Author’s response to reviews

Title: Viral etiology and seasonality of influenza-like illness in Gabon, March 2010 to June 2011

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Author’s response to reviews: see over
Responses to the reviewer 1

Title: Viral etiology and seasonality of influenza-like illness in Gabon, March 2010 to June 2011

Reviewer: Hannah Moore

Major Compulsory Revisions

1. The authors do not appear to have a solid conclusion from this study. Clearly the higher frequency of adenovirus detection in this study should be given more prominence. Both the Conclusions section of the abstract and the manuscript text need to be clearer. For example, the conclusions in the main text of the manuscript state that the responsible viruses were similar to those reported on other continents, however in the discussion it is highlighted that adenovirus was detected in much higher frequencies than other studies.

We added the following paragraphs in the section Conclusions of the abstract and manuscript text.

Abstract (page 3): “Like most studies in the world, the virus PIVs, EV, RSV, Influenza virus, HRV were predominant among children under five years old in Gabon. An exception is made for adenoviruses which have a high prevalence in our study. However adenoviruses can be detected in asymptomatic persons.”

Conclusion (page 16): “The responsible viruses were similar to those reported on other continents except for adenoviruses whose prevalence is higher.”

We added a paragraph in the section discussion in order to explain the high prevalence of adenoviruses.

Page 13: “In young children, asymptomatic porting has been shown for some respiratory viruses such as adenovirus or rhinovirus [53]. More specifically, asymptomatic adenovirus infections are common in young children [53, 54]. In our study, adenoviruses have a predominant prevalence. They are also common in co-infection. Asymptomatic carriage of the virus among young people and their high prevalence in co-infection suggested that they might not be the cause of the disease.”
2. The seasonal distribution analysis needs some further clarification and perhaps further analysis. It is unclear what the chi-squared tests for the seasonality analysis are actually testing. There is clear seasonality as depicted in Figure 2 for all viruses except adenovirus so testing for a linear trend as the authors appear to have done is not all that useful.

For each virus, we compared the overall prevalence obtained during the dry seasons compared to those obtained during the rainy season to determine if there was a significant difference between the prevalence obtained between these different periods.

3. The co-infection analysis also needs some thought. In the tables the authors display the proportion of each individual pathogen involved in a co-infection but no actual data on the prevalence of certain pathogen pairs. The last paragraph of the results state that PIV3/Adv, PIV4/Adv and PIV1/PIV2 pairs were frequent but provide no evidence or numbers and then state that the most common pathogen pairs were Adv/EV and EV/HRV with prevalence’s of 14%. Additionally, in the beginning of that paragraph, the authors state that AdV was one of the most common viruses involved in co-infection but then also state that it was the main virus involved in single infection. I would suggest a re-write of this paragraph and provide some numbers, perhaps in a matrix table to highlight to the reader the most common pathogen pairs.

The number of adenoviruses diagnosed was higher both in single and coinfections. However among all viruses in coinfections, adenoviruses and enteroviruses were the most frequent. We added a table (table 5) with the pathogen pairs and we modified the paragraph about coinfections in the section results (page 11):

“The most common dual co-infections were EV/HRV 28/174 (16.1%) and AdV/EV 25/174 (14.4%) (Table 5).”

4. Discussion, fourth paragraph. The authors state that they found no difference in clinical severity between patients with single and multiple virus detections. There was no data displayed to back this up. Either the authors need to provide some data or explain in further detail in the discussion stating data not shown.
We modified the last paragraph of the discussion (page 15): “Several studies showed coinfections were associated with an increase in disease severity, but the majority of studies didn’t show clinical differences between single and coinfections [69, 70]. Thus we compared the clinical data of the patients in single infections with co-infections (data not shown). Our findings do not allow us to conclude that there was a difference in clinical severity between patients with single and multiple infections.”

5. Discussion. Can the authors shed any light on why there would be any differences between the different geographical areas in particular for RSV? Is this linked to different climates between the various regions?

