Author's response to reviews

Title: Comparative estimation of chikungunya prevalence in Reunion Island

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Author’s response to reviews: see over
The survey study on pregnant women is not well described. The age of the pregnant women subgroup and of the total population are not mentioned. The housing type (urban/rural) of the subgroup is not described. The geographic location of the labs collecting the blood samples, is missing moreover the geographic origin of the women is missing too. This constitutes a problem knowing the CHIK seroprevalence difference among northern, southern, or eastern part of the island (Perrau et al., 2007 INSEE website). The exhaustivity of these 900 pregnant women included here is not mentioned.

The design of the pregnant women study was retrospective, and relied on stored sera of the labs blood banks, no additional information could be added on age, housing, or geographic origin of the women. In the revised paper, we give the amount and the precise location of the participating labs (additional figure, figure 1), as the representativeness of the 888 pregnant women included.

For these reasons and statistical ones, this subgroup (pregnant women) can not be representative of the entire population.

The 19 participating laboratories covered the entire territory and the collected amount of 888 sera was representative enough of the samples available in their bank. As an exact overlap between the area of residence and that of blood collection could not be verified, we think there is no significant cluster effect that could bias the representativeness of the pregnant women study. Moreover, there is no link between toxoplasmosis screening and Chikungunya exposure that could influence serology, and the 888 included sera were representative of the 3888 sera collected for the period. So, the selection (or allocation) bias, although it cannot be ruled out, may be of poor influence. A special statement crossing the repartition of the participating labs and the areas of transmission, as referred in Perrau et al., 2007 INSEE website, was added in the discussion.

The authors should clarify the originality of the population-based survey compared to work performed by Perrau et al. available in the INSEE website (http://www.insee.fr/fr/insee_reunion/prodser/pub_elec/revue/revue129/revue129_Chikungunya). They should also mention in their paper.

However, we don’t know how the data were collected: the questions, the language (French, Creole?), the date of the samples.

The data were collected in French. All field workers were Creole-native speakers and could translate Creole into French and inversely.

There is too little information provided here to compare the two different serological methods used here:
- samples: 100 µl of serum (pregnant) vs drop of whole blood collected on filter paper (cross-sectional study).
- labs (CNR vs GHSR)
- IgM and IgG (pregnant) vs IgG only (cross-sectional study).

The objectives of the paper were redesigned as requested by reviewer#2. In the revised paper, the problem is to draw comparisons between serological and clinical results (serosurvey vs CIRE data). The comparison of serology studies, as those for labs are lapsed.

Result section:

This important section is of poor quality. The data are not clearly presented.

This important section was redesigned. Additional figures and tables were added. The data are now more clearly presented with the text and tables not redundant.
The extrapolation using the pregnant women serological results can not be performed for statistical reasons. This subgroup is not representative of the entire population (not randomly chosen). There are repartition and selection biases. The authors should explain their hypothesis and calculus mode for this “extrapolation”.

The aim of the pregnant women serosurvey was not to be representative of the general population but indeed to give an idea of the attack rate at time of the epidemic expands. At that time, we were neither aware of the asymptomatic/symptomatic rates nor of the representatives of the pregnant women for the general population, which should have presaged of the herd immunity. We had a very hard time to inform public health agencies, practitioners and population on the magnitude of the outbreak, not enough to perform a large academic serosurvey (with CPP agreement, written consent, sampling plan), that is why we chose to design a “quick and dirty” serosurvey in pregnant women using stored sera because they are easy to obtain in this population.

Although, allocation and selection biases cannot be ruled out, as explained above, we think they are of poor influence and unlikely to significantly modify our point estimate of the magnitude of the outbreak. As no random selection was done for the pregnant women study, no 95% confidence interval can surround this rough estimate which was calculated very simply as the multiplication of the seroprevalence in pregnant women × the overall population : \(0.183 \times 787,836 = 143,386\).

The authors should explain how they were able to reconstruct the entire figure 2 with the cross-sectional study data that occurred from August 2006 through October 2006. The authors should explain how they confirmed clinical incidence, and how the serological data was attributed to a particular chik episode before August 6. The authors should precise if more than one chik episode or clinical episode per person existed.

