E. Armah Senior Research Fellow NMIMR, University of Ghana Ghana I have no competing interest
REVIEW RETURNED	22-Jun-2013

THE STUDY	1. The authors cite differentiation between wild type and vaccine strain G! viruses but do not provide methodology for detecting vaccine strain G1 rotaviruses. 2. There has been an update to circulating rotavirus strains from the WHO rotavirus surveillance studies across Africa the authors can source for more data.
RESULTS & CONCLUSIONS	1. Some of the contents of the Tables (Tables 2 and 3) are not necessary or helpful to the paper. The recommendation is to to edit these Tables. 2. The language can be improved with assistance from a native speaker.
GENERAL COMMENTS	Comments to be addressed: 1. Pg.7, Line 12: reference 3 is out of date and authors encouraged to refer to the much more recent publications 2. Pg. 10 line 14: were the samples stored refrigerated or frozen? 3. Pg. 1o line 22: provide location of the regional laboratory 4. Pg 10 line 32: did they test vaccine strain? If so authors should provide the methodology or reference 5. Pg 10 line 35: remove "most prevalent" 6. Pg 10 line 38: authors should explain and be more clear on what the term 'aberrant or unclear pattern' 7. Pg 10, line 51; whilst age of onset (of what) can be recorded, the seasonal distribution can only be determined by analysis of data. This sentence should be rephrased and put in the proper context. 8. Pg. 12, Table 1: Delete row 48-51: live in Abidjan ……. 9. Pg 13, Line 4-12; the Paragraph is not clear, it should be rephrased to bring out more clarity. 10. Pg 13, Table 2: the column with the heading RV missing is not necessary and should be deleted 11. Pg 14, line 30: We can be more certain on seasonality of the

	surveillance was for at least 18 months. This study was for only 13 months and hence can not be very exact in their description of seasonality. They should rather discuss the diarrhoea incidence pattern and the highest month of detection etc. 12. Pg. 15, Table 3: what is the rationale for discussion based on events before hospitalization and the treatment phase. In my opinion this is not relevant and the table should contain events at enrollment into the study. Issues relating to the treatment given and the responses could be discussed in the text. 13. Pg. 17, line 23: Why were only 53 samples genotyped, on what basis were these selected and why were the rest left out. 14. Pg. 18: the prevalence (line 22) of 27.9% observed in this study can not said to be comparable (line 44) as being claimed to the 90% observed in Nigeria (line 37). Pg. 19 paragraph beginning line 44 is not clear and should be re-written,

REVIEWER	Bakeera-Kitaka, Sabrina Makerere University Medical School, Pediatrics
REVIEW RETURNED	15-Jul-2013

GENERAL COMMENTS	The authors should be encouraged to do a WAZ and HAZ analysis on all the patients,and then compare with those who were positive for rotavirus. In addition,one would wish to know if Ivory Coast is already offering Rota virus vaccine and if there was any impact on the prevalence rates.

VERSION 1 – AUTHOR RESPONSE

**You can remove the trial reg section from the abstract as it does not apply.
Response: Trial registration details have been removed.

**Please include the study design (cross-sectional study) in the title.
Response: The study design has been included in the title.

**You don't need to describe cross-sectional as observational. However, the abstract suggests this is a cohort study (talk of enrollment to a prospective study - there's no such thing as a prospective cross-sectional study).
Response: The term ‘prospective’ was removed from the abstract.

**Did you have institutional ethics approval? If so, please state specifically which institution approved the study. If not, please state why this was not necessary.
Response: The study was approved by the national Independent Ethics Committee and this information was added to the Methods section.

**Please make the methods clearer to show whether you were using routinely collected data/samples or samples collected specifically for the study.
Response: Based on the comments, the following modifications were made in the Methods section ‘Stool samples collected within 4–10 days of the onset of GE from all enrolled children as part of the study procedures were labeled, stored at a temperature of -20°C to -70°C and then transported to the Pasteur Institute, Abidjan, within 72 hours of collection for testing.’

**The research question and conclusions discuss the results as if they are applicable to the whole country.
Response: The study was conducted in 5 references hospitals in Abjidan which cater to over 1,300 AGE cases annually, as per the WHO generic protocol. Although the entire Republic of Ivory Coast population was not considered, we believe the sample size chosen is indeed a representative of the whole country.

**Can the acknowledgements be clearer about the 'contributions' made by the GSK staff?
Response: The acknowledgement section was modified and all the contributions of GSK were described.

