PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Urogenital Schistosomiasis (UGS) and Female Genital Schistosomiasis (FGS) in Cameroon: An observational assessment of key reproductive health determinants of girls and women in the Matta Health Area
AUTHORS	Makia, Christine Masong; Fesuh, Nono Betrand; Amabo, Elvis; Gamba, Victoria A; Oluwole, Akinola Stephen; Stothard, Russell

VERSION 1 – REVIEW

REVIEWER	Claire Bourke Blizard Institute, Queen Mary University of London, UK
REVIEW RETURNED	28-Apr-2022

GENERAL COMMENTS	This study by Masong and colleagues addresses a much neglected area of health research – female genital schistosomiasis (FGS) - for which the detailed clinical analyses that they have undertaken could add significant value to our understanding of the health burden, distribution and risk factors. Inclusion of both parasitological and clinical examination for schistosomiasis, particularly among women and girls, has been rarely done and I applaud the study team for this work. Though I support this work for ultimate publication, in my opinion, it requires major revisions to do justice to these important data. I list my major, minor and line-specific comments below to support these revisions and encourage the authors to submit a revised version of their manuscript. Major comments/concerns 1. No research question/ or hypothesis/es are outlined (e.g. Abstract Line 30-31 and Line 120- 123) which means that the main purpose of the study can be inferred but isn't fully clear; this means that sample size calculation estimates, choice of statistical tests, results reporting and discussion are hard to follow 2. How UGS and FGS were defined is unclear – though methods are provided, how these were used is not explained (e.g. was haematuria included in the definition of UGS? what was considered a minimal clinical indication for FGS?); given that the premise of the study is that UGS and FGS are related but the nature of this relationship is unclear in the existing literature, clarity and precision with respect to the study definition and reporting of these conditions is essential 3. The initial sample size estimate was 387 women/girls however the actual sample size for the study fell below this (304 for UGS assessments and 67 for FGS); the study still has value with the numbers assessed, but I do not believe that it is justified to restrict analyses by UGS solely to the women examined for FGS. Inclusion

	of the wider cohort for UGS (n=304) analyses would be extremely valuable for statistical power and accuracy of prevalence estimates of reproductive health symptoms in this community. a. A CONSORT diagram indicating how women and girls were included/excluded with details of the number of exclusions for each criterion per STROBE guidelines on reporting clinical studies is warranted. Figure 1 could be modified for this purpose but would need to include the number of participants excluded for each criterion 4. It is unclear how women were recruited (in the discussion, Line 296, 'primary care setting' is stated but no details are provided in the study methods); information on whether these participants were screened at home independent of seeking healthcare or screened after presentation at clinics/for medical care is essential to interpret the results and their generalisability and full details should be provided 5. It is unclear why certain pertinent indicators of reproductive health were omitted from analyses; for example, miscarriage was included but there are a range of other adverse birth outcomes which have been previously evaluated for UGS (e.g. Murenjekwa et al, J Inf Dis, 2019 (disclosure: I co-authored this study) and Mombo-Ngoma et al, Int J Parasitol, 2017) but not for FGS 6. Statistical reporting is inconsistent with much repetition between text and Tables whilst for other variables information is not tabulated; in places it is unclear what the added benefit of some tests are to interpretation (e.g. Chi-squared versus univariable regression), how quality checks were incorporated into interpretation (e.g. analysis of deviance tests for multivariable models), or how variables were systematically selected for (e.g. Line 273-274 suggests that some collinear variables were excluded from multivariable models without adequate consideration of which to include/exclude); it would also be preferable to avoid reporting p-values without context in-text since it is recognised that this can be misleading without associated test statistics and variance/intervals (suggest referring to the appropriate table for in-context results). 7. Some pertinent information, for example urinalysis results (proteinuria, haematuria and grading (i.e. % +/++/++), if the dipsticks allowed for quantitative analysis), whether microscopy was performed on a single sample or consecutive samples, the volume of urine used (if standardised), overall infection intensity (not just for age strata) per 10mL urine and full quantitative characteristics of FGS assessments (i.e. number of women with each characteristic, severity indicators if quantified etc; preferably in a separate Table); representative images are informative but insufficient 8. A paragraph acknowledging study limitations is warranted in the Discussion Minor comments 1. There is a lack of appropriate referencing throughout the Introduction and Discussion; though a general impression of local risk status, and health-care provision may be clear to an in- context professional, supporting literature is warranted for readers 2. Statistical analysis methodology lacks clear indication of exposure/independent and outcome /dependent variables (e.g. were categorical measures (e.g. present/absent) or continuous measures (e.g. infection intensity/number of sandy patches…) included in statistical models? which 'univariable tests' were used?)

