Author’s response to reviews

Title: Risk Factors for Mycobacterium ulcerans Infection (Buruli Ulcer) in Togo ─ A Case-Control Study in Zio and Yoto Districts of the Maritime Region

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Author’s response to reviews:

COVER LETTER FOR SUBMISSION OF REVISED MANUSCRIPT

16.10.2017

Dear Editor,

We hereby submit a revision of our manuscript entitled “Risk Factors for Mycobacterium ulcerans Infection (Buruli Ulcer) in Togo ─ A Case-Control Study in Zio and Yoto Districts of the Maritime Region” by Issaka Maman as corresponding author to be considering for publication as Research article in the BMC Infectious Diseases.
With reference to the report from reviewers including Minor and Major revision after the first version submitted of this article, we have made some changes in our manuscript which are presented below point-by-point:

Reviewer 1: Kingsley Nnanna Ukwaja, M.D.

Abstract

* "bathing with water from open boreholes (p = 0.01)"

Please be consistent in using either OR or P-value:

The OR replaced the p and use now consistently. However this sentence has been deleted and the results section was revised including the multivariate results as stated by the author

* Please in multivariable analysis, only three factors: age (<10 years (OR =11.48 (3.72-35, 43) and 10- to 14 years (OR = 3.63 (1.22-10.83), receiving insect bites near a river (OR = 10.76 - 74.27)) and bathing with water from open borehole (OR = 5.77 (1.11-29.27)) were independent predictors of acquiring BU infection. I feel only these factors should be included in the Abstract:

The results section and conclusion have been revised including the multivariate results as stated by the author

Introduction and Methods Section

These sections are well-written

Thank you so much for appreciating these two sections

Results Section

* This is fairly well-written

The English language writing has been reviewed by a professional

* The OR obtained following multivariate logistic regression analysis should be labeled adjusted odds ratio (aOR): In multivariate results, we changed the labeling of OR into aOR.

* Table 5: what is the meaning of "ans" in Table 5?
We apologize of the use of “ans” This word was dragged by mistake. It’s a French word that mean year and has been translated to years

Discussion

*Page 14, line 35-42: “Our study also identified other water sources of M. ulcerans infection such as swimming in a river, frequently crossing a river, receiving insect bites or injuries of cuts near the rivers. The same results were found in Ghana [12,38], Ivory Coast [13], Cameroon [14] and Benin [11].” There is need to highlight that after adjustment for potential confounders, this factors were no longer risk factors for BU infection.

While reviewing this paragraph, we found that after adjustment for potential confounders only receiving insect bites was associated with increasing risk of BU infection. Then, we reviewed the discussion by separating studies where factors such as swimming in a river or frequently crossing a river were significant risk from studies they were no longer risk factors.

Therefore, it is important to discuss the differences with studies who found them significant and reasons why they did not remain predictors of BU following adjustments

To discuss the difference with other studies, we looked for any potential effect of age because the risk of BU infection was higher in <10 years and 10-14 years than other age groups. Then, we calculate the aOR by crossing insect bites among cases and controls by age groups. We found the effect modification of age that increases the risk of BU only in 10-14 years group (aOR=7.8, 95%CI=1.48-41.21) while receiving insect bites.

The main reason of the difference between these studies was the age. We made cross tabulations between because after the following adjustments, the insect bite was increase significantly BU infection risk in 10-14 years age

*Page 16 line 18-19: "This study had some limits. We did not reach all participants especially some BU cases due to their unavailability during the survey time."

Please change "limits" to "limitation".

Why were the controls very few since you used the 1 case: 2 controls in enrolment for the study?

Explain the effect of inadequate controls as limitations. There are differences in Age, Sex and educational level between cases and controls. It is important to highlight this in the limitations and how it may affect the study findings.

