Author's response to reviews

Title: Use of genotyping based clustering to quantify recent tuberculosis transmission in Guadeloupe during a seven years period: analysis of risk factors and access to health care

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Version: 2 Date: 27 June 2013

Author's response to reviews: see over
Reviewer's report
Title: Use of genotyping based clustering to quantify recent tuberculosis transmission in Guadeloupe during a seven years period: analysis of risk factors and access to health care
Version: 1 Date: 18 May 2013
Reviewer: Catherine Arnold

Reviewer's report:
Minor Essential Revisions
The authors have carried out a reasonably large study on Mycobacterium tuberculosis strains in Guadeloupe over a number of years and have analysed both epidemiological and molecular data. The molecular methods have evolved considerably during the study period and the authors have adapted the study to accommodate this. The conclusions are sound. This study could be improved by being more concise as it is sometimes difficult to follow the results section. The report is well written but somewhat long and seemed to be over analysed in places.

Author: We would like to thank the reviewer for her positive comments. We have now carefully addressed all the minor comments as suggested (including deleting whatever sentences could be deleted without altering the message conveyed).

Specifically:
1. The word 'Precariousness' is not commonly used in English and a suitable alternative should be used.
   Author: Done. “Precariousness” used in text p7 and p10 has been replaced by “precarious living conditions” and “poor living conditions”

2. Table 1: there is no indication of what the figures are in brackets, i.e. need to specify percentages.
   Author: Done, the percentage symbol appears now in Table 1.

3. There is no mention of the 24-loci scheme in use in the UK and of this study in relation to the use of Next Generation Sequencing for the ultimate resolution of related strains and how whole genome sequencing will affect the use of this type of marker. Personally I feel that they will still be relevant in some contexts and situations but perhaps the number of loci studied would need to be increased.
   Author: We already mentioned in the “discussion” section that: “The suggested minimal requirement based on our study is 12-loci MIRUs, although the optimal format for molecular epidemiological investigations today is a new 15-loci format [33, 34].” Indeed, the use of 24-loci MIRU format is rather meant for phylogenetic analysis.
   However, we have now added the following sentence at the end of the discussion which refers both to 24-loci scheme as well as Next Generation Sequencing as recently reported by walker et al. in UK:
   “Ultimate comparison of clusters retained after 12- and 15-loci MIRUs should be done using 24-loci MIRU-VNTRs [34] followed by the whole genome sequencing [37] of specific clones, in order to shed light on epidemiological and phylogenetical links between strains involved in active transmission, as well as to quantify how much M. tuberculosis isolates vary at the genomic level between epidemiologically linked patient clusters. [38].”

Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests
Reviewer's report
Title: Use of genotyping based clustering to quantify recent tuberculosis transmission in Guadeloupe during a seven years period: analysis of risk factors and access to health care
Version: 1 Date: 23 May 2013
Reviewer: Sebastien Gagneux

Reviewer's report:
This paper reports on a molecular epidemiological study of TB conducted in Guadeloupe between 1999 and 2005. During these 7 years, the authors recruited a total of 129 culture-positive TB patients from which M. tuberculosis (Mtbc) isolate could be obtained. Among the main findings are that 51% of these cases where foreign-born (mainly from Haiti), and that among these foreign-born patients the proportion of TB/HIV and extrapulmonary TB was higher than in French patients. The proportion of recent transmitted TB was estimated at ~18%. No risk factors could be associated with clustering.

My comments and suggestions for (essential) revision are as follows:
1) Ethics: under the Methods section the authors claim that the TB patients provided “oral consent” but under “Ethical considerations” it is stated that the study participants signed and agreement letter. Please clarify. Also, was this study reviewed (and approved) by a formal ethics committee (i.e. what are the French National Order of Physicians and the French National Commission of Informatics and Liberties)?