	3. The results section is disorganised (e.g. parasitology data is reported in the demographics section, switches between prevalence data and statistical analyses) and would benefit from a clearer order (e.g. cohort characteristics, UGS features and prevalence, FGS features and prevalence, UGS and symptoms, FGS and symptoms) with appropriate sub-headings 4. Vague language (e.g. 'bottlenecking', 'obscure epidemiological associations', 'typically hidden or cryptic sequelae', 'Based on several plausible biological determinants', 'individualise praziquantel treatment needs', 'one the one hand…on the other' etc.) should be avoided throughout the text to ensure clarity 5. Throughout the discussion, limitations of previous studies are highlighted but the same issues have not been clarified for this study (e.g. praziquantel treatment history, residential history, water contact behaviours, number or urine samples); suggest adding this information in the results section to inform the discussion Additional line-specific comments: 6. Line 33: Specify age range 7. Line 34: Since age-based selection was used it is not accurate to say 'unbiased'; specify sub- group selection method 8. Line 38: This prevalence estimate does not match that reported in Line 34; please clarify 9. Line 63-65: The goal of the project appears to have been to identify relationships with symptoms rather than to develop a diagnostic screening tool/questionnaire; the latter would require validation of sensitivity for detection for roll-out. Rephrase this statement to more closely reflect your analyses 10. Line 68-69: Ultimately this research could be beneficial elsewhere so it is not clear why anticipated benefits/aims are regionally restricted 11. Line 85: Reference to diagnostics of active UGS with relevant references are warranted here as low intensity infections may be missed by microscopy; weak associations reported between UGS diagnosed by microscopy (particularly based on a single sample) and FGS could therefore plausibly reflect a lack of sensitive tests rather than a lack of biological association 12. Line 91: Provide references to the evidence that FGS lesions are slow to resolve/non- responsive to treatment to support this statement 13. Line 101-102: Provide references for country-specific differences and the nature of the lag to support this statement 14. Line 106: Clarify how 'at-risk status' is defined according to clinical/public health protocols in Cameroon 15. Lines 113-119: These statements would be more appropriate in the discussion as they are speculative and unsupported by references 16. Line 166 and Line 193: Describe any internal validation of examinations used (e.g. by blinded/external team members using the photographic records) 17. Line 181-182: It is unclear why marriage age was used to determine age range for the study; please clarify with data to support the statement and justification for this approach 18. Line 183: Clarify how the age strata were chosen with justification 19. Line 259: 'FGS infection' is misleading since, as explained in the introduction, FGS can persist after an infection has resolved 20. Line 361: clarify how do you define 'strong' in this context

REVIEWER	Michael Hsieh Children's National Medical Center
REVIEW RETURNED	11-May-2022

GENERAL COMMENTS	This is an interesting and important manuscript addressing relationships among urogenital schistosomiasis, female genital schistosomiasis, and a number of patient factors. It is unclear whether the following important data mentioned in the Methods was collected for the study population: "Apart from reporting symptoms for UGS such as blood in urine, for FGS specifically included vaginal itching..." The manuscript would also benefit from a more fleshed-out discussion of study limitations. Minor issues: Discussion: "healthMore" should be "health. More"

REVIEWER	W Secor Centers for Disease Control and Prevention
REVIEW RETURNED	11-May-2022

GENERAL COMMENTS	This manuscript addresses an important question and presents some initial intriguing results but what the authors did and how they went about doing it are not fully developed or adequately described. Although they present a sample size calculation, they do not preface it with the hypothesis or a priori assumptions of the question they are asking. Similarly, they do not present the initial enrollment criteria or how the study participants were recruited. In figure 1, they present some exclusion criteria from the overall study group that mentions males, non-residents, < 5 years age, and no consent. It seems like these groups should never have been part of the overall sampling in the first place. Further, it is not very clear how they selected the 67 persons who received gynecologic exams for FGS and the exclusion criteria for this stage did not actually exclude anyone from the final study population. The final group of 67 participants is only 17% of the calculated sample size, suggesting the authors do not have a sufficient enrollment to conduct the study but these issues are never addressed. Without a clear description of participant recruitment and selection (e.g., is it representative of the village that 90% of the population lives within 200m of the lake?), it is very difficult to assess the validity of the results. The paper could be re-written as a short report or preliminary findings paper that can inform hypothesis generation and sample size calculation for a larger study but does not qualify as "an in-depth assessment of reproductive health determinants" as suggested by the title. Other concerns include: --could sexually transmitted infections be a co-factor or confounder for the observed MI and LAP associations? --line 185-186 indicates that micro- and macrohematuria and proteinuria were measured but no results are presented --do UGS negative but FGS positive participants have a history of infection or antibodies to schistosome antigens? --what is the history of MDA in the area? --women are not "infected with FGS" (lines 255, 282, 326, 341) but have manifestations of FGS resulting from schistosome infections

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Claire Bourke, Blizard Institute, Queen Mary University of London, UK
Comments to the Author:
Thank you for your work on this study which I have now reviewed. I am recommending Major Revisions and attach my full report (pdf) to provide my reasoning and to support you in these revisions. I encourage you to resubmit a revised version.

This study by Masong and colleagues addresses a much neglected area of health research – female genital schistosomiasis (FGS) - for which the detailed clinical analyses that they have undertaken could add significant value to our understanding of the health burden, distribution and risk factors. Inclusion of both parasitological and clinical examination for schistosomiasis, particularly among women and girls, has been rarely done and I applaud the study team for this work. Though I support this work for ultimate publication, in my opinion, it requires major revisions to do justice to these important data. I list my major, minor and line-specific comments below to support these revisions and encourage the authors to submit a revised version of their manuscript.

**Dr Bourke, thank you for this precise and clear summary of our manuscript, and for the in- depth review which you have offered. We have keenly gone over the manuscript with your detailed and directive comments as guide, and have reorganized and added key information highlighted.

Major comments/concerns

1. No research question/ or hypothesis/es are outlined (e.g. Abstract Line 30-31 and Line 120- 123) which means that the main purpose of the study can be inferred but isn't fully clear; this means that sample size calculation estimates, choice of statistical tests, results reporting and discussion are hard to follow

** Dr Bourke, thank you for highlighting this. We have now established a clear study purpose within the abstract and within the introduction to direct our methods, reporting and discussion more clearly. This can be found from lines 30-33, 118-121.