The sample size was calculated based on the proportion of households using water from unprotected sources which was higher than the prevalence of BU. However, due to the lack of the exact prevalence of BU disease and with knowing that water sources was reported to increase risk of BU infection, we used this parameter to estimate the sample size. The number of newly BU cases confirmed in Togo every year is low and varied from 30 to 65 patients. During the study period, we found 91 BU cases but 8 patients were not available at the survey time. The main concern with the number of controls was due to the fact that in many household, there were often two or three patients and even in one home up to six. In these household, it was difficult to enroll two fold of controls. Moreover, as most of patient were under 10 years, it was difficult to interview children who were not capable to describe their own activities which are usually driven by their parent’s duties. The reason we had decided to use parents for some cases as their controls.

Conclusions

* "This study found some major significant risk factors for M. ulcerans infection. The major risk factors were age (<15 years), bathing with water from open boreholes, swimming or frequently crossing a river, as well as receiving insect bites or cuts near rivers. Factors identified as protective from BU include wearing pants or hats during agricultural activities, frequent use of detergents for washing clothes or dishes and hygiene practice."

These were not the factors that were independent predictor of BU following multivariable regression analysis. The conclusion should reflect the final analysis please.
The conclusion has been reviewed including the multivariate results as stated by the author and stated as below:

This study identified age, bathing with water from open borehole and receiving insect bites near a river as potential risk of acquiring BU infection in Zio and Yoto districts of the Maritime Region in south Togo.

Reviewer 2: Lance A. Waller

General Comments:

1. Thank you to the authors for an interesting study. I found the results interesting, but I do have many suggestions for making the language more precise. I do feel it is important to qualify associations by their statistical significance, especially because the study did not quite attain the desired sample sizes to achieve the desired statistical power.

   The association has been requalified using the statistical significance and we will address to the issue of the statistical power related to the sample size in suggestion 2.

2. More specifically, on page 9, the authors’ sample size calculations require 100 cases and 200 controls, but the study ended with 83 cases and 128 controls. Does this mean the study is underpowered? The sample size was calculated based on the proportion of households using water from unprotected sources which was higher than the prevalence of BU. However, due to the lack of the exact prevalence of BU disease and with knowing that water sources was reported to be associated to the BU infection, we used this parameter to estimate the sample size. The number of newly BU cases confirmed in Togo every year is low and varied from 30 to 65 patients. During the study period, we found 91 BU cases but 8 patients were not available at the survey time. The main concern with the number of controls was due to the fact that in many household, there were often two or three patients and even in one home up to six. In these household, it was difficult to enroll two fold of controls. Moreover, as most of patient were under 10 years, it was difficult to interview children who were not capable to describe their activities which are driven by their parent’s duties. The reason we had decided to use parents as their controls.
The potential consequences of not meeting the sample size required for the desired power and should be addressed in the Results on page 10 and in the Discussion.

The potential consequences have been addressed in page 10 and in discussion especially in the limitation of the study.

I am particularly concerned about the small proportion of controls in the under 10 age group which may be driving results (see General Comment 4 below).

The main concern with the number of controls was due to the fact that in many household, there were often two or three patients and even in one home up to six. In these household, it was difficult to enroll two fold of controls. Moreover, as most of patient were under 10 years, it was difficult to interview children who were not capable to describe their activities which are driven by their parent’s duties. The reason we had decided to use parents as their controls.

3. The paper needs to be carefully edited for wording. In multiple places the authors claim high risk or no risk but these should be expressed as statistically significant increases or decreases in risk and the comparison group needs to be carefully defined. Many specific instances are mentioned below, but I also suggest careful proofreading to see if any other corrections are necessary.

In multiple places where we employed the term “high risk” or “no risk”, we revised the discussion by expressing the association or not with the term “statistically significant increases” or decreases in risk” and carefully defined comparison groups where it was necessary.

4. Comparing Tables 4 and 6, there is a large change in the odds ratio associated with receiving an insect bite near a river between the univariate model (OR = 2.16) and the multivariate model (OR = 10.76, adjusting for age and bathing in an open borehole). I believe this may be due to the limited number of controls in the under 10 year age category (11.7% of controls compared to 47% of cases). This discrepancy in the number of controls under age 10 years should be discussed.

We discussed the above this concern about the limited number of controls in the <10 years category.
I suggest presenting some cross tabulations of the insect bite and bathing variables by age group to help interpret any potential age confounding or effect modification in the multivariate model in Table 6.

