PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Assessing herd immunity against rubella in Japan: A retrospective seroepidemiological analysis of age-dependent transmission dynamics
AUTHORS	Kinoshita, Ryo; Nishiura, Hiroshi

VERSION 1 - REVIEW

REVIEWER	Pedro Plans-Rubio Public Health Agency of Catalonia, Spain
REVIEW RETURNED	16-Sep-2015

GENERAL COMMENTS	The paper assesses the epidemic dynamics of rubella in Japan. To do this analysis several sources of data are combined, including CRS information, seroprevalence data and vaccination coverage. The herd immunity level was assessed using two measurements: serroprevalence m1 and seroprevalence m2. The herd immunity threshold estimated assuming that Ro=6,1 was 83.6%. The paper has the following problems: 1. The paper is quite similar to that published in the International Journal of Epidemiology in 2015, and it is not clear what is adding the new paper. 2. The assessment of herd immunity levels in the population is a key information to guide vaccination programs and epidemiological surveillance activities. However the approach followed in the paper is not clear, and the results presented are not different than those presented in the paper of IJE 2015. It is not clear, for example, how was the vaccination coverage taken into account in the model. 3. I recommend to the authors two papers: Plans-Rubió P. Evaluation of the establishment of herd immunity in the population by means of serological surveys and vaccination coverage. Human Vaccines Immunotherapeutics 2012; 8:184-188. Plans P. New preventive strategy to eliminate measles, mumps and rubella from Europe based on the serological assessment of herd immunity levels in the population. Eur. J Clin Microbiol Infect Dis 2013; 32:961–966. The herd immunity threshold in terms of prevalence of positives obtained by Plans ranged from 83% to 94%, depending on Ro, and the sensitivity and specificity of the serologic test. 4. It is not clear what is the modeling adding to the herd immunity analysis, apart from determining seroprevalence levels in each year from 2003 to 2013. Results presented on page 7, however, compare

	only results obtained in the seroprevalence studies carried out in Japan in 2003 and 2013. 5. In 2013, most rubella cases occurred in males aged 20-49 years and females aged 20-29 years, but all population groups had a prevalence of seropositive lower than the herd immunity threshold of 83.6% in males. Therefore this threshold does not explain the distribution of cases in Japan. . 6. Concerning the method used to assess the seroprevalence of positives, most studies consider HI titrers ≥.8 IU/ml as positive, while in the study the prevalence of positives was determined using HI titer ≥.32. Shekarchi et al. (J. Clin. Microbiol. 1981; 13: 850-854) found a good correlation between an HAI titer ≥8 and ELISA ED values of 51.2, and these threshold values were recommended for deciding positivity to rubella. The Laboratory Standards of the National Committee for Clinical Laboratory Standards of the USA consider a rubella HAI titer ≥8 as positive.

REVIEWER	Prof Richard Aspinall Academy of Health and Wellbeing UK
REVIEW RETURNED	22-Sep-2015

GENERAL COMMENTS	This paper complements the work from these authors published recently in the International Journal of Infectious Disease. Although the data is new some of the points made by the authors for example the presence of susceptible pockets in the population and the elevated age of infection were made in the original paper. Some of the new data in the paper would merit more discussion, for example the authors point out that the “peak of CRS cases took place 33 weeks after the peak of the rubella notifications”, but Fig 1B shows that there were about 160 cases in weeks 42, 45, 46, 47 and 50 of 2012 but there were very few cases of rubella notified in week 9, 12, 13 14 and 17 of the same year. In Figure 2 A and B the authors chart the change in the percentage of seropositive individuals in different age ranges in different years. So a female aged 45 in 2003 would find herself in a group which was about 70% seropositive but 5 years later would be in an age group which was 86% seropositive and in 2013 would be in a group which was almost 90% seropositive. Whereas a male aged 45 in 2003 would be in a group which was about 84% seropositive which drops 5 years later to 75% seropositive and stays at that level in 2013.