2. How UGS and FGS were defined is unclear – though methods are provided, how these were used is not explained (e.g. was haematuria included in the definition of UGS? what was considered a minimal clinical indication for FGS?); given that the premise of the study is that UGS and FGS are related but the nature of this relationship is unclear in the existing literature, clarity and precision with respect to the study definition and reporting of these conditions is essential

**Dr Bourke, we have now with precision clearly established a definition for the determination of a case of infection with urogenital schistosomiasis, and manifestation for female genital schistosomiasis amongst the sample population (Lines 81-85, and further within the manuscript), sourced from literature, and from our local site determination for the purpose of this study. A minimal clinical indication for FGS was determined by presence of sandy patches, abnormal blood vessels and/or homogenous yellow patches, while UGS was diagnosed by the presence of microhaematuria in urine, with at least one terminal-spined ovum seen. Thank you.

3. The initial sample size estimate was 387 women/girls however the actual sample size for the study fell below this (304 for UGS assessments and 67 for FGS); the study still has value with the numbers assessed, but I do not believe that it is justified to restrict analyses by UGS solely to the women examined for FGS. Inclusion of the wider cohort for UGS (n=304) analyses would be extremely

valuable for statistical power and accuracy of prevalence estimates of reproductive health symptoms in this community.

**Yes Dr Bourke, we had limited our sample for this manuscript to just 67 girls/women assessed for female genital schistosomiasis after assessment for Urogenital Schistosomiasis. But we have now considered the wider complete sample for UGS (304) in our analyses, and present this within our results (lines 247-345) and have benefited from the extended results to refocus our discussion (348-414).

a. A CONSORT diagram indicating how women and girls were included/excluded with details of the number of exclusions for each criterion per STROBE guidelines on reporting clinical studies is warranted. Figure 1 could be modified for this purpose but would need to include the number of participants excluded for each criterion

Dr. Bourke, as requested, we have now modified Figure 1 to show clearly exclusion/inclusion details. Thank you for raising, and proposing this.

4. It is unclear how women were recruited (in the discussion, Line 296, 'primary care setting' is stated but no details are provided in the study methods); information on whether these participants were screened at home independent of seeking healthcare or screened after presentation at clinics/for medical care is essential to interpret the results and their generalisability and full details should be provided

**Women for this study were mostly recruited and screened within the community, mostly in their homes or a 'safe' house prescribed by the women themselves, or the village leader. Due to the secluded nature of the case study settings, and the preference of a participative nature of recruiting (involvement of formal/informal health workers other stake holders within community), recruitment was contextualized within each community. Participants were met at their homes or selected spots within the community. This has been further explained in the methods on line 184-185. Thank you for highlighting this.

5. It is unclear why certain pertinent indicators of reproductive health were omitted from analyses; for example, miscarriage was included but there are a range of other adverse birth outcomes which have been previously evaluated for UGS (e.g. Murenjekwa et al, J Inf Dis, 2019 (disclosure: I co-authored this study) and) but not for FGS

** Apart from miscarriage, we collected information on lower abdominal sensitivity /pelvic pain, sub-fertility which we counted from age of marriage, age of last child and/or infertility. Other determinants such as adverse birth outcome could not be measured for this study due to the limited measurement criteria (more than 90% of women delivered at home without medical assistance- reported in ethnographic recounts in https://doi.org/10.1371/journal. pgph.0000007 ; and thus, very limited records existed of birth outcomes for new-born at the health center, and these as well mostly were not related to study participants). This study was limited to self-described symptoms and socio-demographic characteristic, as well as clinical examinations. We have now added information on other pertinent indicators not previously reported (Lines 247-333), considered for UGS and FGS. These can be found in this manuscript within the results. Thank you for bringing this up.

6. Statistical reporting is inconsistent with much repetition between text and Tables whilst for other variables information is not tabulated; in places it is unclear what the added benefit of some tests are to interpretation (e.g. Chi-squared versus univariable regression), how quality checks were incorporated into interpretation (e.g. analysis of deviance tests for multivariable models), or how variables were systematically selected for (e.g. Line 273-274 suggests that some collinear variables

were excluded from multivariable models without adequate consideration of which to include/exclude); it would also be preferable to avoid reporting p- values without context in-text since it is recognised that this can be misleading without associated test statistics and variance/intervals (suggest referring to the appropriate table for in-context results).

** Statistical reporting has now been adjusted to ensure consistency. We used Chi-squared alongside univariate logistic regression to ensure consistency in results, one consolidating the other. In effect, Chi-squared checks for dependence while univariate logistic regression tries to explain the (Lines 229-237). Analysis of deviance tests were displaced to show the global significance of the variables included in the final models. This information has now been highlighted in the text. We have also explained how variables were systematically selected in the final models (285-298; 326-329). We have also avoided the reporting of p- values without context in-text. Thank you for highlighting this.

7. Some pertinent information, for example urinalysis results (proteinuria, haematuria and grading (i.e. % +/++/++), if the dipsticks allowed for quantitative analysis), whether microscopy was performed on a single sample or consecutive samples, the volume of urine used (if standardised), overall infection intensity (not just for age strata) per 10mL urine and full quantitative characteristics of FGS assessments (i.e. number of women with each characteristic, severity indicators if quantified etc; preferably in a separate Table); representative images are informative but insufficient

** Dr Bourke, dipstick analysis was not collected for quantitatively, we report just the presence of haematuria and proteinuria from this. Also, microscopy was done and reported on a single urine sample of 10ml, and graded as per ≥50 (high intensity) or < 50 (low intensity). Now we have clearly reported this within the manuscript on line 255-257. Also, full quantitative characteristics of all FGS assessments as well, has now been reported separately within this manuscript results. Thank you for highlighting this.