The entire figure 2 (in the revised paper, figure 3) was reconstructed according to the declarations of the people sampled in the SEROCHIK population-based survey. In the revised paper, errors in the dating of declarations potentially due to a memory bias were corrected, in order to fit as close to the CIRE data.

People who declared more than one Chik episode or clinical episode per person were classified as Chik+. Relapses of Chikungunya were much less noisy than inaugural episodes and therefore easier to remember. We don’t know any subject classified Chik + after a relapse for who the inaugural episode was asymptomatic. The distribution of relapses was as follow: 36% in people who declared the Chikunungya, 30% in IgG+ people, 36% in people who declared the Chikungunya but were IgG- (false positives).

Discussion section:

The sentence line 7 is not supported by the data presented here: there may be other transmission spots missed by the 28 out of 45 labs included in this study.

The discussion was entirely redesigned. The sentence line 7 was dropped. We hope that there is no longer assertion not supported by our results.

The “congruent” or “relevant” result is due to a random effect, the labs and tested pregnant women are not representative. The authors can not over interpret their results.

We agree with the possibility of a random effect and the non-representativeness of both labs and pregnant women. However, we continue to believe this selection bias was of poor influence. We added a new paragraph discussing the selection phenomenon: since more than 50% of the sera of pregnant women came from south and west labs at a time when transmission was still predominant in theses regions… we think the allocation bias produced an unexpected geographical adjustment on the transmission level, as the amount of sera collected in the participating laboratories was correlated to the regional progression of the transmission.

The potential biases due to pregnant women recruitment are higher than the ones due to false positive or false negative.

We agree with this remark but recall the pregnant women study goal that was not to produce precise estimates of the seroprevalence in pregnant women but just a point estimate of the magnitude of the outbreak, assuming pregnant women would behave like everyone else. In fact, it is puzzling that pregnant women would be better protected against Chikungunya, a vector-borne disease, than anybody else, because they share many risk factors such as high body mass index, reduced motility, etc… and protective factors such as younger age or better protection. Indeed, the behavior of pregnant women is not so easy to presage, the allocation bias not so implicit!
Recent publications such as Sergon et al. 2007 Am J Trop Med Hyg would provided interesting elements of comparison.

The seroprevalence observed in La Réunion Island was far inferior to those reported recently from the Kenyan island of Lamu (75%) and the Grande Comore Island (63%) from the publication by Sergon et al., in 2007, as now stated in our revised paper. The absence of a case-control study on risk factors of Chikungunya transmission in Sergon survey don’t allow explanations beyond simple hypotheses, as far as the population and the urban development between Grande Comore and La Réunion are very different.

Conclusion section:

The last sentence “In conclusion…” is not supported by their own datas.

The conclusion was entirely redesigned. Assertions not supported by our own data were dropped.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct).

The background section is too long compared to the result section.

The IC95% is missing for many data: 18.2%, 12.6%, 88.5%, 90.3%, 75.9%. The number N is also missing in the population based study results.

95% CI were added only for the population-based survey, as the absence of random selection in the pregnant women study preclude any possibility to give information on the variability (variance) of the estimates.

Table 1

The number N is missing

Effectives were given for each tables which present now the data as the numbers of persons questioned and (row) or [column] percentages. It must be stressed that extrapolating the data in the sample questioned to the general population in the SEROCHIK survey implies a recovery (redressement statisitque in French) weighted on age, gender, geographical area, and type of habitat. This produces different results than simple extrapolations from rough estimates.

Lines in the manuscript margin should be removed.

Lines in the manuscript margin can be removed by unchecking the option “follow-up of the correction” in the word program.

Discretionary Revisions (which the author can choose to ignore)

Figure 1 and 2 may be grouped.

Figure 2 and 3 cannot be grouped, for they have different ordinate scales and figure 2 is now under copyright.
PATIENTS AND METHODS – PREGNANT WOMEN

1) Were these outpatients or inpatients, or both?

