Author's response to reviews

Title: Seroepidemiology of pandemic influenza A (H1N1) 2009 virus infections in Pune, India

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Version: 2 Date: 15 June 2010

Author's response to reviews: see over
The Editor,

BMC Infectious Diseases Journal

Ref: MS: 1264084635376677

Seroepidemiology of pandemic influenza A (H1N1) 2009 virus infections in Pune, India

Sir,

The manuscript has been revised as per the comments and suggestions by the editor/reviewers. We have presented data using a threshold titre cut off of 1:40 for international comparisons. We have modified the text as per the reviewer Laurie’s queries. The sampling strategy has been described in the methods section. Serological data in relation to the key groups is presented in the results section and Table 1. We have provided more information regarding the epidemiology of seasonal influenza in Pune, anticipated levels of seasonal vaccine uptake and overall experience of the pandemic in the introduction, methods and discussion sections in the revised manuscript. We have also clarified about the exemption of study procedures for ethical approval. We have addressed all the comments in the revised manuscript along with point-by-point response to the concerns raised by the editor/reviewers. All the changes have been indicated in ‘tracked changes’. Results section has been modified considering a cut off of 1:40. The major changes include modifications in Table 1, Table 2 and Figure 1. Also, we have added new references and cited them in introduction and discussion sections in the revised manuscript.

We are sure that BMC Infectious Diseases journal is the appropriate medium for communicating the findings for larger dissemination and impact on public health practice and research.

Thanking you,

Faithfully yours,

Dr. AC Mishra (Corresponding author)
Author responses to the editorial/referee comments

MS: 1264084635376677

Seroepidemiology of pandemic influenza A (H1N1) 2009 virus infections in Pune, India
Babasaheb V Tandale, Shailesh D Pawar, Yogesh K Gurav, Mandeep S Chadha, Santosh S Koratkar, Vijay N Shelke and Akhilesh C Mishra

Associate Editor comments:
In order to facilitate international comparisons, please attend to the reviewers’ comments regarding the threshold titre of 1:40 as an accepted correlate of protection, against which the seropositive proportion in your sample should be assessed.

Authors: We agree that during the current pandemic, the titre cut-off of 1:40 is an accepted correlate of protection for international comparisons. We have presented the findings considering 1:40 as the cut off titre in the revised manuscript. In India, cross-reactivity is not reported as yet. Hence, we have provided data of 1:10 in addition to 1:40 cut off.

Please also address reviewer Laurie’s queries regarding the need for more detailed description of the sampling strategy employed, and the need to present serologic data in relation to the key groups studied. More information regarding the epidemiology of seasonal influenza in Pune, anticipated levels of seasonal vaccine uptake and overall experience of the pandemic would help readers to place your findings in context, particularly in relation to the experience of other countries.

Authors: We have described in detail the sampling strategy in study design and sampling subsection of the methods section in the revised manuscript. We have presented serologic data in relation to the key groups studied as these were considered to be at higher risk of infections as
compared to the community. Information regarding the epidemiology of seasonal influenza in Pune is provided in the study area subsection in the methods section. The level of seasonal vaccination uptake is negligible in India. This is presented in the discussion section of the revised manuscript. Overall experience of the pandemic has been presented in the introduction section in the revised manuscript.

Editorial request: - Please clarify why ethical approval for this study was exempt.

Authors: The study protocol was reviewed and approved by the institutional ethical committee for research on human subjects. It was considered exempt by the committee as the study was undertaken in an outbreak situation for better understanding of the epidemiology of the ongoing pandemic and thereby guiding implementation of community mitigation activities.
Reviewer's report

Title: Seroepidemiology of pandemic influenza A (H1N1) 2009 virus infections in Pune, India

Version: 1 Date: 1 May 2010

Reviewer: Ted Ross

Reviewer's report:

Major Compulsory Revisions

These studies are necessary and it is nice to see that they are being conducted in multiple areas of the world in order to give a complete picture of the pandemic.