8. A paragraph acknowledging study limitations is warranted in the Discussion
** Thank you for highlighting this Dr. Bourke. We have now set a paragraph for the study limitations. (Lines 416-441).

Minor comments
1. There is a lack of appropriate referencing throughout the Introduction and Discussion; though a general impression of local risk status, and health-care provision may be clear to an in- context professional, supporting literature is warranted for readers

**Thank you, Dr Bourke, we have gone through the manuscript and added more references to provide evidence and clarity throughout the manuscript, especially in the Introduction and Discussion.

2. Statistical analysis methodology lacks clear indication of exposure/independent and outcome /dependent variables (e.g. were categorical measures (e.g. present/absent) or continuous measures (e.g. infection intensity/number of sandy patches...) included in statistical models? which ‘univariable tests’ were used?)

**We have now added for more clarity clear indications of independent and dependent variables included in statistical models within the methods (lines 226-243). Though we also clarify here intensity and number of counts of some variables, others like sandy patches were not quantified per participant/individual, but per the study population for prevalence.

3. The results section is disorganised (e.g. parasitology data is reported in the demographics section, switches between prevalence data and statistical analyses) and would benefit from a clearer order

(e.g. cohort characteristics, UGS features and prevalence, FGS features and prevalence, UGS and symptoms, FGS and symptoms) with appropriate sub-headings

**Thank you for highlighting this Dr Bourke. We have now restructured the results section in this manuscript (lines 245-345) to show UGS/FGS reports differently, as well as general participant characteristics graded as: socio-demographic, syndromic, clinical. Thank you.

4. Vague language (e.g. ‘bottlenecking’, ‘obscure epidemiological associations’, ‘typically hidden or cryptic sequelae’, ‘Based on several plausible biological determinants’, ‘individualise praziquantel treatment needs’, ‘one the one hand...on the other’ etc.) should be avoided throughout the text to ensure clarity

**Thank you for pointing this, we have now removed and rephrased this vague language everywhere within the manuscript.

5. Throughout the discussion, limitations of previous studies are highlighted but the same issues have not been clarified for this study (e.g. praziquantel treatment history, residential history, water contact behaviours, number or urine samples); suggest adding this information in the results section to inform the discussion

** Thank you, Dr. Bourke. We have considered your comment and have included these aspects considered within this study and in our manuscript which were raised within the discussion. As such, we have presented participants treatment history with praziquantel, linking this with analysis and in the discussion (lines 259-260). Also, residential history and water contact have now been incorporated more clearly in the results (lines 248-251) and discussion (362-364), in-line with raised observations in other studies and in the literature.

Additional line-specific comments:

6. Line 33: Specify age range

** Thank you. We have now specified the age range of the 304 total participants. The line now reads “From a population of 304 females aged 5- 89 years all examined for UGS by urine filtration and microscopy”. Line 36, 248.

7. Line 34: Since age-based selection was used it is not accurate to say ‘unbiased’; specify sub-group selection method.

**Thank you, Dr. Bourke, for highlighting this. We reviewed this statement as you requested, and have detailed the sub-group selection method for the FGS sub-group, which consists of “non-virgin women and girls aged >13 who had had been diagnosed for UGS within the study, were not pregnant and consented for gynecologically examinations”. (Line 35-37; 176-183)

8. Line 38: This prevalence estimate does not match that reported in Line 34; please clarify

**As requested, Dr. Bourke, we have reviewed and clarified better the differing UGS estimates on line 34 and 38 which for the earlier (63.8%) reports the UGS prevalence estimates for the entire sample population of 304 girls and women, and for the later (59.7%) for women also diagnosed for FGS. (Line 44-45)

9. Line 63-65: The goal of the project appears to have been to identify relationships with symptoms rather than to develop a diagnostic screening tool/questionnaire; the latter would require validation of sensitivity for detection for roll-out. Rephrase this statement to more closely reflect your analyses

**Yes, our goal is to identify relationships with symptoms and not to develop a diagnostic or screening tool questionnaire. This latter, is only a suggestion or proposal which we offer from our results. Thank you for clarifying this, thus we have rephrased accordingly, to reflect this objective. (Lines 122-125).

10. Line 68-69: Ultimately this research could be beneficial elsewhere so it is not clear why anticipated benefits/aims are regionally restricted

**Yes, Dr. Bourke. We have now changed this to reflect a broader coverage for the benefits of our research, which is to better improve women's health within S. haematobium endemic regions. (Line 50-52)

11. Line 85: Reference to diagnostics of active UGS with relevant references are warranted here as low intensity infections may be missed by microscopy; weak associations reported between UGS diagnosed by microscopy (particularly based on a single sample) and FGS could therefore plausibly reflect a lack of sensitive tests rather than a lack of biological association

** Thank you, Dr. Bourke. We have added on references referring to the validity of microscopy for UGS diagnosis even without PCR, especially in low resource settings. Line 81,83,84,360,361

12. Line 91: Provide references to the evidence that FGS lesions are slow to resolve/non- responsive to treatment to support this statement

**Thank you for raising this Dr Bourke. We have clarified further with evidence this statement within this manuscript. Such references include:
- Kjetland EF, Mduluza T, Ndhlovu PD, et al. Genital schistosomiasis in women: a clinical 12month in vivo study following treatment with praziquantel. Trans R Soc Trop Med Hyg. 2006;100(8):740-52.
- Treatment of Female Genital Schistosomiasis (FGS) With Praziquantel: A Proof-of-Concept Study (NCT04115072) [Available from: https://clinicaltrials.gov/ct2/show/NCT04115072]. NIH: ClinicalTrials.gov archive. 2020[cited 31/01/2022].
Line 95.