The patients enrolled in our two studies were most outpatients. In the rapid serosurvey, pregnant women were screened in outpatient laboratories. It was added in the first sentence of the first paragraph of the discussion.

2) In participating laboratories, were all sera collected from pregnant women during 15 jan-15feb 2006 included in the study? If not, how sera were selected?

888 sera were collected which were representative of the 3888 sera collected for the period.

A map locating the 28 participating laboratories and the 19 which provided the 888 validated sera out of the 46 covering the Reunion Island territory is given with the additional figure 1.

PATIENTS AND METHODS – GENERAL POPULATION

3) How was the stratification on “population size and type of habitat performed”? What does type of habitat mean?

The stratification was not done on population size as previously said but on age, gender, geographical area and type of habitat. Precisions are given about the regional boundary and the municipalities included in the four regions of Reunion island, as also illustrated in Figure 1.
We mean for type of habitat collective or individual housing.

4) A reference should be provided for the “Kich method” and it should be described in simple terms.

The Kish method was described in simple terms and referenced.

5) How many potential subjects were lost to each reasons for exclusion?

580 subjects (3022 – 2442) were lost for analysis, they are presented as follow:
3032 subjects randomly selected by INSEE.
- (212 refusals + 144 absences at home + 29 wastes of blood collection + 142 other reasons) + 8 duplicates (errors on ID) leaving 2513 subjects to be questioned by a field worker.
- 61 for insufficient blood collection leaving 2452 to participate
- 15 eliminated for duplicates or missing data + 6 duplicates leaving 2442 subjects to participate.

6) What does “redressed by INSEE…” mean?

“Redressed” means recovered. In our survey sampling, the recovery of our sample aims at improving the representativeness of the sample questioned on age, gender, geographical area and type of habitat. From the sample questioned, estimates are provided for the general population leading the results to be expressed from effectives into percentages. Therefore, in the section Results of the first draft, all the data presented were estimates expressed in percentages that took into account the sampling plan. In the revised paper, effectives for the sample questioned are provided in addition to the row and column percentages.

7) What was included in the questionnaire? What timeframe were subjects asked to consider in answering the questions?

In the population-based survey, standardized structured questionnaires were administered at home, in face to face, by INSEE field workers to collect data on demographics, way of life, current and past symptoms, treatment given, knowledge about Chikungunya, behaviour to avoid mosquito bites, type of habitat and environment.

Subjects were asked to declare any Chikungunya (or dengue-like illness) episode for which the onset of symptoms occurred between March 2005 and August 2006. The symptom module considered current symptoms (still present at the time of questionnaire) and past symptoms (vanished since the episode)
This article is only interested in the statement of Chikungunya and serology. Aspects on risk factors which are alluded to in conclusion will be developed in a case-control study, which would be the subject of another paper.

RESULTS – PREGNANT WOMEN

8) Several prevalence estimates are given in the text that could be assessed more efficiently in a table. For example, a table of test result (+, -) by antibody (IgG, IgM, IgG &/or IgM), stratified by study pregnant women, general population), would convey the key results of the manuscript.

An additional Table is given (Table I) presenting the Chikungunya serological status (positive/negative) stratified by study. In the pregnant women study, IgM and IgG results are pooled assuming the absence of previous circulation of Chikungunya virus in La Réunion and the absence of previous infection (or IgM-/IgG+ asymptomatic case) in the 27 women native from Comoros islands (including Mayotte) let unimportant the question to distinguish IgM and IgG status (i.e., all seropositive cases, whether assessed by IgM or IgG were recent infections contracted during the Reunion island outbreak).

9) A point estimate should be provided to accompany the range of possible number of infections based on results in pregnant women (154,000 to 190,000) and the type of range used should be identified (is it a 95% CI?).