Author: It has now been appropriately explained and detailed in the text as follows:

“The study protocol was presented to the Conseil National de l'Ordre des Médecins (http://www.conseil-national.medecin.fr/qu-est-ce-que-l-ordre-1206), which guarantees the quality of healthcare provided to the patients. As recommended, it was thereafter submitted to the Commission nationale de l'informatique et des libertés (CNIL; http://www.cnil.fr/english/the-cnil/operation/#c1556) which ensures the protection of personal data. Patients were duly informed about the description and aims of the study in writing, and those enrolled after their oral consent were asked to sign an agreement letter, returned to investigators. The study was approved by CNIL under a formal agreement number 999343 (modalities of registration, storage and analysis of epidemiological, clinical and socioeconomic data are available online from: www.pasteur-guadeloupe.fr/tb/projects/tuberculose.pdf).”

2) The authors also looked at “access to healthcare” and observed significant delays, which I think is interesting and important. However, these findings are not referred to in the abstract. It would also be interesting to look in a stratified manner comparing foreign-borns and French.

Author: We have now added the following text in the abstract and discussion sections, respectively:

“The study revealed an important delay in access to healthcare with a median delay of 74.5 days between the 1st symptoms and clinical suspicion of TB.”

“Interestingly, the management of patients improved in the period 2006-2011 [23] with a median delay of 55 days between the 1st symptoms and clinical suspicion of TB instead of 74.5 days in 1999-2005; as well as the delay for clinical suspicion of TB after a 1st medical consultation (18 days vs. 26.5 days). Comparison of foreign-born vs. French patients did not reveal significant differences in access to healthcare.”

2) The socio-economic assessment and the finding of 824 EUR/month is difficult to interpret in absence of a reference. Please provide some data that help put these figures in perspective.

Author: It is mentioned as follows on p10:

“Furthermore, TB patients in our study were characterized by a very high rate of unemployment (70.7%; Table 1) and a below average income close to the official limit of poverty in France (monthly income of 824 EUR vs. 812 EUR/month in 2001; [29, 30]).”
3) Foreign-borns were more likely to be TB/HIV co-infected and also showed a higher proportion of extrapulmonary disease. These observations are likely not independent which has not been assessed since only univariate analyses have been performed. TB/HIV are generally more likely to have extrapulmonary TB so it would be important whether these variables are independently associated with foreign-borns.

**Author:** Stratified analysis was not performed so it is not possible to be conclusive; nonetheless, we have added the following sentence on page 10:

“In our study, foreign-born patients were more likely to be TB/HIV co-infected and also showed a higher proportion of extrapulmonary disease. These observations are likely not independent, although stratified analysis was not performed to be conclusive.”

4) The authors refer to the “ill-defined” T spoligotype, and as shown in Figure 1, these strains are indeed paraphyletic. So what is the justification to group them into one group?

**Author:** This phylogenetical discussion is unfortunately beyond the scope of our own paper.

5) On multiple occasions the authors refer to “contamination” which would better be described as “infection”. Please change accordingly.

**Author:** Done.

6) The discussion starts with the claim that this is the first prospective molepi study of TB in Guadeloupe. However, the authors published a similar study in 2012 where they looked at patients recruited between 2006-2011 (Ref. 23), hence this claim is not entirely correct. I just would delete it.

**Author:** It is not the case since the recently published work corresponding to ref 23 (Cadelis et al.) was not based on genotypic profiles of *M. tuberculosis* isolates, so our is still the first prospective MolEpi study.

7) Overall, it is not quite clear what this new study adds in terms of new knowledge compared to the previously published one. Overall, the authors should stress these novel aspects more.

**Author:** See response to the point 8. In addition, it is the first time that risk factors are studied in a prospective manner in conjunction with genotyping data in Guadeloupe.

8) Among the clustered cases, where there instances where foreign-borns infected French patients or vice versa? What was the proportion of such “mixed” clusters?

**Author:** Added on page 11:

“In our study, mixed clusters involving both foreign-born and French patients concerned 11/18 or 61% of clusters by spoligotyping and 5-loci VNTRs; however after retrospective 12-loci MIRU typing their number was reduced to 5 mixed clusters containing a total of 18 cases. Thus the suggested minimal requirement based on our study is 12-loci MIRUs, although the optimal format for molecular epidemiological investigations today is a new 15-loci format [33, 34].”

Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

**Author:** We hope that this revised version will now be acceptable for publication in BMC Infectious Diseases.

Kind regards,
Nalin Rastogi
(Corresponding author)