13. Line 101-102: Provide references for country-specific differences and the nature of the lag to support this statement

** As requested, we have added two research articles from studies carried out in Cameroon which give evidence to the lag limited involvement of FGS diagnostics and control in Cameroon, as well as the lack of research or advocacy activities related to FGS in this schistosomiasis endemic Country. Thank you for highlighting this. Line 97-98

14. Line 106: Clarify how 'at-risk status' is defined according to clinical/public health protocols in Cameroon

**At risk populations for urogenital schistosomiasis according to the World Health Organization include school- aged children in endemic areas; adults considered to be at risk in endemic areas, people with occupations involving contact with infested water such as fishermen, farmers, irrigation workers, and women, whose domestic tasks bring them into contact with infested water. The Cameroon protocol of schistosomiasis and soil transmitted helminths considers these same groups of people, as well as entire communities living within highly endemic areas. Such people can be found

within several regions of Cameroon, such as the Far North Region and some areas of the South West and West regions amongst others. We have now clarified this further within this manuscript with references to support this point (line 113). Thank you

15. Lines 113-119: These statements would be more appropriate in the discussion as they are speculative and unsupported by references

**Dr Bourke, thank you for noticing, and suggesting this. We have now moved these statements highlighted from the introduction to the discussion part of the manuscript, where we have used them to emphasize better our self-based discussion on treatment limitations, and possible solutions and further action needed. (Lines 353-358)

16. Line 166 and Line 193: Describe any internal validation of examinations used (e.g. by blinded/external team members using the photographic records)

**As requested, we have explained further the cross examination of photo-colposcopy images by external group members in addition to verification/comparism to the WHO FGS pocket atlas, as seen on line 203-209. Thank you.

17. Line 181-182: It is unclear why marriage age was used to determine age range for the study; please clarify with data to support the statement and justification for this approach

**Marital age was used to determine age range within this study on two levels. Firstly, this was used to select age for start of sexual activity. For visual examination purposes for FGS detection, non-virgins were excluded from our second cohort participants, due to the invasive nature of the gynecological examination. The age of marriage for most participants was used to set the minimal age for gynecological examinations (with assent from spouses and/parents), opening the possibility to ask girls above 13 if they had or were sexually active. Though the possibility of younger girls manifesting some FGS symptoms have been shown https://doi.org/10.1371/journal.pntd.0002104, within the context, and as prescribed elsewhere (cervical cancer examinations for girls= above 15yo) no gynecological examination was carried for girls younger than 14. Secondly, age of marriage was used as a determinant for sub-fertility: the age at marriage; age of first child; and age of last child, was used to determine sub-fertility in women. As reported in https://doi.org/10.1371/journal. pgph.0000007 amongst others (line 198), similar context specific cultures required a woman to have children every two years at least, and if she went for more than two years, she was considered not well. We have clarified this a bit further within the manuscript (lines 198-203) with the relevant references for evidence sited. Thank you.

17. Line 183: Clarify how the age strata were chosen with justification

** Age strata within this study were formed firstly by the minimal limit (above 13 years) based on recommended reproductive health determinants (12 years by some recommendations e.g WHO SRH), and context specific characteristics, where we determined general age of marriage and first prenatal visit from 13 years for girls. This was a necessary prerequisite for this study considering the invasive nature of colposcopy examination which requires a non-virgin for examination. Thus, from this, 3 groups were set (adolescents, young adults, and older adults) open enough to fit the different age groups found within the study population, and as per consideration of reproductive health grouping in females. (Line 198-2005)

18. Line 259: ‘FGS infection’ is misleading since, as explained in the introduction, FGS can persist after an infection has resolved

**Thank you for raising this Dr Bourke. We have revised the term “FGS Infection” to currently read as “FGS manifestation”. Yes, “FGS infection” is misleading, as infection could clear with UGS, but manifestations of FGS persist. We have changed this elsewhere within the manuscript as well, as Dr. Secor pointed out as well.

19. Line 361: clarify how do you define ‘strong’ in this context

**We consider the epidemiological associations raised here as ‘strong’ per our case study context where our results show within the study population tested for UGS and FGS, significant relations found between some reproductive health variables and UGS/FGS which could be exploited further for precise diagnosis and management in low resource S. haematobuim endemic settings.

Reviewer: 2
Dr. Michael Hsieh, Children's National Medical Center
Comments to the Author:
This is an interesting and important manuscript addressing relationships among urogenital schistosomiasis, female genital schistosomiasis, and a number of patient factors. It is unclear whether the following important data mentioned in the Methods was collected for the study population: "Apart from reporting symptoms for UGS such as blood in urine, for FGS specifically included vaginal itching..."

**Thank you for this review, Dr. Hsieh.
Yes, as mentioned in the methods, vaginal itches amongst other symptoms were collected, apart from the other reproductive health determinants reported. We have now included details on all symptoms collected and responding figures within this manuscript. From Lines 245-323.