1. Infection with Influenza virus causes significant seasonal morbidity and mortality worldwide. In addition, influenza strains differ in their burden on age groups and socioeconomic populations and National policy agendas are assisted by serosurveillance data, understanding transmission rates and immune reactivity. Do the high rates of school aged children were also seropositive for the representative seasonal flu strain? Past vaccinations may contribute to cross-reactivity against seasonal strains. Are there HAI cross-reactivity between H1N1 strains induced by vaccination or infection with strains circulating between 1957-2008 and the novel H1N1 2009 strains.

Authors: Yes. School children experienced high rates of seropositivity for the representative seasonal flu strains like H3N2 and H1N1 viruses. Seasonal flu vaccination uptake is almost negligible as it is not included in the national immunization programmes and is also not recommended by the physicians except in special circumstances like requirements for Hajj pilgrimage and optionally for international travel. Also, the seasonal flu vaccines are not widely available in Indian market. As on today, there are no reports of cross-reactivity from India. We are working on this issue.
2. A titer of 1:10 in HAI is too low to determine seroprevalence. The authors should reanalyze the data for a 1:40 titer cut-off and present the data. The commonly accepted positive HAI titer is 1:40.

Authors: We agree with the referee that a titer of 1:10 in HAI is too low for determining seroprevalence. We have reanalyzed the data and presented the findings considering a commonly accepted positive HAI titer of 1:40. However, we are also mentioning data with 1:10 cut off as cross-reactivity has not yet been reported in India.

3. Were there higher rates of novel H1N1 positives in the highest age group (greater than 80 years of age)? The author broke out the school children into smaller increments, they should do the same with the group over 65 years of age. Published data indicates that the oldest age groups may have pre-existing antibodies to these new 2009 strains of H1N1.

Authors: Rates of novel H1N1 positives in the highest age group (greater than 80 years of age) could not be studied as the sample size was not sufficient. We did not find any difference in seropositivity in those aged 60-69 and 70-79 years. This break up is now provided in the revised manuscript.

4. The authors should comment on the following to give the reader a full picture of the pandemic in this area of India. A) When did the second wave of infections hit India and when did they peak? B) How does the peak in infections correspond to the collections of samples by this group? C) When did the novel H1N1 vaccine become readily available in this part of India? D) How many healthcare workers were vaccinated and what was the vaccine history of the workers in this study? E) Since infections in children are associated with time of the school year, the authors might let the readers know the time of school in India.
The first case of pandemic H1N1 2009 virus in India was reported on 16th May 2009. Pune reported the first case on 22nd June 2009. The first peak was reported in mid-August 2009. The cases decreased slightly at the end of August and low activity continued in September. As such, the second wave was not identified in India.

The collection of samples in the first survey was done in August and it represents the period which was coincident with the start of indigenous transmission. The surveys undertaken in September-December may be considered to be at the end of the first peak in activity.

The novel H1N1 vaccine in this part of India became available in March 2010 only for healthcare workers engaged in pandemic activities. The vaccine is being planned to be made available for purchase on physician prescription in India in June 2010.

The healthcare workers were not vaccinated at the time of survey as the vaccine became available only in March 2010.

The time of school year in India is June through March next year.
Reviewer's report

Title: Seroepidemiology of pandemic influenza A (H1N1) 2009 virus infections in Pune, India

Version: 1 Date: 7 May 2010

Reviewer: Karen Laurie

Reviewer's report:

The manuscript describes a serological survey assessing infection with pandemic influenza 2009 in various populations from Pune, India. The study encompassed a number of professions and analysed factors associated with risk of infection with this novel strain. Analysis by the authors revealed significantly different proportions infected with pandemic influenza compared to other recently published serological studies. As a different cut-off for the serological assays was used in this study compared to other published studies, it is unclear what these findings mean in a broader context.

Authors: We have reanalyzed the data and presented the results and comparisons with reported literature in the revised manuscript. In our study, the proportions infected with pandemic influenza were less as compared to other recently published serological studies. Also, the elderly subjects experienced the lowest infection rates as compared to children and adults. We used a cut off of 1:10 whereas most of other studies used 1:40 cut off. However, in the revised manuscript, we have analyzed data using both the cut offs separately.