REVIEWER	Gulam Khandaker University of Sydney, Australia
REVIEW RETURNED	28-Sep-2015

GENERAL COMMENTS	This is an important paper assessing the herd immunity against rubella in Japan in the context of recent rubella epidemics. Japan has implemented Rubella immunisation in the 80s and routine rubella immunisation was implemented in the 90s. However, the recent rubella epidemics in Japan reinforces the need for appropriate immunisation strategies and this manuscript will be an

	important contribution to the knowledge of rubella epidemiology and infection dynamics. Overall, the manuscript is well written and the methods are scientifically sound. I have only few minor comments as stated below. 1. Page 1 (line 19); Abstract objective add "rubella" before epidemic from 2012-14. 2. Page 3 (line 58); ....risk of having fetus with congenital rubella infection (not syndrome, considering not all will develop CRS) 3. Page 3 (line 73); during the 2012-14 epidemic (to make it consistent) 4. Page 4 (line 85); Replace cases with "Rubella". i.e. Rubella and CRS data rest on .... I strongly recommend to accept this manuscript with these minor corrections.

VERSION 1 – AUTHOR RESPONSE

[Responses to Reviewer 1]
Reviewer: 1: Reviewer Name: Professor Pedro Plans-Rubio
The paper assesses the epidemic dynamics of rubella in Japan. To do this analysis several sources of data are combined, including CRS information, seroprevalence data and vaccination coverage. The herd immunity level was assessed using two measurements: serroprevalence m1 and seroprevalence m2. The herd immunity threshold estimated assuming that Ro=6,1 was 83.6%. The paper has the following problems:

1. The paper is quite similar to that published in the International Journal of Epidemiology in 2015, and it is not clear what is adding the new paper.
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The submitted manuscript is different from the published short note (Nishiura et al., International Journal of Infectious Diseases, 2015) in many respects. While the IJID paper focused on estimating the impact of the most recent epidemic on age-specific seroprevalence, the present study comprehensively assessed herd immunity. Specifically, the present study: (i) characterizes the longitudinal achievement towards rubella control in Japan, (ii) explicitly identifies the failures of the vaccination strategy change, (iii) clearly describes the increasing age at rubella infection, and (iv) estimates the number of live births at risk for CRS. These points have been emphasized in the first paragraph of the Discussion (Page 10, Lines 218-224).

2. The assessment of herd immunity levels in the population is a key information to guide vaccination programs and epidemiological surveillance activities. However the approach followed in the paper is not clear, and the results presented are not different than those presented in the paper of IJE 2015. It is not clear, for example, how was the vaccination coverage taken into account in the model.
3. I recommend to the authors two papers:
Plans-Rubió P. Evaluation of the establishment of herd immunity in the population by means of serological surveys and vaccination coverage. Human Vaccines Immunotherapeutics 2012; 8:184-188. Plans P. New preventive strategy to eliminate measles, mumps and rubella from Europe based on the serological assessment of herd immunity levels in the population. Eur. J Clin Microbiol Infect Dis 2013; 32:961–966.
The herd immunity threshold in terms of prevalence of positives obtained by Plans ranged from 83% to 94%, depending on Ro, and the sensitivity and specificity of the serologic test.
>> (Response to comments 2 and 3)
The vaccination coverage has been plotted in Figure 1A to indicate that there was a drastic change in

the vaccination strategy in 1995. These points have been emphasized in the Methods (Page 5, Lines 105–107) and Results (Page 7, Line 155). Our model did not incorporate vaccination coverage because a substantial fraction of immune individuals in Japan (especially adults who have been the main driver of the recent epidemic) acquired their immunity from a natural infection rather than from vaccination. This point has been added on Page 6, Lines 133–135.