The manuscript would also benefit from a more fleshed-out discussion of study limitations.

** Thank you for raising this Dr. Hsieh. We have now included a separate and detailed paragraph on the study limitations within this manuscript. Lines 417-429

Minor issues:
Discussion: "healthMore" should be "health. More"

**Dr. Hsieh, we have corrected this within the manuscript. Thank you.

Reviewer: 3
Dr. W Secor, Centers for Disease Control and Prevention
Comments to the Author:
This manuscript addresses an important question and presents some initial intriguing results but what the authors did and how they went about doing it are not fully developed or adequately described.

**Dr. Secor, thank you very much for this very extensive review. Sir, we have now a more fully developed and detailed description of the methods within this revised manuscript, especially as regards sampling and presentation of study results.

Although they present a sample size calculation, they do not preface it with the hypothesis or a priori assumptions of the question they are asking.

**Sir, indeed, certain a priori assumptions/hypothesis were established before estimating sample size. In effect, considering the main indicators of interest in this study are UGS and FGS prevalence, the

formular for computing sample sizes in prevalence studies was used. For a start, there exists/existed no recorded information on adults (females specifically) prevalence for schistosomiasis within our case study area. Since such information is needed in estimating the sample, an estimation was made on the prevalence of urogenital schistosomiasis amongst target population, using existing prevalence data from school-aged children. We have clarified this further within this manuscript on lines 156-165. Thank you for highlighting this.

Similarly, they do not present the initial enrollment criteria or how the study participants were recruited. In figure 1, they present some exclusion criteria from the overall study group that mentions males, non-residents, < 5 years age, and no consent. It seems like these groups should never have been part of the overall sampling in the first place. Further, it is not very clear how they selected the 67 persons who received gynecologic exams for FGS and the exclusion criteria for this stage did not actually exclude anyone from the final study population. The final group of 67 participants is only 17% of the calculated sample size, suggesting the authors do not have a sufficient enrollment to conduct the study but these issues are never addressed. Without a clear description of participant recruitment and selection (e.g., is it representative of the village that 90% of the population lives within 200m of the lake?), it is very difficult to assess the validity of the results. The paper could be re-written as a short report or preliminary findings paper that can inform hypothesis generation and sample size calculation for a larger study but does not qualify as "an in-depth assessment of reproductive health determinants" as suggested by the title.

**Sir, we have now presented clearly the enrollment criteria, specifying eligibility and reasons for exclusion and reasons for final participant number for FGS analysis. We have also rephrased and clarified better our sampling decisions. Furthermore, we considered your feedback and that of Dr Bourke and have presented now an analysis of the total population examined for UGS, Symptomatic analysis for initial FGS assessment, and clinical assessment for conclusive FGS diagnosis. This has now increased the overall number of assessed participants in the study, as well as provided more analytic dimensions. Still, we have rephrased our study title to exclude' in-depth', considering a number of reproductive health determinants related to UGS (such as birthweight, ectopic pregnancies, reasons for this detailed within the manuscript) were not considered within this manuscript. We still will very much prefer this manuscript to be considered as the full-length original research paper, which it is, and hopefully with these amendments, this comes through as such. Thank you for highlighting and proposing the alternative adjustments, and helping us restructure this manuscript.

Other concerns include:
--could sexually transmitted infections be a co-factor or confounder for the observed MI and LAP associations?

**Yes Sir. Sexually transmitted infections were considered for an alternative explication of some of the symptoms such as lower abdominal pain and vaginal itches in participants. Participants were tentatively (questioned on sexual habits, with the cervix observed visually for 'straw berry markings') assessed for Trichomonas vaginalis; and for hr-HPV. Possibly due to the insensitive nature (no microscopy), no cases were found of T vaginalis, and also no cases for hr-HPV (possibly due to faulty collection and preservation of samples, thus not reported in this paper. This was considered a possible confounder for vaginal itches, abnormal discharges and LAP. Though, conclusive diagnosis for UGS/FGS verified these symptoms as related to genital schistosomiasis.

--line 185-186 indicates that micro- and macrohematuria and proteinuria were measured but no results are presented.

**Thank you for highlighting this Sir. Yes, we measured for micro and macro-hematuria, as well as proteinuria, and we have added these to our results section as seen on line 248-267.

--do UGS negative but FGS positive participants have a history of infection or antibodies to schistosome antigens?

Yes Sir. Actually, the case study site (Matta Health area) for this manuscript is a hot spot for schistosomiasis with a steady infection rate of more than 40% over the past decade. This is due to the household and economic dependence of most surrounding communities on the lake (Mape Dam), which is the main source of schistosomiasis infection in this area. With this high reinfection rate, possibly daily/weekly, a concomitant immunity from extreme reinfection possibly exists amongst older women resident here not shedding schistosome haematobuim eggs in urine but having FGS manifestations. Thus, possible asymptomatic persistent infection of UGS. Alternatively, this also increases the chances of neglect with its asymptomatic nature, necessitating a crucial need for treatment uptake, especially early on, in such areas. Thank you for highlighting this.

--what is the history of MDA in the area?