No data proved the existence of different climates between regions. But the ecosystem is different. Northern and Southern of Gabon are composed of forest and savanna. We added a paragraph in the discussion to explain the differences between the different geographical areas.

Page 14: “Some viruses are predominant in urban areas, others in rural areas. Some studies about epidemiology of RSV in Kenya show that the all-cause community-based incidence of SARI among infants is higher in the rural study site, compared to the urban study site [62, 63]. In Gabon, Franceville which is a semi rural area has a lower density of population than Libreville and the fact that there is only one regional hospital where patients go, explain why viruses associated with infections in very young children such as the SIB or RSV are more important in this region where the spread would be faster.”

Minor Essential Revisions

1. Background first paragraph. Is SRAS-CoV meant to be SARS-CoV?
Ligne 38 page 4: The word “SRAS-CoV” was replaced by “SARS-CoV”.

2. Reference 35. This reference appears to be missing details.
This reference has been completed (reference 25 now).
3. Figure 1. These graphs are on the same scale as Figure 2 and yet Figure 1 is labelled temporal distribution and Figure 2 is labelled seasonal distribution. The authors need to be consistent on their interpretation and presentation of the data. The legend to the figure 1 was modified: “Temporal distribution of ILI by town”. The figure 1 showed the variation of number of cases throughout the study in the four towns. Whereas the figure 2 showed the prevalence of the different viruses diagnosed throughout the study in children under five years old.

4. I would combine Tables 1 and 2 into a single table. I would also provide a more descriptive title to the table. We combined tables 1 and 2. We changed the title: “Demographic characteristics and prevalence of viral infections in ILI patients.”

5. Tables 1-3. ]0-5[ should be [0-5] The age group was composed of children < 5 years old. So we changed ]0-5[ by [0-4] in the tables 1 and 2.

6. Tables 1-3. The final column should be labelled 95% CI and 95% CI (%) which is confusing to the reader. We modified the final column. We label 95% CI in the tables 1-4.

7. Tables 3-5. These tables would greatly benefit from having a foot note of the abbreviations for the different viral pathogens. In each table, the order of the pathogens seems quite random. I would suggest they be listed in order of declining prevalence. We added a foot note of the abbreviations for the viral pathogens and we changed the order of the pathogens in order of declining prevalence (Table2-4).

8. P-values should only be reported to 2 dp. We modified the p-values in order to be reported to 2 dp.

9. Table 4 and 5. Remove the final 2 columns as they do not add any valuable information and are repeats of information in earlier tables. The last two columns were different in the three tables. The prevalence values varied due to the total number of patients. This explains why we chose to integrate them in the three tables.
Discretionary Revisions

1. It would be useful for the reader if the authors could provide some information on the proportion of ILI patients that consented to have a specimen collected rather than only stating the number of specimens that were collected.

Patients with ILI were automatically enrolled in the study. Thus the number of specimen collected correspond to the number of patients that consented to have a specimen collected.

2. Results, Epidemiological findings. I would delete the sentence “Children less than 5 years old represented 77.8% of patients” as the age distribution is mentioned at the end of that paragraph.

We removed this sentence (page 8).

3. Results, Virus identification. I don’t feel that stating the total number of viruses that were identified (869) is very useful. Considering deleting.

We removed the number of viruses (869) in the sentence (page 9).
Responses to the reviewer 3

Title: Viral etiology and seasonality of influenza-like illness in Gabon, March 2010 to June 2011

Reviewer: Adam Meijer

Reviewer's report:

There are no page numbers and line numbers to refer to, therefore page numbers are taken as the pages appear in the downloaded manuscript and lines are not mentioned.

We mentioned the page numbers and line numbers.

Major Compulsory Revisions

1. In the results section frequently most of table contents are repeated. If the information is included in a table limit the text to the most important results in the table without listing the data again in the text.

We removed in the text, informations that were contained in table 2 (page 9).

2. Page 2, Abstract: clarify whether the PCR was one multiplex PCR for all pathogens or whether multiple single target PCR assay were used.