We thank the reviewer very much for recommending these two papers to us. We have read the suggested papers and both greatly advanced our knowledge about the epidemiological assessment of herd immunity. Both papers have been added to the reference list (as references 25 and 26). Moreover, we have adopted 94% as an alternative threshold for all relevant figures in the revised version of our manuscript. This point is now mentioned in the Methods (Page 6, Line 122–127). Additionally, based on the reviewer's helpful suggestions, we have utilized the sensitivity and specificity of the serological testing (Page 6, Lines 125–127 and Page 8, Lines 181-183).

4. It is not clear what is the modeling adding to the herd immunity analysis, apart from determining seroprevalence levels in each year from 2003 to 2013. Results presented on page 7, however, compare only results obtained in the seroprevalence studies carried out in Japan in 2003 and 2013.
>>
Our model has allowed us to simply compare the herd immunity threshold against a representative value of the seropositive proportion (Figure 4A). This point has been emphasized in Page 9, Lines 194–196. Because of the inconsistency in the age grouping and to keep the data in Figure 2 visually comparable, we have decided to maintain the comparison among only three time points (2003, 2008 and 2013) in Figure 2.

5. In 2013, most rubella cases occurred in males aged 20-49 years and females aged 20-29 years, but all population groups had a prevalence of seropositive lower than the herd immunity threshold of 83.6% in males. Therefore this threshold does not explain the distribution of cases in Japan. .
>>
We thank the reviewer for this comment because we believe that our revised analysis has greatly improved the finding on the suggested point. We have drawn a new Figure (Supplementary Figure 1) using a cut-off value of HI ≥ 8 and a herd immunity threshold of 94%, and only adult males aged ≥30 years appeared to be below the threshold. This finding is consistent with the observed data (Results in Page 8, Lines 176–183).

6. Concerning the method used to assess the seroprevalence of positives, most studies consider HI titrers ≥.8 IU/ml as positive, while in the study the prevalence of positives was determined using HI titer ≥.32. Shekarchi et al. (J. Clin. Microbiol. 1981; 13: 850-854) found a good correlation between an HAI titer ≥8 and ELISA ED values of 51.2, and these threshold values were recommended for deciding positivity to rubella. The Laboratory Standards of the National Committee for Clinical Laboratory Standards of the USA consider a rubella HAI titer ≥8 as positive.
>>
We agree with the reviewer that the use of 8 IU/ml has been conventionally accepted and that we should consider not only HI titers ≥32 but also those ≥8. In the revised manuscript, we have analyzed the seroprevalence using both cut-off values (Page 6, Lines 122–127).

[Responses to Reviewer 2]
Reviewer: 2. Reviewer Name: Professor Richard Aspinall
This paper complements the work from these authors published recently in the International Journal of Infectious Disease. Although the data is new some of the points made by the authors for example

the presence of susceptible pockets in the population and the elevated age of infection were made in the original paper.
>>
The submitted manuscript is different from the published short note (Nishiura et al., International Journal of Infectious Diseases, 2015) in many respects. This point has been emphasized in the first paragraph of the Discussion (Page 10, Lines 218–224).

Some of the new data in the paper would merit more discussion, for example the authors point out that the “peak of CRS cases took place 33 weeks after the peak of the rubella notifications”, but Fig 1B shows that there were about 160 cases in weeks 42, 45, 46, 47 and 50 of 2012 but there were very few cases of rubella notified in week 9, 12, 13 14 and 17 of the same year.
>>
We apologize for the confusion. To more clearly convey what we meant in the earlier version, that sentence has been rewritten as follows: “The peak in CRS cases took place in the second week of 2014 with n = 4 reported CRS events, which was 33 weeks after the peak of the reported rubella cases in 21st week of 2013. (Page 7, Lines 162–164). To avoid similar confusion, the legend for Figure 1B (with two vertical axis measures) was also rewritten (Page 17, Lines 382–385).

