Author's response to reviews

**Title:** Seroconversion and asymptomatic infections during oseltamivir prophylaxis against Influenza A H1N1 2009

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

We thank you and the reviewers for the prompt review and invaluable comments on our manuscript. We have provided a point-by-point response to the comments for your kind perusal. Changes to the manuscript have also been underlined for easy reference.

**Reviewer 1’s Comments**

This manuscript reports on an observational cohort study that evaluated the serologic outcome during oseltamivir prophylaxis and after post-exposure prophylaxis during three nH1N1 outbreaks in the Singapore military. The three outbreaks involved a total of 252 subjects. A strategy of oseltamivir chemoprophylaxis with cohorting of the affected unit was used to limit the spread of the nH1N1 virus. A 10 day course of oseltamivir prophylaxis was administered at the onset of the outbreak. NP swabs were collected 3 times per week and tested for nH1N1 by RT-PCR. Screening was maintained until no new cases were identified for three days. All subjects positive for nH1N1 by RT-PCR were given a minimum of a 7 day home medical leave. Also up to three blood samples for HI tests were collected at start of the outbreak, 2-3 weeks after completion of chemoprophylaxis and 4-6 weeks after completion of chemoprophylaxis. The last blood sample was obtained after the peak of nH1N1 activity in Singapore. There were 11 index cases, 16 subjects who developed >4 fold-rise in HI between 1st and 2nd blood samples and 23 subjects who seroconverted between the 2nd and 3rd blood samples. GMTs were comparable between the subjects who seroconverted shortly after end of chemoprophylaxis compared to those subjects who became infected after chemoprophylaxis. 8 of 16 subjects who seroconverted during or shortly after chemoprophylaxis were asymptomatic during the entire period. Chemoprophylaxis appeared to have curtailed spread of nH1N1 within the affected units and did not appear to have increased the attack rate after end of chemoprophylaxis.

**Major Compulsory Revisions**

The timing of the blood collections is unclear. There were three outbreaks that started 1-2 weeks of each other. Perhaps in Table 2 information can be provided on the mean (SD) duration for each period. Also a seroconversion rate based on person-day should also be provided. This would be most helpful in comparing rates between the two time periods and would strengthen the discussion on the infection rate after post-exposure chemoprophylaxis. As currently described it is difficult to determine if there exists a beneficial or detrimental impact from the chemoprophylaxis strategy on the rate of infection post-chemoprophylaxis.

We have included the exact dates of the blood collections for each outbreak in the manuscript (1st paragraph of the results) and in Table 2 for greater clarity on the timing and duration of the study. The blood samples for each outbreak were all taken on the same day, hence there is no variable seroconversion rate based on different time to blood taking.

**Minor Essential Revisions**

It is unclear when subjects were allowed to return home for the weekend. Were they allowed to visit their families during chemoprophylaxis or
post-chemoprophylaxis? Please describe in text.

The subjects were allowed to return home for the weekend both during and post-chemoprophylaxis. As mentioned in the methods, military personnel in general would stay in camp for the week and return home on weekend. We have now described this in clearer detail in the manuscript (1st paragraph of the Epidemiological Investigations section).

It is unclear if the subjects who seroconverted during the post-chemoprophylaxis period had symptomatic and/or asymptomatic infection. Please describe in text.

23 (12.1%) subjects seroconverted during the post-chemoprophylaxis period. 4 (2.1%) were symptomatic. However, this low rate of symptomatic infections may be due to recall bias as between 2nd and 3rd blood sample (ie seroconversion post-prophylaxis), only one questionnaire collecting symptoms was administered after the 1 month or greater duration. On the other hand, between the 1st and 2nd blood sample (ie seroconversion during prophylaxis), regular questionnaires (3 times per week) were administered to collect symptoms.

We have included this and the limitations in the manuscript.

It would be helpful if the nomenclature to describe the periods during and after chemoprophylaxis were consistent throughout the manuscript. That used in Table 3 would be appropriate.

We thank the reviewer for his comments and have made the relevant changes in the manuscript using the nomenclature “during prophylaxis”, and “post-prophylaxis” or “after cessation of prophylaxis” where relevant.

Were nH1N1 positive subjects who were sent home treated with oseltamivir? Did these individuals have serologic analysis performed even if they did not have an acute blood sample at baseline? If yes, it would be helpful to know their convalescent GMT. I would predict that they will have higher GMT compared to those subjects who developed breakthrough infection during chemoprophylaxis. This is because ill subjects who are treated are likely to have a higher total viral load compared to subjects who receive chemoprophylaxis and develop a breakthrough infection.

