Reviewer's report

**Title:** Mycobacterium tuberculosis ecology in Venezuela: Epidemiologic correlates of common spoligotypes and a large clonal cluster defined by MIRU-VNTR-24

**Version:** 1  **Date:** 17 February 2009

**Reviewer:** Viviana Ritacco

**Reviewer's report:**

- Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)

1. **ABSTRACT.** There is certain ambiguity in the attributes ascribed to SIT 605. The statement that this SIT is “infrequent in regions other than Valencia” seems to disagree with the previous one saying that “all six SITs were the most common in almost all regions” and the conclusion stating that SIT 605 “appears to be spreading throughout the country”.

2. **ABSTRACT.** The statement “Six SIT’s cause 49% of cases of tuberculosis in Venezuela and are the most common in all regions” in the Conclusions, is a repetition of Results.

3. **ABSTRACT.** Again in Conclusions, a repetition of Results is found in the first clause of the sentence “Patients with SIT 53 were older and more commonly smear negative, suggesting that…”. This could be simplified as follows “SIT 53 appears to be less virulent and associated with reactivation of past infection in older people.

4. **ABSTRACT.** Only 3 of the 6 most common SITs are commented in the abstract. Any comment on the other 3, in particular SIT 93, the second most frequently found in the study?

5. **BACKGROUND.** Were the genotypes differently distributed through time in the 10 yr period of study? Is there any temporal evidence of some genotypes emerging and others disappearing as suggested in the last paragraph of this section (page 6, line 8)?

6. **DISCUSSION:** Please, fundament the assertion “SIT 605…. appears to be spreading around the country” in page 11, last 2 lines, which is reiterated in the abstract.

7. **DISCUSSION.** (page 12) According to supplementary data, all 5 strains of the Beijing family were isolated in Caracas, the most cosmopolitan of the areas in this study. There is some evidence of Peru being a South American reservoir of Beijing strains (see Aristimuño JMM 2007 & Ritacco MIOC 2008). It would therefore be of interest to know if patients with Beijing strains in this study were born in Venezuela, in other South American country or overseas.

8. **DISCUSSION.** (page 12, line 10) How would drug resistance as such explain
the extensive local transmission of a genotype?

9. Results and Discussion are lucid, fluent and stimulating but there is some overlapping and some information is presented for the first time in the discussion. Perhaps both sections of the manuscript could be merged under a joint heading “Results and Discussion”.

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

1. TITLE PAGE. Affiliation of corresponding author is camouflaged because an enter is missing before it
2. TITLE PAGE. What is the contribution to the work of authors marked with symbols other than “*”?
3. TITLE PAGE. The initial E for the author named Howard Takiff is either missing in the authors list or redundant in the list of emails.
4. ABSTRACT. Mycobacterium tuberculosis is abbreviated the first time it is mentioned in the abstract and written in full the second time. Please invert this order.
5. THROUGHOUT BODYTEXT. Different abbreviations (“ST” and “SIT”, singular; “SITs” and “SIT’s”, plural) are used for the same concept; when mentioned individually, some SIT numbers are preceded by a space and others are not (“SIT605” and “SIT 605”). Please, unify style.
6. THROUGHOUT BODYTEXT. Some spaces are missing, particularly before references in brackets.
7. INTRODUCTION. page 5, line 4: replace “100.000 (WHO Report 2.006) by “100,000 (WHO Report 2006)”; line 15: replace “Laboratory” by “Laboratorio”.
8. BACKGROUND. First paragraph. Mycobacterium tuberculosis should be written in full the first time it is mentioned in the bodytext.
9. BACKGROUND. page 4, last paragraph: Suggested modification: “The more recent technique of microsatellite analysis (34), MIRU-VNTR 24 loci, is less technically demanding than the IS6110 RFLP typing technique, can discriminate at strain level and (in place of but) is also promising for use in phylogenetic studies.”
10. BACKGROUND. Page 5, line 15. Please, clarify the expression “…have been working in national networks with the National TB Control Program …”(does it mean “in collaboration with”?)
11. DISCUSSION: page 11, line 3: Replace “Camaroon” by “Cameroon”.
12. CONCLUSIONS: (page 14, line 19: “…of the Venezuela..” (delete “the” and duplicated full stop). “The only two strains with ST605 described outside of Venezuela were isolated in New York in patients from Colombia, and (suggested modification: …”were confirmed by MIRU-VNTR to belong to the Valencia”… instead of “ST 605”) cluster.”
13. METHODS: Could you please make clear if every strain corresponded to a
different patient?

14. METHODS (Page 15, line 12): The statement “The strains were obtained by culturing sputa or non-pulmonary clinical specimens that were positive for Acid Fast Bacilli by microscopy.” is not congruent with results of correlation of clustering with epidemiological analysis (page 9) where only 75% of the patients were found to produce AFB positive sputa and “…patients with SIT 53 were less likely to have bacilli seen in their sputa compared to patients with SIT 17 and SIT 605”. Also, the abbreviation AFB appears several times in the bodytext without previous decoding.

15. METHODS (Page 16, line 12): Please clarify the statement “For some of the patients whose isolates were included in the study, the information on these parameters was not available” in the light of this other in RESULTS (Page 8, line 5) “…for several parameters the information was recorded for 25% to 50% of the patients”. The validity of results on correlation with epidemiological parameters can be argued if the study sample is not clearly described.

16. METHODS (page 18) & RESULTS (page 7) the description of SNP method is exiguous, compared with the description of methods more widely used, like spoligotyping and MIRU-VNTR genotyping. Likewise, readers would welcome some clarification on SNPs analysis and also information on how many/which isolates with SIT 605 were analyzed, given that some MIRU-VNTR variation within this SIT is observed in the study.

17. METHODS (Page 15, line 7): Replace “capitol” by "capital”.

18. METHODS (Page 16, line 18): Replace “(31, 32, 34) (33)” by “(31-34)”

19. METHODS. Page 17, line 1: replace “Megative” by “Negative”; line 7: replace “dendograms” by “dendrograms”

20. REFERENCES. The list of references included under this heading does not fit citation numbers in the bodytext. Please replace it by the alphabetical list (without heading) found misplaced in pages 30-34.

21. TABLE 1. The table appears truncated at SIT ranked 27 while, according to its heading, it should present up to SIT ranked 36, the last SIT with a frequency of 5.

22. FIGURE 1. In captions for Caracas and Sucre, years are truncated and illegible.

23. FIGURE 2. Graphics look elongated

24. FIGURE 3. Two slightly different headings are found for the same figure, one in figure list and the other in the figure itself. The box including SIT 605 strains is displaced downwards.

Please note: Items 20 to 24 can be distortions produced upon pdf conversion.

- Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

none
Level of interest: An article whose findings are important to those with closely related research interests

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