Author's response to reviews

Title: Molecular identification of adenoviruses associated with respiratory infection in Egypt from 2003 to 2010

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Version: 3 Date: 3 October 2013

Author's response to reviews: see over
Dear Editor:

Please find enclosed our revised manuscript for consideration for publication in *BMC Infectious Diseases*. The article entitled “Molecular typing of adenovirus associated with respiratory infection in Egypt from 2003 to 2010” by Pola Demian, et al. has been modified per your requests.

We have updated the ethics statement in line with the request from the Editorial staff. We have also modified the manuscript based on suggestions and comments from reviewers. We have modified the title per the request of one of the reviewers, the new title is "Molecular identification of adenovirus associated with respiratory infection in Egypt from 2003 to 2010".

During the revision process I became aware that we did not have access to all of the sequence data due to several circumstances beyond the authors control. Therefore, we have decided, and discussed with the editorial staff, a slight change in direction in this revised manuscript. The PCR typing utilized in this paper was the basis for all of the typing and results and was based on previously published methods (Metzgar, D., et al., *PCR analysis of egyptian respiratory adenovirus isolates, including identification of species, serotypes, and coinfections*. J Clin Microbiol, 2005. 43(11): p. 5743-52.). The sequencing data served as confirmation, and another method of analyzing the data. However, given the lack of access we have chosen to remove all mention of sequencing because without the full dataset we cannot adequately address the concerns of the reviewers. Therefore, In order to give more weight to the PCR typing we have added a more thorough statistical and epidemiologic analysis of the data that we feel rounds out the manuscript nicely. Lastly, we have added one additional author due to her role in revising this manuscript and contributing the statistical and epidemiological analysis.

This letter, the response to the reviewers and the accompanying modified manuscript and figures should serve to address the concerns raised earlier. If there are any further questions or concerns, please feel free to contact me.

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Sincerely,

Anne M Gaynor
Reviewer 1: Josefina Garcia

1. Page 1, Line 1: The authors should consider changing the title as the words “molecular typing” do not reflect the focus of the study. No phylogenetic information is given. This is more a epidemiological surveillance study.
   Response: The reviewer raises a useful thought about the name “molecular typing” in the absence of phylogenetic information. We have chosen to change the term “typing” to “identification” which we hope will alleviate any concerns about overstating the focus and results of this study.

2. Page 5, Line 93: the authors state that a total of 550 patients met the WHO criteria but in page 7, line 154 they state there were 3,106 non influenza specimens tested. This is misleading.
   Response: We appreciate the reviewer noticing this discrepancy. We have chosen to remove all denominator data and focus on the 99 positive samples with 105 total isolates of HAdV.

3. Page 7, Line 137: The authors describe the alignments and software used, but do not provide any information about the alignments in the results or figures. Have the sequences been submitted to Genbank? Are they available online? The authors should provide this information.
   Response: We have removed all mention of sequencing and accompanying analysis due to lack of access to the full dataset.

4. The authors should describe the statistical methods used to compare prevalence in the results.
   Response: Statistical methods have been added to the materials and methods and results to address this point.

5. The authors should provide in all results, figures and tables the total amount of samples collected, as the information in tables could be misleading. It is unclear when using proportion if the authors refer to the total amount of samples, the ILI samples, the non-influenza samples etc. The authors should clearly state this information in the tables or in the legends. Also, the statistical analyses used for prevalence’s should be in the results section, and the p values provided
   Response: Statistical data, and denominator data has been added where available and applicable.