**Sir, the Matta Health Area consists of remote fishing camps or communities, naturally limiting access especially to most health interventions. In Cameroon, the National program for the control of schistosomiasis and soil transmitted helminths carries out deworming campaigns twice annually within schools and in highly endemic communities (such as Matta Health area) within communities. Within our case study site, due to its remoteness, MDAs are inconsistent, as captured within questionnaire, showing less than 40% treatment adherence/reach of the population; especially amongst women and girls. This is as part is due to access; and on the other part due to adherence. Women specifically, had less access to treatment as they were dependent on their husband's decision of whether to take treatment or not, in most cases where MDAs were carried out. Specifically, from participants accessed for FGS, less than 20% had received praziquantel in the last 24 months. This has now been included within this manuscript. (Line 259-260)

--women are not "infected with FGS" (lines 255, 282, 326, 341) but have manifestations of FGS resulting from schistosome infections.

**Sir, thank you for highlighting this, and for giving the appropriate term. We have corrected this within the manuscript everywhere FGS manifestation was referred to as "infection with" and have replaced this with "FGS manifestations/having manifestations of FGS". Thank you for pointing this out.

VERSION 2 – REVIEW

REVIEWER	W Secor Centers for Disease Control and Prevention
REVIEW RETURNED	04-Sep-2022

GENERAL COMMENTS	Thank you to the authors for your revisions. Unfortunately, the paper, while still having potential to be a meaningful contribution to the field, is still very far from being publication ready. Many of the previous comments by reviewers have not been addressed or have not been addressed adequately and the paper is quite confusing in many sections. I would encourage the first author to employ the assistance of the more senior authors before resubmitting the paper. Many of the issues just need to be presented more clearly. Line 45--shouldn't this be ascending age? Line 47--MI had not been defined. Presumably menstrual

	irregularities? Line 47--use "symptomatic indicator" rather than "epidemiological flag" Line 14 and throughout--it is not always clear when < 14 year old girls were included and when they were not. Clearly colposcopy was not performed on them but diagnosis for UGS was. Was the questionnaire administered to them? Line 68 and throughout--it is never clear what the UGS/FGS breakdown is for the 67 women who received colposcopy. At some point the authors should note the number of FGS+/UGS+, FGS+/UGS-, FGS-/UGS+, and FGS-/UGS- women from this subset. They do indicate (line 367) that 34/67 had FGS and 40/67 had UGS but the breakdown and overlap information is important to include. --Lines 78, 110, 121, 158 and throughout--it is not necessary to capitalize the name of the disease in the middle of the sentence. --Line 79 and lines 213-214--are the authors required that both eggs and hematuria be present for a diagnosis of UGS or should the "and" be an "or" in line 79 and the "with" should be an "or" in line 214. --Line 106--a period seems to be missing after [27,28]. --Line 162 to 170--the calculation of sample size description is still inadequate. Were the authors trying to determine the prevalence of UGS or FGS? If FGS, why were girls <14 even enrolled if they were not eligible for assessment of clinical FGS? The authors never address the ramifications of failing to reach their sample size on the results. --line 194--please include the definition of menstrual irregularities here rather than waiting until line 309. --Figure 1, Table 1, and associated text--it is never clear where the diagnosis of UGS comes in and how it affects the ongoing engagement of participants. In some places (table 1 and text), it sounds like all 304 enrollees had UGS, but Table 1 indicates that not every participant is egg positive. Please clearly define the criteria for UGS (egg OR hematuria or both) and include this information in the Figure 1 flowchart. --Line 246--use "enrolled" rather than "met" to describe the sample population. The calculated sample size was not met. --Line 251-252--what is the microhematuria sensitivity and specificity calculated against? Egg positivity? --Lines 259 to 263--this sentence does not make sense. Compared to what? Use "included" rather than "showed" in line 260 and "fewer miscarriages" rather than "lower miscarriages" to differentiate that "lower back pain" clearly indicates a location of pain rather then relative frequency. --Table 1 uses a different denominator in the different sections. While the denominator is clear in some sections, it is not in others. Please indicate the n associated with each variable in the left column. This is especially important for the syndromic responses as in Tables 2 and 4, the denominator seems to vary from symptom to symptom. Please be clear for the denominator in every table either in the overall description or at each variable. --lines 270, 272, 273, 280, 295 (authors check for elsewhere), just like FGS is the name of the disease and not the infection, UGS is the name of the disease and not the infection. Thus, constructs like "UGS infection" and "infection with UGS" are incorrect. --Tables 2 and 4--as mentioned above, the number of respondents seems to be different for each symptom (lower abdominal pain, coital pain, etc.)--why is this? How are the authors clearly differentiating between a non-response from a "no" response? --Line 377--presumably the authors mean "menstrual health" rather

	than "mental health"? --Line 385-386--the authors suggest an analysis of proximity to the lake and disease manifestation (which disease? UGS or FGS) in Table 5 but Table 5 does not include this information.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 3

Dr. W Secor, Centers for Disease Control and Prevention

Comments to the Author:

Thank you to the authors for your revisions. Unfortunately, the paper, while still having potential to be a meaningful contribution to the field, is still very far from being publication ready. Many of the previous comments by reviewers have not been addressed or have not been addressed adequately and the paper is quite confusing in many sections. I would encourage the first author to employ the assistance of the more senior authors before resubmitting the paper. Many of the issues just need to be presented more clearly.

**Thank you Sir, for your time and consideration in reviewing this once more. Also, for highlighting the changes needed and proposing clear recommendations to move forward. We have now once more reviewed our manuscript and incorporated the changes requested with the very constructive feedback.

Line 45--shouldn't this be ascending age?

**Yes Sir, this is ‘ascending’ rather than ‘descending’ age (line 45). We have now corrected this. Thank you for highlighting.

Line 47--MI had not been defined. Presumably menstrual irregularities?