To determine any potential effect of age, we calculate the aOR by crossing insect bites among cases and controls by age groups as well as for the bathing with water from open borehole. For the variable insect bite, we found the effect modification of age that increases the risk only in 10-14 years group (aOR=7.8, 95%CI=1.48-41.21)

For the bathing variable, no significant aOR was not found in any age group suggesting the potential confounding effect of age.

Specific Comments: (note, line numbers refer to actual lines of text, not the line numbers in the margins (which don't correspond to lines of text)

1. Abstract, paragraph 1, line 6. "cases was" to "cases were". Correction done

2. Abstract, paragraph 1, line 9. "matched by the sex" to "matched by sex". Correction done

3. Abstract, paragraph 1, line 10. "and be conditional logistic" and "and conditional logistic". Correction done

4. Abstract, paragraph 2, line 1. Are the percentages necessary in this sentence? The percentage were removed

5. Abstract, paragraph 2, line 6. "crossing a water" to "crossing water". This line was deleted
6. Abstract, paragraph 2, line 7. "trouser" to "trousers". This line was deleted. The results section in abstract has been revised with multivariate analysis data after adjustment on potential confounders.

7. Page 5, line -7. "of BU have been" to "of BU were". Correction done

8. Page 5, line -2. "study on" to "study of", "confirmation of BU" to "confirmed BU", and "proved that prevalence of BU" to "established prevalence of BU". Correction done

9. Page 6, line 10. "used the PCR" to "used PCR". Correction done

10. Page 6, line -3. "mine careers" to "mining", "to the environmental" to "to environmental", and "and the spreading of" to "and may contribute to the spread of". Correction done

11. Page 7, line -6. "place of residence". Is this home, village, etc? The place of residence was referred to the home where lived the BU case or in the neighbor home but in the same village.

12. Page 7, line -3. "originated from" to "originate". Correction done

13. Page 7, line -2. "a population estimated to" to "an estimated population of". Also, "1,767.518" to "1,767,518". Correction done

14. Page 8, line 3. Is the "depression of Lama" indicated on the map? No

15. Page 8, line 4. Move "(Figure 1)" to line 6, after "lad Togo". Correction done

16. Page 8, line 7. "seasonal variations" to "seasonal variations in participation"? It's mean seasonal variations of precipitations
17. Page 9, line 9. "was set at 2. Hence, we calculated a sample size" to "was wet at 2, yielding a sample size…". Also see General Comment 1 above. Correction done

18. Page 10, line 2. "as dependent variable" to "as the dependent variable". Correction done

19. Page 10, line 3. "The exact Fisher test" to "Fisher's exact test". Also, it is not clear to me why Fisher's exact test is used to compare mean or median age…this should be a t test or a non-parametric comparison of medians, shouldn't it? You’re right; we change the Fisher's exact test by the t-test

20. Page 10, line 4. "significant level was set" to "with significance level set". Correction done

21. Page 11, lines 6-7. "The median age was significantly different between cases and controls (p=0.001)" Please describe the difference (which group has a higher median age?). This paragraph has been revised by describing which group has a higher median age

22. Page 11, lines 7-8. "women (60%) were more frequently affected than men (40%) (p=0.01)." This seems to be different than the p-value reported in Table 3. In this section, we only present data among BU cases by comparing women and men. This data did not show in the table 3 that gave the p-value different to the p in Table 3.

Also, the p-value is associated with a test comparing the percentage of women between cases and controls, not comparing 60% to 40%. This should be clarified. Yes, in Table 3 you are right, but the result showed just compared the proportion of BU cases between women and men

23. Page 11, line 10. "pump (p=0.49) was not" to "pump (p=0.49) were not". Correction done

24. Page 11, line 9. "open borehole was a high risk" to "open borehole was associated with higher risk". Correction done
25. Page 11, line -7. "did not provide any protection against BU" to "was not associated with reduced risk of BU". Correction done

26. Page 11, line -4. "mud was not associated with the risk" to "mud did not significantly increase risk". Correction done

27. Page 11, line -2. "in a river was" to "in a river were". Correction done

28. Page 12, line 6. "compared to other parts of the body". Can we tell from the data whether the body location of the insect bites correspond to the body location of the ulcers? No. The comparison here means that some parts of body are more exposed than other parts.