Yes, the index cases were sent home treated with oseltamivir (75mg twice daily for 5 days). 10 of the 11 index cases had a post-seroconversion blood sample taken. The post-seroconversion GMT for these index cases was 65.0 (SE 6.8) compared to 59.1 (SE 6.1) in those who seroconverted during prophylaxis. The difference is not statistically significant (p=0.590). We have included this in the manuscript.

We agree with the reviewer’s logical argument, but the data unfortunately does not support any large difference in GMT between the 2 groups.

Reviewer 2’s Comments
Post-exposure oseltamivir prophylaxis for close contacts such as household
members or as seasonal prophylaxis in the community was shown to be effective in preventing clinical influenza in healthy adults. The current manuscript described the results of evaluation of the number of asymptomatic influenza infections which occurred during oseltamivir post-exposure prophylaxis and after cessation of prophylaxis. The study was performed in 3 outbreaks in Singapore military between 22 June and 16 July 2009.

Major Compulsory Revisions:
1. The findings that 3.5% seroconversion occurred in participants who were RT-PCR negative required additional experimental evidence. RT-PCR is a very sensitive method and the antibodies can rise only in response to influenza virus infection. Additional tests must be conducted with sero-positive (but RT-PCR negative samples) to confirm these findings. The authors must analyze the samples in virus neutralization assay in MDCK cells or Western Blot or ELISA.

There are several reasons why seroconversion may occur in PCR negative individuals besides issues with RT-PCR sensitivity – particularly true asymptomatic (subclinical) infections without shedding and therefore lack of virus particles in the nasopharyngeal samples, or delayed sampling (particularly in an outbreak setting where symptomatic individuals might have stopped shedding by the time sampling is done) which we tried to minimize by sampling 3 times per week, but this is still the most likely explanation.

In a separate study of ours which is the subject of another publication, we have already compared this same HI technique with viral micrownturalisation (VM) technique in 45 Influenza A H1N1 2009 RT-PCR positive patients. 82% (37/45) seroconverted by HI assay compared to 89% by VM (39/44). At least two-fold or greater increase in titer was observed in 96% (43/45) by HI compared to 98% (43/44) by VM. That is VM and HI were found to be equally as sensitive in detecting serological detection of infection with the pandemic H1N1 virus.

2. Description of materials and methods lack essential experiments details. Description of results required clarifications.

We have now added additional experimental detail on the RT-PCR testing in the methods section of the manuscript.

The HI tests was performed by the WHO Collaborating Center for Reference and Research for Influenza in Melbourne, Australia and have been validated in another paper (see JAMA 2010; 303:1383-91). We have now added information on control sera used for the HI assays.

Minor Essential Revisions:
1. The authors are using not appropriate expressions for some of the study elements. For example “serological infection”. The authors determined “serological confirmation of influenza infection”. The authors must modify the Abstract and text of the manuscript accordingly. It is also not correct to use “non-infectious seroconversion”. Development of specific antibodies must be in response to influenza virus infection and this expression must be corrected.
We thank the reviewer for her comment and have made the relevant changes in the abstract and manuscript.

2. Abstract - indicate that HA gene primers were used for RT-RCR. Indicate the prophylaxis oseltamivir regimen (75 mg once daily). What is the major conclusion from these results – to recommend oseltamivir post-exposure prophylaxis or not?

We have included the relevant portions in the abstract. Post-exposure prophylaxis is effective as a measure in mitigating pandemic influenza outbreaks and we are recommending that it be considered as one of the possible measures during influenza outbreaks. We have included this in the abstract and manuscript.

3. Methods – details of the experiments must be provided, e.g. composition of the transport medium, virus purification, virus inactivation, primers for RT-PCR, cell culture for virus isolation, control samples for HI assay. “Swab-positive cases” is not correct expression. The authors meant “virus-positive samples”? The abbreviations must be spell out, e.g. SWH1 Forward/Reverse primer set; IZP-A/California/7/2009. What is the level of detection in RT-PCR used in the study?

We thank the reviewer for her comments and have corrected the incorrect expression and spelt out the abbreviations. As mentioned above, additional details of the laboratory methods is now included in the manuscript.

4. Results – all subtitles in the results section are confusing. What does it mean “seroconversion during outbreaks”? The title “antibody titers with and without prophylaxis” must be change into “antibody titers during and after completion of oseltamivir prophylaxis”. The description of results is unclear.

We thank the reviewer for her comments and have made the relevant changes

We thank you again for the comments, and hope that your will consider our manuscript as an important addition to the literature on this issue.

Sincerely,
Dr Vernon Lee
On behalf of the authors