Reviewer's report

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

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Reviewer: Karen Laurie

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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.

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.

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.

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.

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 insitutional ethical committee'. This comment is unclear.

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.
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.

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

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.

9. Can the authors please define the test-retest reliability criteria.

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?

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 that general practitioners.

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.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare I have no competing interests