We modified the section methods of the abstract (page 2):

“Nasal swabs were sent for analysis to the Centre International de Recherches Médicales de Franceville, where they were screened for 17 respiratory viruses in a multiplex real-time reverse transcription polymerase chain reaction for all pathogens according the following pairs: adenovirus / parainfluenza virus 4, respiratory syncytial virus / human metapneumovirus, parainfluenza virus 1 / parainfluenza virus 2, pandemic influenza virus A (pH1N1) / seasonal influenza virus A (H1N1, H3N2) / seasonal influenza virus B, human coronaviruses 229E / OC43, human coronaviruses NL63 / HKU1, rhinovirus / human parechovirus, and enterovirus / parainfluenza virus 3.”
3. Page 2, Abstract and page 7, Laboratory analysis: clarify what exactly the targets were for the influenza virus PCR assays. From the references they are: influenza H1pdm09 (hemagglutinin gene) and generic influenza type A (matrix gene). The generic will detect any influenza type A influenza virus and not just A(H1N1) and A(H3N2). Specimens positive for H1pdm09 should also be positive in the matrix PCR. Should be corrected throughout the manuscript.

There was a mistake on the reference concerning the PCR influenza virus A that we used. We modified the reference (reference 31) (page 7). For the diagnosis of influenza A viruses, we did not use a generic type A PCR to diagnose all type of influenza A. The target was the matrix gene. But we used two specific PCR, one using a probe specific of seasonal influenza A (H1N1, H3N2) and the other using a probe specific of pandemic influenza.

4. Throughout manuscript use the WHO naming for the pandemic influenza virus: A(H1N1)pdm09 or if only the H1, use H1pdm09.

We changed “influenza virus A p(H1N1)” or pH1N1 by A(H1N1)pdm09 throughout the manuscript.

5. Page 2 Abstract, and Discussion: In specimens from ILI patients less than 5 years old multiple pathogens can be detected. However, there are many publication showing that in specimens from symptomless persons of the same age also one or more of these viruses can be detected. Although the paper is not on causality, this issue should be discussed as this is especially an issue in young children.

We added the following paragraph about virus diagnosed in asymptomatic children in the section Discussion (page 13):

“In young children, asymptomatic porting has been shown for some respiratory viruses such as adenovirus or rhinovirus [53]. More specifically, asymptomatic adenovirus infections are common in young children [53,54]. In our study, adenoviruses have a predominant prevalence. They are also common in co-infection. Asymptomatic carriage of the virus among young people and their high prevalence in co-infection suggested that they might not be the cause of the disease.”
We added this sentence in the abstract (page 3):

“An exception is made for adenoviruses which have a high prevalence in our study. However adenoviruses can be detected in asymptomatic persons.”

6. Page 4, case definition ILI. As it reads all symptoms should be present. Should it be fever and one or more of the other symptoms?

According to WHO, the case definition of ILI is fever $\geq 38^\circ$C and cough, or fever $\geq 38^\circ$C and sore throat).

7. Page 4, Background and Discussion. There are large overlaps in these sections as similar items are reviewed in these parts of the manuscript. The Background part could benefit of shortening and only mentioning that what is needed to explain why this study was performed. In the Discussion section it could be made more clear how the results described for Gabon correlate to the results achieved elsewhere and what this means for the situation in Gabon. Is the situation in Gabon unique or roughly similar to other parts in the world with similar or other climate etc.

We modified the background and the discussion.

We removed the following paragraph (background page 5) and we added them in the discussion (line 259 page 13):

“In Brazil, ILI sentinel surveillance from 2000 to 2010 showed that the viral etiology of ILI was respiratory syncytial virus (RSV) in 31% of cases, influenza A in 26%, adenovirus (AdV) in 12%, parainfluenza 2 (PIV-2) in 9%, parainfluenza 3 (PIV-3) in 9%, and influenza B in 9% [9].”

