Author's response to reviews

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

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Version: 3  Date: 13 July 2010

Author's response to reviews: see over
To,

The Editor,

BMC Infectious Diseases Journal

Reference: MS: 1264084635376677

Sir,

We have provided the point-by-point responses to the referee/reviewer comments. The figures used in the revised manuscript are correct and consistent. Age and sex distribution of the study subjects among different study groups at various time points including baseline samples is provided in Table 1 in the current revision. We have added 34 baseline sera in the 40-59 years age group to the 188 baseline sera mentioned in earlier revision. Baseline sera now add up to 222 sera in the current revision. We have provided data in the text about the seropositivity data with breakdown of age groups in different study groups at various time points. Table 2 in the earlier version is renumbered as Table 3 in the current revision.

The institutional ethical committee for research on human subjects at the National Institute of Virology, Pune, India provided ethical approval for this study.

The changes made in the current revision of the manuscript are highlighted as ‘tracked changes’. We have also retained the changes made in the earlier revision (15 June 2010) in tracked format. English language corrections are made to the best of our understanding and capabilities.

We sincerely request you to consider the current revision of the manuscript for publication.

Thanking you,

Sincerely yours,

Dr AC Mishra, Corresponding author
Author responses to reviewers’ comments:

Reviewer's report

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

Version: 2 Date: 18 June 2010

Reviewer: Karen Laurie

Reviewer's report:

Major Compulsory Revisions

Original comment : 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.

Re-revision comment: Although much clearer, these revisions have only been partially addressed. Can the authors please include the number of samples that were adsorbed and the spread of these samples amongst the population to indicate if any biases were or were not found.

Authors: In each assay, serum control included testing of serum without test antigen. Sera without non-specific agglutinins showed button formation, whereas sera with non-specific agglutinins showed haemagglutination. Sera with non-specific agglutinins were treated with turkey red blood cells, which removed non-specific agglutinins (included in the Methods section). We treated only 441 sera with turkey red blood cells. These sera were randomly distributed and were few as compared to the large number of sera processed in the study (included in the Results section). We do not consider that these led to biases in the study (included in the Discussion section).
Original comment: 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 timepoint', 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.

Re-revision comment: This has been partially addressed. The timepoint data for Hospital staff and General practitioners is much clearer, but the age distribution data (20-39 year olds vs 40-59 year olds) is not clear for these two groups. What timepoint is described for the aged divisions, this does not add up to the numbers in table 1?

Further, for the school children, Table 2 shows a total of 2528 children sampled, whilst the text describes 2527 (pg 9). The time of collection (September) should also be included in the Table legend. 631 children are sero-positive according to Table 2 and page 9, whilst ‘650’ sero-positive subjects are indicated on page 10. Please clarify.

Figure 1 – The text (pg 10) indicates 56% school children were seropositive for seasonal H1N1 and 27.3% for seasonal H3N2. Can the authors please check this against the figure as it may be reversed. The text (pg 11) indicates 2520 people in the general population, as does Table 1, however Figure 1) shows 2328 people. It appears that the 10-14 year old age group has a higher proportion of sero-positive people to pandemic H1N than the 20-29 year olds. Please indicate in the text when ‘data is not shown’.
Authors: For hospital staff and general practitioners, age distribution data (20-39 year olds vs 40-59 year olds) was earlier provided for the total subjects sampled in these risk groups ignoring the different time points of serum collection. In the section outlining determination of cut-off titre in the earlier revision, we had analyzed all 1599 positive (a titre of 1:10 or more) sera for presenting the percentage of sera among positives detected with the titre of 1:10. This has now been deleted in the current revision as data is presented with a titre cut off of 1:40.

Table 1 has been added in the current revision for outlining the group, time of collection, age and sex for every serum set including baseline samples. Accordingly, the table 1 in the earlier version has been renumbered as Table 2 in the current revision, which includes seropositivity among study groups at various time points during the pandemic.

As mentioned earlier, we had clubbed the data from various time points for age differences in seropositivity in the earlier revision. In the text of current revision, we have considered age divisions for presenting seropositivity in different groups sampled at each time point.

The number of school children sampled was 2527 as indicated in the text in the earlier revision. We have corrected the number 2528 wrongly mentioned in Table 2 in earlier revision as 2527 in the Table 3 in the current revision. The number of subjects sampled in the age-group 10-14 years was wrongly mentioned as 1307 in earlier Table 2 instead of actual 1306 subjects. Due to this correction, we have replaced the row indicating 10-14 years in the current Table 3. We have also included the time of collection as September 2009 in the title of Table 3 in the current revision (Table 2 in earlier revision). Similarly, the number of school children detected seropositive is 631 instead of 650 wrongly mentioned in the text in earlier revision and is now corrected in the current version.

Figure 1 is about the subjects sampled from the community in October 2009. The statement in the text (pg 10) indicating 56% school children were seropositive for seasonal H1N1 and
27.3\% for seasonal H3N2 is correct and is retained in the current revision. The number of subjects sampled from the community in October 2009 was 2520 as mentioned in the text. Only 2328 subjects were considered for presenting data in Figure 1. Remaining 192 subjects were excluded as age data was not available for 17 and remaining 175 subjects had no serology results available for seasonal H1N1 and H3N2 viruses. We agree that the 10-14 years age group had higher seropositivity of 7.2\% to pandemic H1N1 than 5.5\% in 20-29 years age group. This has now been clearly mentioned in the current revision. We have also indicated clearly as ‘data not shown’ wherever applicable.