Neither valid (unbiased) point prevalence estimate nor 95% confidence intervals can be provided to know the possible number of infections in pregnant women by February 15, 2006. The reason is that we have no idea on the variability (variance) of the calculated estimates because there was no random sampling in the pregnant women study. The range of 154,000 to 190,000 was given as a window of possibilities calculated from low- and high-progression of positive IgM observed between week 3 (January 15, 2006) and the incomplete week 7 (February 15, 2006) at a time of exponential progression of the outbreak. According to the CIRE data, the number of cumulative cases at week 6 (February 6-12, 2006) was 110,000 (www.invs.sante.fr/presse/2006/le_point_sur/chikungunya_160206) and 157,000 at the end of week 7 (February 13-19, 2006) (www.invs.sante.fr/presse/2006/le_point_sur/chikungunya_230206) approximating the daily number of cases at 6714 for this critical period and the number of people infected by February 15, 2006 at 110,000 + 3 × 6,714 = 110,000 + 20,142 ≅ 130,000.

However, to fulfill the remark, we now give a point prevalence estimate (acknowledging it is probably biased) to standardize the presentation of our results. The point prevalence of 18.2% by February 15, 2006, led us to roughly estimate at 143,000 (787,836 × 0.182) the number of persons infected at that time.

10) Table I would be easier to interpret if either the row or column margins totaled to 100%. For example, if the row margins totaled 100%, one could read directly from the table % seropositive among those who answered “No” and those who answered “Yes”. In its current form, the Table does not allow comparison of seropositivity by questionnaire response or of questionnaire response by seropositivity, without calculation. Also, I suggest dropping the 95% CI from table and adding sample size for each cell (e.g., each cell could have the cell sample size and in parentheses, the %, based either on the row or the total sample size). Footnotes “a” and “b” could be dropped. Sample sizes are now given for each cell with row and column percentages. Footnotes “a” and “b” have been dropped.

The data following the sentence in question are now presented in the revised Table 1 (Table 2).

12) The data presented in the last paragraph of the Results section are a bit confusing. Since 88.5% of people who self-reported chikungunya fever also reported fever and arthralgia, there must have been separate questions on whether subjects had chikungunya and more specifically on what symptoms subjects had. These data would be easier to assess in a table, showing questionnaire answers on symptomatology by serological results.

The data presented in the last paragraph of the Results section are now presented in a new table (Table 3) showing questionnaire answers on clinical signs by serological results.

13) In fact, Table 1 could be expanded to include both the question it currently has as well as questions on symptoms. Demographic and other questionnaire data also may be helpful to see by serology, either in the same table or separately.
We acknowledge demographic and other questionnaire data would be interesting to see by serology. Nevertheless, at this point of the revision, we want to remind our paper goal. The purpose of the current study was really to refine and to discuss the surveillance-system estimates at two critical periods of the 2005-2006 outbreak. That is why we propose for the moment not to fulfill this remark arguing that answering the remark would pose more new questions. We acknowledge the question is important and merits further consideration. It should be done thoroughly using multivariate regression model analysis. Indeed, we are preparing a new manuscript that will present a multivariate (thorough) analysis of Chikungunya serology. This article will be ready for submission by December 31, 2007. To date, and until we have submitted our new paper, we are puzzled to fulfill the current remark, afraid to loose homogeneity in multiplying objectives, and definitely to frustrate BMCID readers with an incomplete analysis. In fact, we strongly believe multiplying objectives will deserve the current paper.

DISCUSSION

14) The possibility that some “asymptomatic” or “atypical cases” might have been infected before the current epidemic should be discussed, especially since only IgG was tested in this general population.

The rapid survey in pregnant women showed that only one serum among the 162 (0.6 %) collected was IgM-/IgG+. Thus, it seems very unlikely that a significant proportion of the population sampled by our population-based survey was infected before the current epidemic. Moreover, none of the 27 women native from Comoros islands sampled by the population-based survey was IgG+. In a retrospective study such as the SEROCHIK survey, the “asymptomatic” or “atypical” cases should evoke first a memory bias for which false negatives in symptoms (in fact “paucisymptomatic” cases) were not enough afflicted to remind their illness long time after the episode. We must keep in mind that the SEROCHIK survey was performed between August and November 2006, 2 to 15 months after the onset of symptoms.