We removed the following paragraph (background page 5) and we added them in the discussion (line 263, page 13):

“In others countries African countries as Kenya, Ghana, in India, or Europe, the prevalence of RSV in children under 5 years of age with ILI ranges between 12% and 60% [13, 42, 46-48], while that of PIV and adenovirus ranges between 3-22% and 5-25% respectively [13, 15, 42,
46-50]. In Europe, Rhinovirus (HRV) is found in 8% to 12% of young children with ILI [18, 37, 51].”

We removed the following paragraph (background page 5) and we added them in the discussion (line 251, page 12):

“Indeed, in Cameroon, 28.2% of specimens was influenza virus, however, the most common viruses found in children under 5 years of age were RSV (83.9% of specimens) PIV (76.9%) and HRV (64.6%). In the Central African Republic, respiratory viruses were detected in 14.9% of children aged 0-15 years with ILI or acute respiratory illness, including influenza virus in 8.8%, PIV1/3 in 3.3% and RSV 2.7%”

We added the following paragraph in the section background (line 79, page 5):

“Before, the circulation of influenza viruses had been little studied. In Gabon, the circulation of respiratory viruses was not a major public health interest because of the other infectious diseases that cause a febrile illness such as malaria. In a pandemic context, it proved important to know the circulation and prevalence of influenza viruses and others respiratory viruses.”

We modified the discussion. We explained that the situation of Gabon was similar to tropical countries or others countries in Africa or Europe (page 12-13).

8. Page 13. Do the authors have an explanation for the far higher AdV prevalence in Gabon and Kenya compared to the other studies? This is a general limitation of the manuscript. The discussion often mentions the results for Gabon, the results from elsewhere (comprehensive!), but stops there. E.g. here, what does it mean that results are similar to Kenya and different from many other studies. Another example on the same page: why is only Peru mentioned to illustrate the influenza A/B proportion distribution? WHO FLUNET would give you much more regional and global information. So why Peru?

We added the following paragraph in the discussion (page 13, line 271):

“In young children, asymptomatic porting has been shown for some respiratory viruses such as adenovirus or rhinovirus [53]. More specifically, asymptomatic adenovirus infections are
common in young children [53,54]. In our study, adenoviruses have a predominant prevalence. They are also common in co-infection. Asymptomatic carriage of the virus among young people and their high prevalence in co-infection suggested that they might not be the cause of the disease.”

Our results seem similar to those of Kenya on adenoviruses because in both cases, the study population consists of young children who may be infected with adenovirus but where the virus might not be the cause of the disease.

We deleted the informations about influenza in Peru. We added the following paragraph about the global subtype of influenza, worldwide, from November 2010 to February 2011 (WHO FLU NET reference 61), (page 14 and line 289):

“From November 2010 to February 2011, the subtypes of influenza virus which were mostly prevalent in North Africa, the Near East, Europe and Asia, were the subtype B and the A(H1N1)pdm09 [61]. Our results match those described in the world for this time of year and show that these two types of influenza are predominant.”

9. **Figure 1 should illustrate rates. Therefore it would be better to have proportions positive by months.**

We wanted to represent the number of cases for which we obtained a positive diagnosis compared to the number of negative cases in a visual way on the graph that is why we opt for this presentation rather than rates.

**Minor Essential Revisions**

1. **Page 2, Abstract: abbreviation CIRMF can be removed.**

   We removed the abbreviation CIRMF.

2. **Page 4, SRAS-CoV should read SARS-CoV; in addition explain abbreviations SARS and CoV.**

   We changed SRAS-CoV by SARS-CoV and we explain abbreviation SARS-CoV (page 4 and line 39).
3. Page 10, ...no cases of SIA. Looking at the PCR characteristics all influenza type A viruses should have been positive in the H1pdm09 and generic type A PCR. Therefore no other influenza A virus subtypes were detected. The authors should more precisely describe the characteristics of the influenza virus PCRs and reflect that in their description of the results.

For the diagnosis of influenza A viruses, we did not use a generic type A PCR to diagnose all types of influenza A, but we used a specific PCR seasonal influenza A (H1N1, H3N2) on the hand, and a specific PCR pandemic influenza on the other hand. There was a mistake on the reference concerning the PCR influenza virus A that we used. We modified the reference (reference 31).