Major Compulsory Revisions

1. Conventionally, a positive HI titre is accepted to be 40. This is a widely accepted criteria, used by research scientists, epidemiologists, health professionals and governments. Although the authors claim that no pre-pandemic samples had a titre greater than 10, this does not indicate that
the accepted titres should be changed. The authors should reanalyse their data with this limit used.

Authors: We agree that the positive HI titre is accepted to be 1:40. We have presented the data using the titre cut-off of 1:40 in the revised manuscript. We have reanalyzed and presented the data considering a cut-off of 1:40 in the revised manuscript. We are not making a case in this study for change in cut-off. As the levels of cross-reactivity differ in geographical regions, we are providing data on minimum detectable titre of 1:10 in addition to 1:40.

2. It is unclear where the pre-pandemic samples are from. As they are all 'negative' it is imperative to know the age of the donors and any health conditions these donors have. Unless they are children, it is extremely unusual for all samples to be negative and suggests a biased sample collection or assay.

Authors: Pre-pandemic samples were referred for dengue diagnosis in the year 2008. We have described the age of these patients. The details of underlying health conditions in these donors were not available. We did not detect positivity in any of these pre-pandemic sera. We selected 103 sera referred from patients in Pune during the year 2008 as more sera were not available. We have further tested 85 more sera from persons aged 60 years or more making a total of 188 sera from the pre-pandemic period. Similar observation is also reported from Singapore.

3. Laboratory procedures mentioned that 'sera with non-specific agglutinins were treated with turkey red blood cells'. Did this apply to all samples? How was the non-specificity determined? Was this reaction removed by RBC adsorption. This needs to be made more clear to ensure no bias in the assaying process has occurred.

Authors: All sera were not treated with turkey red blood cells. Sera with presence of non-specific agglutinins showed HA activity. Such sera were treated with turkey red blood cells to remove
non-specific agglutinins. This treatment could remove non-specific agglutinins. These sera were then retested in HI assay. We have clarified it in laboratory assays subsection of the methods section in the revised manuscript.

4. In the final paragraph of the study design it is declared that the study was 'exempt for ethical review as per the policies of the institutional ethical committee'. This comment is unclear.

Authors: The study protocol was reviewed by the institutional ethical committee for research on human subjects. It was considered exempt by the committee as the study was undertaken in an outbreak situation for better understanding the epidemiology of the ongoing pandemic and thereby guiding implementation of community mitigation activities.

5. The methods, results and discussion alude to some samples being paired. Paired serum samples should be cleared shown and deemed different from unpaired samples.

Authors: Paired samples have been clearly shown in the concerned study groups in the results section in the revised manuscript. School staff was resampled after 5 weeks, hospital staff after 9 weeks and general practitioners after 13 weeks. Therefore, we could not deal them separately.

6. Samples were collected at multiple times, post-pandemic. The results divide the samples into age brackets, yet there is no outline of the age breakdown in the methods, nor if there is any bias in age group collection at each timepoint. The number of samples for hospital staff and general practitioners in the results text does not equal the number of samples in Table 1. Also, in the section outlining the determination of cut-off titre, '1599 sera with detectable HI antibodies' is mentioned. Where are these sera from? This does not match with the data in the tables. The results text and tabulated results suggest a mix-up of the data and reduces confidence in the analysis. A table outlining the 'group', 'time of collection', 'age at each timpoint', and sex should
be broken down for every serum set so the reader is clear on the samples analysed - including the baseline samples. A separate Table should then outline the proportion positive and negative.

Authors: We have provided age-breakdown in the methods section in the revised manuscript. The subjects were invited to participate and volunteers were selected for sampling after consent. The numbers of samples in hospital staff and general practitioners have been presented in the text and it matches with the data presented in Table 1 in the revised manuscript. We have now clearly provided the number of samples analyzed including baseline samples in the revised manuscript. In the earlier manuscript, we had mentioned a total of 1599 subjects among risk groups and population which were detected seropositive among 8973 subjects sampled in the year 2009. An error in percentage has been corrected in the revised manuscript.