6. The phylogenetic information and comparison with previously published sequences is necessary. As well as the Genbank submission numbers.
   Response: We have removed all mention of sequencing and accompanying analysis due to lack of access to the full dataset.
Reviewer 2: Paola Barrero

1. The authors had a clear objective: to characterize HAdV associated with influenza-like illness in the Nile region of Egypt from 2003-2010. The work presented by Dr. Demian et al. is a sequel of previous work conducted by Metzgar et al., in 2005 that examined isolates in the NAMRU-3 collection from 1999 to 2002. It is relevant to continue the molecular surveillance, as the prevalence of types seemed to change from the first report in 2005 to the present report. This switch in types between studies or years might be statistically assessed to strengthen the conclusions.
Response: We appreciate the reviewer’s statements and questions. We have made comparisons to the prior data from Metzgar et al., 2005, however, without the primary data from Metzgar we are unable to made a full statistical analysis comparing these two studies.

2. Methods are adequate. The trademark of the swabs is a good detail to add because there are many different forms and depending on that is the quantity of cells you recover. In that way, the quality of the sample might be assessed by PCR of a housekeeping gene, like RP, proposed in 2009 by the WHO and CDC (H1N1pdm protocol).
Response: The reviewer raises a useful point about the type of swabs that are used in these types of studies as much is known about potential inhibitors of PCR and better sampling with different material. Unfortunately due to the high turnover of personnel in the surveillance sites and the extended time frame that this study was conducted over, we are unable to verify the type of swabs that were used for each sample. For the second comment, the laboratory routinely assesses sample quality for PCR using RP as per the WHO recommendation; however, these samples were first inoculated into cell lines and then tested by PCR. Therefore, we did not use RP to verify the quality of the original sample.

3. I’m concerned about cell culture: authors might be alert if they are disregarding the effect of selection due to the preference of cell line. It is known that adenovirus species B, C and E, even types from B1 subspecies have different receptors that can be absent or present in different proportions in different cell lines. In this context, the presence of co-infection might be underestimated and might be tested in the sample before cell culture to see if findings agree.
Response: The reviewer again raises a useful point that we have now addressed in the discussion section. It is indeed well known that different HadV species and genotypes utilize different cell surface receptors and that by only utilizing our normal respiratory panel of cell lines that we may be biasing our isolation. We are unable to re-test the original samples by PCR to elucidate whether isolation underestimated the rate of co-infection.

4. Sequence analysis is poor and phylogenetic analysis is absent, there is no tree to infer the relationships between the isolates, or the distance or the likelihood of the
inferences. Nowadays, the sequencing of 600-620 bp in a big genome (~36kb) is not novel. Authors might sequence the PCR products to see whether fragments obtained from early (PCR B-C-E), intermediate (PCR VA RNA) and late (hexon or fiber) blocks of the genome are in concordance. Authors might evaluate phylogeography and Response: We have removed all mention of sequencing and accompanying analysis due to lack of access to the full dataset.

5. The total number of samples is confusing: the authors mention in the abstract 99 isolates, in methods they refer to 550 patients and in results they refer to 99/3106 FluA-negative specimens. It might be better to restrict the population to the 99 adenovirus isolates. 
   Response: We appreciate the reviewer noticing this discrepancy. We have chosen to remove all denominator data and focus on the 99 positive samples with 105 total isolates of HAdV.

6. Figure 2 might be deleted as data is repeated in the text (results).
   Response: Figure 2, the pie chart, has been removed.

7. Figure 1 and Table 1 might be unified.
   Response: Figure 1 has been reformatted to show more useful information. A new table has been created to show all of the pertinent data regarding genotypes and geography, age, year and gender.

8. Patterns related to seasonality might be statistically proved by correlation tests.
   Response: We have added in statistical analysis to examine the trends associated with seasonality.

9. Data related to age is referred to Figure 4 and has to be corrected in results and discussion (Results section "Distribution of HAdV species among different age groups", Discussion second paragraph).
   Response: This has been corrected in all locations.

10. The authors don’t mention if they have performed negative controls, no template controls, heterolog (non HAdV) controls and cell culture controls.
    Response: Information about pertinent controls has been added to the Materials and Methods section.

11. Authors do not mention if they had submitted their sequence data to GenBank.
    Response: We have removed all mention of sequencing and accompanying analysis due to lack of access to the full dataset.