29. Page 12, line 6. "at home was" to "at home were". Correction done

30. Page 12, line 14. "did not provide any protection against BU" to "did not provide any significant reduction in risk of BU". Correction done

Also, I suggest starting a new paragraph with "Farming occupation". Correction done

31. Page 12, line 13. "Wearing frequently trousers" to "Frequently wearing trousers". Correction done

32. Page 12, line 14. "provided additional protection during farming activities" to "during farming activities provided significant reduction in risk". Correction done

33. Page 12, line -6. "that living (p" to "that living with (p" Correction done

34. Page 12, line -5. "did not represent a risk" to "did not represent a significant increase in risk" Correction done
35. Page 12, line -5. "In addition, receiving bites or scratch of" to "Neither did receiving bites or scratches from". And delete "was not at risk of BU disease." Correction done

36. Page 12, line -3. "was not associated with an infection" to "was not significantly associated with increased risk of infection" Correction done

37. Page 13, line 3. "ensured to know" to "were familiar with"? Correction done

38. Page 13, line 4. "Regarding the treatment behavior" to "Regarding treatment behaviors". Correction done


40. Page 13, line 7. "though" to "thought", and "surrounding could be at risk" to "surroundings could increase risk". Correction done

41. Page 13, line -2. "children under 15 years of age were at high risk". Should this be "higher risk"? Is this in comparison to adults? This needs to be specified.

Details provided with comparison to adults in the text

42. Page 14, line 2. "would" to "could".

43. Page 14, line 4. "was a high risk" to "was associated with higher risk" Correction done

44. Page 14, line 6. "bathing were" to "bathing was". Correction done
45. Page 14, line 8. "a high risk" to "an increased risk". Also on lines 8 and 9 there are two references to reference 38 but thy seem to come to opposite conclusions? This needs to be carefully revised. The reference refer to 38 has been reviewed and corrected.

46. Page 14, line -12. "provided from the commercial" to "provided from commercial". Correction done

47. Page 14, line -9. "The same results" to "Similar results". This paragraph has been revised by presenting multivariate results

48. Page 14, line -7. "This would explain the location of wounds..." Does "wounds" refer to insect bites, ulcers, or both? No. Our idea is just to show that where people reported to receive insect bites is also the parts of body where wounds were observed. We deleted this explanation as we don’t have sufficient elements to describe this.

49. Page 14, line -1. "provide protection against Buruli ulcer" to "provide significant reduction in Buruli ulcer risk." Correction done

50. Page 15, line 2. "pants or hats provide protection from the mycobacterial" to "pants or hats are associated with reduction in risk of mycobacterial". Correction done

51. Page 15, line 11. "may cause BU disease" to "may increase risk of BU disease". Correction done

52. Page 15, line 12. "we observed...of contracting BU." to "we did not observe significant increase in risk associated with contact to domestic animals." Correction done

53. Page 15, line 15. "any difference" to "any significant difference". Correction done
54. Page 15, line 16. "lack the impact of the BCG" to "lack a significant association with BCG". Correction done

55. Page 15, line -6. "any association" to "any significant association". Correction done

56. Page 15, line -1. "the situation in five years ago" to "the situation five years ago" and provide a reference. Correction done

57. Page 16, line 4. "The personal poor" to "Personal poor". Correction done

58. Page 16, line 5. "surrounding has been recognized" to "surrounding were recognized". Correction done

59. Page 16, line 5. "potential risk of contracting BU" to "potential risk factor for participants". Correction done

60. Table 1. I suggest adding "%" after 51.9 and 70.5 to make the table to read quickly. Similarly for Table 2. To keep the same formatting for all tables, we decide to use the “%” once time in the table heads.

61. Table 3. The p-value reported for sex is 0.32, which is different than the p-value reported in the text, see Specific Comment 22 above. In this section, we only present data among BU cases by comparing women and men. This data did not show in the table 3 that gave the p-value different to the p in Table 3.

62. Table 4. Should the HIV positive case be excluded? We removed line of HIV positive
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