7. Were the samples collected from the schools taken from the same sites at different times, or from different schools at each timepoint?

Authors: Only the school staff in some schools was sampled at two time points. School children were sampled only once at different time points in September.

8. There is no discussion on the vaccination status of the population. Can the authors please include some background information on the typical proportion of the Indian population vaccinated and the dominant circulating subtypes in recent previous influenza seasons for readers not familiar with the region.

Authors: The population was not vaccinated with the pandemic vaccine at the time of survey. For seasonal influenza, vaccination is not considered in the national routine immunization program and is not recommended by physicians except in special circumstances like pilgrimage to the Hajj and optionally for international travel. The H3N2 seasonal flu strain is the most
predominant virus strain in India followed by H1N1. This data has been provided in the subsections on school children and staff and section on seropositivity in other groups.

9. Can the authors please define the test-retest reliability criteria.
Authors: We tested a subset of 101 sera in HI assay for repeatability. We have provided the reliability data for pandemic H1N1, seasonal H1N1 and seasonal H3N2 strains in the revised manuscript. Most of the sera showed similar titres in repeat tests.

10. The authors should comment on the interactions of adults and smaller children in the household with school aged children. Was there any increased seropositivity?
Authors: It is noted in several studies that the school children are most susceptible for influenza and are able to transmit infections easily to family members and other contacts due to higher rates of transmissibility and longer duration of period of communicability. An increased seropositivity was seen in children than adults in schools and families.

11. The authors should comment on any public health measures or guidelines the Indian government used to control infection, and how this may have influenced their results. Particularly as hospital staff had lower infection rates than general practitioners.
Authors: Indian government used school closure, restriction of crowding in public places and advocated the patients to stay home during the pandemic to reduce transmission. This may have decreased the chances of exposure to the infected patients. Also, the patients and their contacts were prescribed oseltamivir therapy even before laboratory diagnosis, thereby decreasing the transmission. Hospital staff was provided with all the personal protective measures and prophylactic oseltamivir therapy. Whereas, the general practitioners were in direct contact with the patients and the infection rates among them were higher as compared to the hospital staff.
Simple practice of using face-mask and hand-washing was not common in general practitioners, whereas it was strictly followed by hospital staff.

12. The discussion points out that higher seropositivity was noted in young adults but not elderly in India and that this is ‘in contrast to the studies in some other countries’. These data looked at pre-pandemic samples. The authors should recheck the references.

Authors: We did not find higher seropositivity in elderly in our study at baseline and even during the pandemic. Most studies have reported higher baseline seropositivity in elderly in pre-pandemic samples. The infection rates in elderly even during the pandemic were less as compared to children and adults as noted in reported literature.
Reviewer's report

Title: Seroepidemiology of pandemic influenza A (H1N1) 2009 virus infections in Pune, India

Version: 1 Date: 11 May 2010

Reviewer: mohsen moghadami

Reviewer's report:

1) describe type of clustering for dengue fever sampling, what is sample size? is it cluster sampling?
Authors: The sera from Dengue fever cases were referred for laboratory diagnosis and were not sampled during the study. Sample size was 188 subjects as updated in the revised manuscript. It was random sampling from the archived sera.

2) method of HI test need more precise definition. the most important point is source and type of used antigen
Authors: The antigen used for the HI assay in this study was prepared using the pandemic influenza A (H1N1) 2009 virus isolated from human subject. This virus was grown in embryonated chicken eggs and allantoic fluid from the infected eggs was used as a source of antigen. This virus was inactivated using beta-propiolactone. This has been provided in the laboratory assays subsection of the methods section in the revised manuscript.

3) describe limitation of work exactly.
Authors: We have described limitation of work in discussion section in the revised manuscript.

4) references need writing as Vancouver method and more recent articles published and need adding these papers.
Authors: We have rechecked references as per Vancouver method suggested by the referee. We have searched all the recent papers and added these papers in the revised manuscript.