In Figure 2 A and B the authors chart the change in the percentage of seropositive individuals in different age ranges in different years. So a female aged 45 in 2003 would find herself in a group which was about 70% seropositive but 5 years later would be in an age group which was 86% seropositive and in 2013 would be in a group which was almost 90% seropositive. Whereas a male aged 45 in 2003 would be in a group which was about 84% seropositive which drops 5 years later to 75% seropositive and stays at that level in 2013.
>>
We agree with the reviewer’s point; however, the age-grouping was unavoidable owing to the small sample size for each age. This point has been emphasized in Page 5, Lines 115–118 in the revised manuscript. To address this issue, we have added a comparison within the same birth cohort in Figure 2C and 2D. In fact, except for those who were born in the very distant past, the seroprevalence in 2013 is generally greater than that in 2008, and that in 2008 is greater than that in 2003 (Figure 2C and 2D). Moreover, even though the provided 2013 value appears smaller, this could reflect waning immunity over time.

[Responses to Reviewer 3]
Reviewer: 3. Reviewer Name: Professor Gulam Khandaker
This is an important paper assessing the herd immunity against rubella in Japan in the context of recent rubella epidemics. Japan has implemented Rubella immunisation in the 80s and routine rubella immunisation was implemented in the 90s. However, the recent rubella epidemics in Japan reinforces the need for appropriate immunisation strategies and this manuscript will be an important contribution to the knowledge of rubella epidemiology and infection dynamics. Overall, the manuscript is well written and the methods are scientifically sound. I have only few minor comments as stated below.

1. Page 1 (line 19); Abstract objective add "rubella" before epidemic from 2012-14.
>>
The wording in this sentence has been corrected accordingly (Page 1, Line 19).

2. Page 3 (line 58); ....risk of having fetus with congenital rubella infection (not syndrome, considering not all will develop CRS)
>>
The sentence in question has been corrected accordingly (Page 3, Line 59).

3. Page 3 (line 73); during the 2012-14 epidemic (to make it consistent)
>>
The wording in this sentence has been corrected accordingly (Page 4, Line 76).

4. Page 4 (line 85); Replace cases with "Rubella". i.e. Rubella and CRS data rest on ....
>>
The wording in this sentence has been corrected accordingly (Page 4, Line 88).

I strongly recommend to accept this manuscript with these minor corrections.

VERSION 2 – REVIEW

REVIEWER	Pedro Plans-Rubió Public Health Agency of Catalonia, Spain
REVIEW RETURNED	09-Nov-2015

GENERAL COMMENTS	The paper descrive with gereat precision the anti-rubella situation in Japan. The paper has improved significantly and review questions have been answered adequatly. The addition of the two papers I recommended to the paper's references can be of great assisstance to the readers of the paper. It is, however, necessary to do some clarifications in tbe paper, concerning tne use of two antibody level (HI titer >8 and >32) and two herd immunity threhods (84,6% and 94%). The use of both criteria is interesting but it is necessary to do comparisons adequatly. The comparison that must be done is between HI >32 and >8 and the same immunity threhods (83.6% and 94%). The comparison between HI > 32 + 83.6% and >8 + 94% has not a clear meaning. Text on results (page 8, lines 176-180) must be reviewed and corrected. On lines 178-179, "... HI >32 along with 94% herd imm. thres. only the adult male population aged >30 years appeared to be vulnerable" is not correct. The difference between using HI >32 and >8 must to detect population groups bellow the herd imm. thresh. must be indicated with more precision. In 2013, usng HI >8 + 94% threshold, males aged >30 years are also vulnerable, while using HI >32 + 83.6%, males aged >34 years are vulneravle. This difference is important, and it should be indicated in the text. Suplementary figures 1 A and B should be included as Figure 2 C and D, instead of actual Figures 2 C and D. Figures 2 C and D should be included as Supplementary Figures 1 A and B in the text. Results presented in supplementary Figures 1 A and B are of great importance for understanding the immunity situation in Japan and should be threfore presented as main results of the study. Minor comments: Line 166: the sentence "..increased pver time from 2003-13" must be corrected. Line 173: the sentence "...pocket born in those born..." must be