4. Page 12, EV/HRV 24 (134.8%): how sure are the authors about co-infection? The HRV PCR targets the 5'-NCR, which is known to have high similarity with certain enteroviruses and hence can generate a positive signal with enterovirus. Have the authors thought about this when analysis the presumed EV/HRV co-infections?

Despite the fact that the 5' non coding region of the HRVs and EVs contains highly conserved sequences, there is some variable regions that contain a signature T that distinguish HRVs from EV (reference 34 and 36). The choice of primers was done in order to distinguish the two pathogens. We can therefore suppose that HRV/EV are co-infections.

5. Page 15, ILI symptoms should read ILI diagnoses. The authors did not collect symptoms. In addition, the conclusion is wrong that the highest prevalence of ILI diagnoses was found among children under 5 years of age. Most specimens have been collected from children with ILI under 5 years of age.

We changed the sentence page 16 line 337: “The highest prevalence of viral infections concerned AdV, EV, RSV, HRV, PIV3, SIB. Theses pathogens have the highest prevalence among children under five years old.”

6. Tables 1 and 2 can easily be merged. Both tables: square brackets around age group 0-5 should be switched.

We combined tables 1 and 2. We changed ]0-5[ by [0-4].
7. Figures 1 and 2, mark the months with outside tick marks on the X-axis, rotate the label vertical to allow each month name being plotted. In Figure 2 the X-axis month labels could only be plotted in the lowest graph, similar to the year scale.

We changed the figures 1 and 2.

8. Figure 1: what is special with the Nov 10 results for Koulamoutou? High number of specimens and low number of positives compared to the other cities and also months of Koulamoutou. Was the ILI case definition not correctly applied?

In November 2010, there were some cases of ILI in patients who came from a locality near Koulamoutou. We diagnosed the circulation of A(H1N1)pdm09 in this group. Thus a lot of patients with ILI decided to do diagnosis because they were afraid of the propagation of A(H1N1)pdm09. The ILI case definition was applied. The difference is that most people decided to go visit a doctor because they were afraid to be infected by A(H1N1)pdm09.
Responses to the reviewer 2

Title: Viral etiology and seasonality of influenza-like illness in Gabon, March 2010 to June 2011

Reviewer: Yavuz Uyar

Minor Essential Revisions

*Few corrections should be add to text*

1) *There are grammatical errors on the text. I marked them on the PDF doc.*

*The grammatical errors have been corrected:*

Line 17: The word “Resultats” was replaced by “Results”.
Line 39: The word “SRAS-CoV” was replaced by “SARS-CoV”.
Line 265: We modified the sentence with “between 3-22% and 5-25%, respectively”.
Lines 72 and 257: The word “RDC” was replaced by “DRC”.
Line 102: We have no paper describing the seasonality of Gabon. These data come from the General Direction of meteorology of Gabon.
Line 138: “national ethics committee” was replaced by “National Ethics Committee”.
Table 1 and 2: ]0-5[ was replaced by [0-4].

2) *On the other hand, discussion section could be extended with using compare of the rural and urban areas’ result of Gabon. Why some virus ratio higher or lower than the other urban or rural area?*

Page 14 and line 294: We added in the discussion section the following paragraph mentioning the comparison between the results of rural and urban areas:

“All some viruses are predominant in urban areas, others in rural areas. Some studies regarding epidemiology of RSV in Kenya show that the all-cause community-based incidence of SARI among infants is higher in the rural study site, compared to the urban study site [62, 63]. In Gabon, Franceville which is a semi rural area has a lower density of population than Libreville and the fact that there is only one regional hospital where patients go, explain why
viruses associated with infections in very young children such as the SIB or RSV are more important in this region where the spread would be faster”.

3) The neighbours country and other African countries' seasonal results (2010-2011) could be give on the table for compare.

Line 251 to 267: We added in the discussion section a paragraph mentioning the comparison between neighbouring countries (Cameroon, DRC and the Central African Republic), others African countries’ (Ghana, Kenya) and another continent (Europe).