15) I’m not sure how the Positive Predictive Value for clinical diagnosis “at the peak of the outbreak”, given as > 95%, was calculated. The data in this paper from the peak of the outbreak comes from pregnant women, for whom clinical information is not provided. The general-population study did include self-reported clinical information, but 1) it is not clear that patients were asked to report whether they had received a diagnosis of chikungunya fever, or whether they were asked to report symptoms and their own assessment of whether they had chikungunya fever; 2) this study occurred after the epidemic, not at its peak; and 3) PPV for self-report of chikungunya fever based on Table 1 would be 31.4%/35.9% = 87.5%.

Patients were asked to declare a Chikungunya episode or any symptoms compatible with such a diagnosis. In consequence, people who declared the Chikungunya were composed of serologically (IgM+ or IgG+), virologically (PCR+), or medically confirmed cases, and self-reported cases. The PPV for the ten most frequent self-reported symptoms is now given in the new Table 3. Ditto, the PPV for the combination of sudden fever and incapacitating arthralgia is corrected to 87.5%.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Title:

1) The tile is ambiguous – it suggests that prevalence estimates based on different methods, or in different populations, have been compared, but does not identify those methods or populations. It may be more helpful to identify the study as seroprevalence study – e.g., “Seroprevalence of Chikungunya virus infection in Reunion Island”, or something like that.

A new and unambiguous title is given.

Abstract:

2) Background: the meaning of the sentence, “However, it was not known…” is unclear. This sentence could probably be omitted, and the following sentence revised to introduce the present study as a serological assessment that complements previous clinical diagnosis-based prevalence estimates.

Two unambiguous sentences situating the context and the aim of our study are now given.
3) Methods: the questionnaire should be mentioned and key questions identified.

As stated above, this article is only interested in the statement of Chikungunya and serology, the comparison of clinical and serological-based estimates. The questionnaire is not necessary to be mentioned. Nonetheless, key questions are now identified.

4) Results: Prevalence should be given separately for IgM+, IgG+, and IgM+/IgG+. If space permits, comparison of ELISA results to questionnaire results should be included. A range of number of cases in Reunion based in pregnant women is provided (154,000 to 190,000), but a point estimate based on general-population results is provided (300,000). These results should be communicated consistently – e.g., point estimate alone for both.

Prevalence is now given separately for IgM+, IgG+, and IgM+/IgG+. Comparisons of serology and declarations results have been included. The range of number cases for pregnant women was replaced by a point estimate, whilst the point estimate on general population was surrounded by a 95% confidence interval, as appropriate. More details on estimators and their calculation are now given in the text and in the current rebuttal letter.

5) Conclusions: The seroprevalence estimates should be compared to previous estimates by CIRE using clinical diagnosis alone.

Seroprevalence estimates are now compared to previous estimates by CIRE in the Result sections, reserving the conclusion for the major issues drawn from our paper.

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RESULTS:

1) The sentence, “the rate of asymptomatic cases combined to atypical cases…” might be clearer if rephrased as “The prevalence of seropositivity with negative self-report of chikungunya fever diagnosis was 5.0% (95% CI 3.9-6.3%)” or something like that.

This sentence was rephrased as requested.

DISCUSSION:

2) The authors mention that their results “allow evaluating the order of magnitude of seroprevalence and herd immunity”. Their estimate of seroprevalence certainly is much better than an order-of-magnitude estimate. But there’s no direct assessment of herd immunity, so I suggest dropping reference to it here.

3) I don’t see that references 13 and 15 provide the figures given in parentheses for asymptomatic infection rate in West-Nile and dengue, respectively. It’s possible that calculation is required or that I’ve misses where these papers provided those numbers, but the authors may wish to verify.

Calculation is required to deduce the rate of asymptomatic dengue fever cases. For instance, in the reference 13, it can be deduced from the inapparent/acute dengue infections ratio = 4.3% / 3.6% ≅ 1.2 ≅ 54.6 % / 44.4 > 50%.