	corrected

VERSION 2 – AUTHOR RESPONSE

[Response to Reviewer 1]
Reviewer: 1. Reviewer Name: Professor Pedro Plans-Rubió
The paper describes with great precision the anti-rubella situation in Japan. The paper has improved significantly and review questions have been answered adequately. The addition of the two papers I recommended to the paper's references can be of great assistance to the readers of the paper. It is, however, necessary to do some clarifications in tbe paper, concerning tne use of two antibody level (HI titer >8 and >32) and two herd immunity threhods (84,6% and 94%). The use of both criteria is interesting but it is necessary to do comparisons adequatly. The comparison that must be done is between HI >32 and >8 and the same immunity threhods (83.6% and 94%). The comparison between HI > 32 + 83.6% and >8 + 94% has not a clear meaning.
>>
We thank the reviewer for this comment. We agree that it is more appropriate to describe all possible combinations of results using two different cut-off values and two different threshold values of herd immunity. Accordingly, the corresponding section was substantially rewritten (Page 8, Lines 168-188).

Text on results (page 8, lines 176-180) must be reviewed and corrected. On lines 178-179, "... HI >32 along with 94% herd imm. thres. only the adult male population aged >30 years appeared to be vulnerable" is not correct.
>>
We thank the reviewer for picking this error. The corresponding part was concerned with the result using HI>8 and thus the sentence was rewritten
(Page 8, Lines 178-181).

The difference between using HI >32 and >8 to detect population groups bellow the herd imm. thresh. must be indicated with more precision. In 2013, usng HI >8 + 94% threshold, males aged >30 years are also vulnerable, while using HI >32 + 83.6%, males aged >34 years are vulneravle. This difference is important, and it should be indicated in the text.
>>
We agree and made revisions to more precisely discuss age groups and birth cohorts that are below thresholds (Page 8, Lines 168-188).

Suplementary figures 1 A and B should be included as Figure 2 C and D, instead of actual Figures 2 C and D. Figures 2 C and D should be included as Supplementary Figures 1 A and B in the text. Results presented in supplementary Figures 1 A and B are of great importance for understanding the immunity situation in Japan and should be threfore presented as main results of the study.
>>
We appreciate the reviewer's comment. However, we are afraid that the original Figures 2C and 2D have great insights into past vaccination policy in Japan. Namely, susceptible pockets among male are beautifully identified, specifying clearly which birth cohorts were left unvaccinated due to inadequate switching of past vaccination policies. The cohort specific seroprevalence has even exhibited time-dependent increase in Figures 2C and 2D and less vulnerable to time-dependent analyses. Due to our scope of the paper in understanding the current situation in relation to past vaccination policy, we would like to maintain Figures 2C and 2D as part of main results in the main text.

Minor comments:

Line 166: the sentence "..increased pver time from 2003-13" must be corrected.
>>
We appreciate this comment, and correction with better precision was conducted accordingly.

Line 173: the sentence "...pocket born in those born..."must be corrected
>>
The error has been corrected (Page 8, Line 177)

VERSION 3 – REVIEW

REVIEWER	Pedro Plans-Rubio Public Health Agency of Catalonia, Spain
REVIEW RETURNED	18-Dec-2015

GENERAL COMMENTS	line 186. Add the following: If we adopt the 94% as the threshold with cut.off of HI >8, male adult population aged >30 years were vulnerable.

VERSION 3 – AUTHOR RESPONSE

[Response to Reviewer 1]
Reviewer Name: Dr. Pedro Plans-Rubio
Please leave your comments for the authors below
line 186. Add the following: If we adopt the 94% as the threshold with cut.off of HI >8, male adult population aged >30 years were vulnerable.
>>
We have revised the corresponding sentence as suggested by the reviewer:

“Additionally, when we adopted the cut-off value of HI ≥ 8 along with a 94.0% herd immunity threshold for the birth year cohort, only the adult male population of those born later than 1979–83, or all males aged 30 years or older in 2013, appeared to be vulnerable (Supplementary Figure 1A and 1C).”