Comments from Reviewer: Prof. George E. Armah

1. The authors cite differentiation between wild type and vaccine strain G! viruses but do not provide methodology for detecting vaccine strain G1 rotaviruses.
Response: The methodology for detecting wild-type and vaccine strains was similar and has already been published by van Doorn LJ et al., J Clin Microbiol 2009;47:2704–12. The methodology followed in our study was similar to the one in the indicated reference. Therefore, we have already cited this reference in the text and the full description was not included to avoid redundancy.

2. There has been an update to circulating rotavirus strains from the WHO rotavirus surveillance studies across Africa the authors can source for more data.
Response: The indicated reference from WHO African rotavirus surveillance was included in the discussion section under the relevant topic.

3. Some of the contents of the Tables (Tables 2 and 3) are not necessary or helpful to the paper. The recommendation is to edit these Tables.
Response: Table 2 was removed from the manuscript and has now been presented as a figure for easy comprehension of the presented data.
Table 3 was modified to present only characteristics at admission.

4. The language can be improved with assistance from a native speaker.
Response: The manuscript was reviewed by a native English Speaker and her name has been included in the acknowledgement section.

Specific Comments:

1. Pg.7, Line 12: reference 3 is out of date and authors encouraged to refer to the much more recent publications
Response: The indicated reference has been replaced by a more recent reference.

2. Pg. 10 line 14: were the samples stored refrigerated or frozen?
Response: Based on the comments, the following modifications were made in the Methods section ‘Stool samples collected within 4–10 days of the onset of GE from all enrolled children as part of the study procedures were labeled, stored at a temperature of -20°C to -70°C and then transported to the Pasteur Institute, Abidjan, within 72 hours of collection for testing.’

3. Pg. 10 line 22: provide location of the regional laboratory
Response: The location of the regional laboratory was updated as ‘Ivory Coast National laboratory for rotavirus, which is affiliated to the WHO regional reference laboratory located in Accra-Ghana [West Africa], at the Noguchi Memorial Institute for Medical Research.’

4. Pg 10 line 32: did they test vaccine strain? If so authors should provide the methodology or reference
Response: Indeed, the vaccine strain was tested in the study. The methodology for detecting wild-type and vaccine strains was similar and has already been published by van Doorn LJ et al., J Clin Microbiol 2009;47:2704–12. This methodology followed in our study and has already been cited in the text and the full description was not included to avoid redundancy.

5. Pg 10 line 35: remove "most prevalent"
Response: The term ‘most prevalent’ was removed from the indicated section.

6. Pg 10 line 38: authors should explain and be more clear on what the term 'aberrant or unclear pattern'
Response: The rotavirus genotyping was based on PCR followed by reverse hybridization on a strip (one for G/VP7 and one for P/VP4). This strip comprises of probe line on a membrane, and after hybridization, a pattern of lines is visible. Each genotype is recognized by a specific pattern. If an unclear pattern was observed, we analyzed the PCR product by an alternative method, i.e., sequencing. The term ‘aberrant’ meant ‘unclear’, which has now been removed from the text in order to provide more clarity to the statements made in the text.
The indicated section was modified as ‘A random subset of RV positive stool samples were subsequently tested using reverse transcriptase-polymerase chain reaction (RT-PCR) of the VP7 and VP4 genes followed by reverse hybridization on a strip at DDL Diagnostic Laboratory (Rijswijk, the Netherlands) to identify G and P genotypes and to differentiate the presence of wild-type G1 RV from the vaccine strain virus.[23] A visible pattern is observed on the strip after hybridization which is specific to each genotype. As a complementary tool, sequence analysis was performed to identify the genotypes for samples that yielded an unclear pattern that could not be recognized. The resulting sequence was analyzed by BLAST search against the GenBank database to determine the RV genotype.[23].’

7. Pg 10, line 51; whilst age of onset (of what) can be recorded, the seasonal distribution can only be determined by analysis of data. This sentence should be rephrased and put in the proper context.
Response: The indicated section was modified as ‘The occurrence of severe dehydrating RVGE (based on the Vesikari scale,[22]) administered treatment (including intravenous [IV] re-hydration), duration of hospitalization and outcome were all reported, together with the seasonal distribution of RVGE and the occurrence of RV types.’

8. Pg. 12, Table 1: Delete row 48-51: live in Abidjan
Response: The indicated row was deleted from Table 1.

9. Pg 13, Line 4-12; the Paragraph is not clear, it should be rephrased to bring out more clarity.
Response: The document was reviewed by a native English speaker and indicated paragraph was modified for clarity.

