



SDI Review Form 1.6

Journal Name:	International Journal of TROPICAL DISEASE & Health
Manuscript Number:	2015_IJTDH_16321
Title of the Manuscript:	Mycobacterial Infections in Humans: Buruli Ulcer in West Africa
Type of the Article	Original Research Article

General guideline for Peer Review process:

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound.

To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Compulsory REVISION comments	The title is misleading. "Molecular diversity of environmental Mycobacterium ssp". In this study only M. ulcerans was worked on. Further no sequencing was done to confirm molecular diversity. The use of MIRU1-VNTR profiling alone is not enough to justify the title. The same applies to the running title "Mycobacterial infections in Humans". Only BU was worked on. Both titles should be rephrased or the authors should provide enough evidence to justify these. There are major grammatical and punctuation errors in the text. I presume the authors are French speaking and would recommend that they find an English speaking person to review the correctness of the sentences in the entire document. Line 58-59: The authors will do well to provide reasons for the failure mentioned in this sentence. Table 3: present the ct values for the positive and negative control samples. Positivity and bacterial loads can only be determined in relation with the	Answer : We agree with the reviewer and have revised a new title: Clonality of Mycobacterium ulcerans by using VNTR-MIRU typing in Ivory Coast (Côte d'Ivoire). For the running title, we have changed as Environmental Mycobacteria infections: Buruli ulcer in West Africa. Answer :We agree with the reviewer and review the entire document by English speaking person. The new version was been revised. Answer:The failure of molecular test by IS2406 or IS2404 is doing because PCR inhibitors in some environmental samples, and clinical and environmental samples often contain complex mixtures of DNA that may generate false positives. Answer: We agree with the reviewer, all



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	positive and negative control results. Line 152: 85.7% Table 4: present the VNTR profile for the positive control sample. It would be good to combine Tables 3 and 4 so the reader could see the profiles together with their locations. What do the results mean in terms of the genetic variability in <i>M. ulcerans</i>? It would be good if the authors could explain their results further. Studies have suggested patho-adaptation (Narh et al., 2015 published in Plos NTD, as an example). Besides the VNTR profiling, the study does not provide enough evidence to conclude that the circulation of different profiles or genotypes of <i>M. ulcerans</i> for BU in Côte d'Ivoire explains the endemicity of the disease in the country. How is the endemicity related to the profiling? In the results only one VNTR profile could be determined for 6 out of 21 samples. If the samples had been worked on correctly and the profile for the rest of the samples could not be determined,	samples were determined in relation of positive control (ITM) and negative control . Answer : Because the size of both tables, we add a new column for the location from table 1 in table 4 by the suggestion of the reviewer for the profile and their locations. Answer: The genetic variability in <i>M. ulcerans</i> was determined by the existence of genotyping markers like MIRU, ST1, or VNTR or tandem repeats in genome. The method is well validated for clinical and environmental samples. By the writing of the present article, the results presented by Narh et al 2015 were not published. Futher studies with whole genome sequencing of <i>M. ulcerans</i> will elucidate the entire diversity of M.U in West Africa by patho-adaptation or existence of new virulence genes. Answer :We agree with the reviewer, VNTR profiling does not providence enough evidence but suggest the evidence of genetic diversity of the strains. The sequencing projet will elucidate the correlation of clinical manifestation of BU and strains diversity in endemic sites. Ivory Coast has the highest confirmed cases of BU in West Africa.
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	sequencing the samples would have helped in identifying the causes, such as SNP changes. This has not been done. Perhaps the unidentified profiles are new? Presenting the profile for 6/21 (28.6%) of the samples is not enough to say that Profile C is the most common in Cote d'Ivoire. Similarly, the investigators mentioned in the abstract that different genetic strains were circulating in the country. The data provided is not enough to come to that conclusion. The authors also provided results without giving explanations. For example, they mentioned that <i>The strain 2070 with the profile (3,1,1,2) and the strain 2071 with the profile (1,-, 2,2) were collected in the same site Djekanou and have distinct genetic diversity. It would have been good to let the reader know why these profiles are distinct. What makes them distinct?</i>	Answer : For the studies, all samples were analysed with the same method, and the inhibition in the PCR was done. Our results were consistent because all controls have been included; we believe that the sequencing of the strains will give the detection of SNP changes by deletion or insertion. 28.6 % is not enough to be most profile. We have changed it the Line 245 . Answer: The strain 2070 and 2071 were from the same endemic site Djekanou, both were isolated from ulcer. By PCR typing of the strain, the strain 2070 has amplify for 3 copies MIRU while the strain 2071 has amplify one copy MIRU1 and no amplify VNTR locus 6. Epidemiology investigation in Djekanou has not identified the source of transmission in the environment (unpublished Data)
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<u>Minor</u> REVISION comments	Line 11: delete a mycobacterium called Line 21: first analysed in molecular diagnosis of BU using Line 22: ...we have analysed the strains ... Line 24: ... clinical samples were positive... Line 31: strains of M. ulcerans Line 32-33: revise the sentence. Line 37: caused by ... Line 41-43: revise sentence... I presume the authors are referring to the usefulness of molecular methods in diagnosis, due to the low growth of the bacteria in culture medium. Line 44: revise sentence Line 48-51: Revise sentence Line 52: aquatic insect (7) and ... Line 55: have not been elucidated Line 56-57: revise sentence Line 64: fingerprint Line 66: (13). Tandem repeats ... Line 67: copy numbers Line 67: backbones? Line 68: enterobacteria (16-17). Line 69: Mycobacterium marinum, a fish pathogen, and ... Line 71: for typing M. ulcerans and other environmental mycobacteria allows the discrimination between clinical and ... Line 77-79: revise sentence Line 81: discriminate between ... MPMs (14-15 Line 83: Mycobacterium sp Line 86: swabs Lines 87-88: males and females Line 88: first clinically confirmed for BU Table 1: The origin is not necessary as all sample origins are human. Replace nd with unidentified. Line 97: swab Line 98: mixed for 2 min	Answer: We have revised the article with all minor comments from the reviewer.
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	Line 99: genomic DNA extraction Line 102: write the common name of $\text{CH}_3\text{CO}_2\text{Na}$ Line 103: washed ... The control strain Line 111: annealing at what temperature? Line 113: real-time PCR machine Expand all ONE_SENTENCE paragraphs, or merge them with other paragraphs	
<u>Optional/General</u> comments		