10. Pg 13, Table 2: the column with the heading RV missing is not necessary and should be deleted
Response: Table 2 was removed from the manuscript and has now been presented as a Figure. The information on RV missing was not included in this Figure.

11. Pg 14, line 30: We can be more certain on seasonality of the surveillance was for at least 18 months. This study was for only 13 months and hence can not be very exact in their description of seasonality. They should rather discuss the diarrhoea incidence pattern and the highest month of detection etc.
Response: Our study describes the RV circulation during only one season. This has been mentioned in the limitations of the study. The following text was included in the limitation section under

Discussion ‘Lastly, firm conclusions regarding the seasonality of RV and the variation in circulating RV strains could not been drawn as the surveillance activities were conducted for just one year following recommendations from WHO.[43] However, routine surveillance is warranted as rotavirus circulation might vary from one calendar year to another.’ Additionally, diarrheal incidence pattern and the proportion of RV positive cases among these have already been described in text and Figure 2.

12. Pg. 15, Table 3: what is the rationale for discussion based on events before hospitalization and the treatment phase. In my opinion this is not relevant and the table should contain events at enrollment into the study. Issues relating to the treatment given and the responses could be discussed in the text.
Response: Table 3 was amended based on the comments provided. Only characteristics at enrollment were included along with the Outcomes and Nutritional status.

13. Pg. 17, line 23: Why were only 53 samples genotyped, on what basis were these selected and why were the rest left out.
Response: For quality purposes, only a random subset of RV positive stool samples was typed at DDL Diagnostic Laboratories. The relevant section in the Methods was modified as ‘A random subset of RV positive stool samples were subsequently tested using reverse transcriptase-polymerase chain reaction (RT-PCR) of the VP7 and VP4 genes followed by reverse hybridization on a strip at the DDL Diagnostic Laboratory (Rijswijk, the Netherlands) to identify G and P genotypes and to differentiate the presence of wild-type G1 RV from the vaccine strain virus.[23] A visible pattern is observed on the strip after hybridization which is specific to each genotype. As a complementary tool, sequence analysis was performed to identify the genotypes for samples that yielded an unclear pattern that could not be recognized. The resulting sequence was analyzed by BLAST search against the GenBank database to determine the RV genotype.[23].’

14. Pg. 18: the prevalence (line 22) of 27.9% observed in this study can not said to be comparable (line 44) as being claimed to the 90% observed in Nigeria (line 37).
Response: The indicated statements in the comment above are of two different comparisons made. First, the 27.9% prevalence in a previous study in Ivory Coast was comparable to the RV positivity rates among hospitalized and visiting ER (30.1% and 26.9%, respectively) in the present study. On the other hand, the 90% RVGE positive cases in Nigeria were among children aged <2 years which is comparable to our study where over 90% children with RVGE were aged up to 23 months. However, minor modifications have been made in the text to reflect this.

Pg. 19 paragraph beginning line 44 is not clear and should be re-written
Response: The indicated paragraph was modified as ‘Data presented in this study highlight the magnitude of RV-associated disease burden and might allow policy makers to determine the need to implement of prevention strategies in the Republic of Ivory Coast. Indeed, the inclusion of a RV vaccine, as a prevention strategy, might help to reduce the burden of AGE and AGE-associated hospitalizations..’

Comments from Reviewer: Sabrina Bakeera-Kitaka

The authors should be encouraged to do a WAZ and HAZ analysis on all the patients, and then compare with those who were positive for rotavirus.
Response: The WAZ analysis was indeed done for all enrolled children and the results are presented in text and in the Table (Classification of nutrition). To clarify this, the methodology was added in the Analysis section and the following footnote was added to Table 3 ‘Note 2: The nutritional status was derived from the weight for age z-score (WAZ)’. However, HAZ was not performed in this study.

In addition, one would wish to know if Ivory Coast is already offering Rota virus vaccine and if there

was any impact on the prevalence rates.
Response: As indicated in the Discussion section (In the Republic of Ivory Coast, RV vaccines are available in the private sector; however, no RV vaccine is currently included in the universal mass vaccination program), RV vaccine is not included into the universal mass vaccination program and hence data on the impact of these vaccines is not available.

VERSION 2 – REVIEW

REVIEWER	Prof. George E. Armah Senior Research Fellow NMIMR, University of Ghana Ghana I have no competing interest
REVIEW RETURNED	20-Aug-2013

The reviewer completed the checklist but made no further comments.