**Yes Sir, MI indicates Menstrual Irregularity. We have now added this (line 47). Thank you.

Line 47--use "symptomatic indicator" rather than "epidemiological flag"

**Thank you suggesting this, we have replaced “epidemiological flag” with “symptomatic indicator” (line 47).

Line 14 and throughout--it is not always clear when < 14 year old girls were included and when they were not. Clearly colposcopy was not performed on them but diagnosis for UGS was. Was the questionnaire administered to them?

**Girls younger than 14 were diagnosed for UGS, and also, questionnaires (for FGS related symptoms) were selectively administered to this age group. This was dominantly dependent on age, amongst other factors. For example, for girls younger than 14, questions linked to sexual health were avoided, and questioning involved young girls who either had a parent/guardian for present, for aiding or complementing their responses after assent, or responding directly for them. Thus, the reason for the differing denominators within Tables 1, 3 and 4. We have rephrased and explained this more clearly within the manuscript as seen on lines 194-201, 210-211 and 228-232. Also, the sample size of females eligible for each question has been added to Table 1 for clarity. Thank you for bringing this up.

Line 68 and throughout--it is never clear what the UGS/FGS breakdown is for the 67 women who received colposcopy. At some point the authors should note the number of FGS+/UGS+, FGS+/UGS-,

FGS-/UGS+, and FGS-/UGS- women from this subset. They do indicate (line 367) that 34/67 had FGS and 40/67 had UGS but the breakdown and overlap information is important to include.

**Sir, the UGS/FGS breakdown for the 67 women who received colposcopy has now been included in the results of this manuscript. This is seen on line 322-323. Thank you.

--Lines 78, 110, 121, 158 and throughout--it is not necessary to capitalize the name of the disease in the middle of the sentence.

**Thank you, we have relooked and de-capitalised the disease name everywhere when found within the sentence (lines 27, 28, 83, 123, 134, 161, 169).

--Line 79 and lines 213-214--are the authors required that both eggs and hematuria be present for a diagnosis of UGS or should the "and" be an "or" in line 79 and the "with" should be an "or" in line 214.

**Yes please, we required an egg-positivity “or” hematuria for diagnosis for UGS, and have rephrased now (lines 79 and line 255) as recommended. Thank you.

--Line 106--a period seems to be missing after [27,28].

**Thank you for highlighting this, we have now added the missing period on line 106 (now line 115).

--Line 162 to 170--the calculation of sample size description is still inadequate. Were the authors trying to determine the prevalence of UGS or FGS? If FGS, why were girls <14 even enrolled if they were not eligible for assessment of clinical FGS? The authors never address the ramifications of failing to reach their sample size on the results.

**Sir, the sample size estimation for this study was based on UGS prevalence (considered as key indicator for the study. Diagnosis for FGS is a secondary objective. So, our original sample size is based on diagnosis for UGS, where diagnosis for FGS is secondary, and depends on this population. We have now addressed this lack of clarity and explained further on the sample size determination and calculation, and also some context for our decisions (on line 150-163, 173-182, and 197-200). Also, we have added on as a study limit, the reduced FGS diagnosed population on the general and findings (lines 64, and 438-439). Thank you for this important remark.

--line 194--please include the definition of menstrual irregularities here rather than waiting until line 309.

**Thank you, we have now moved the definition of menstrual irregularities to line 204 (previously 194), to set the pace for subsequent text which mentions “menstrual irregularities”.

--Figure 1, Table 1, and associated text--it is never clear where the diagnosis of UGS comes in and how it affects the ongoing engagement of participants. In some places (table 1 and text), it sounds like all 304 enrollees had UGS, but Table 1 indicates that not every participant is egg positive. Please clearly define the criteria for UGS (egg OR hematuria or both) and include this information in the Figure 1 flowchart.

**UGS is diagnosed in this study by hematuria or egg-positivity (line 224-225). From this UGS diagnosed population, a sub-group was diagnosed for FGS based on some criteria, and further limited by certain constraints. We have now reviewed our manuscript and included this information, as well as criteria for UGS diagnosis in the Figure 1 flowchart. We have now presented the UGS prevalence amongst the enrolled population at the beginning of the results (line 263). Thank you.

--Line 246--use "enrolled" rather than "met" to describe the sample population. The calculated sample size was not met.

**Thank you for highlighting this. We have now rephrased our use of these terms to now read “A total sample population of 304 females were enrolled, and all diagnosed for UGS...” (line 258).

--Line 251-252--what is the microhematuria sensitivity and specificity calculated against? Egg positivity?

**Yes, sir, we evaluated dipstick diagnosis of S. haematobuim infection to standard egg-count parasitology. Thus, microhaematuria sensitivity and specificity was calculated against egg positivity alone. We have now specified this on line 265-267.

--Lines 259 to 263--this sentence does not make sense. Compared to what? Use "included" rather than "showed" in line 260 and "fewer miscarriages" rather than "lower miscarriages" to differentiate that "lower back pain" clearly indicates a location of pain rather than relative frequency.

** Thank you, Sir, we have identified this error within the text, and reviewed this sentence to now report “miscarriages, lower abdominal pain and lower back pain…”, instead of “lower miscarriages, abdominal pain, and lower back pain…”. Line 273-277.

--Table 1 uses a different denominator in the different sections. While the denominator is clear in some sections, it is not in others. Please indicate the n associated with each variable in the left column. This is especially important for the syndromic responses as in Tables 2 and 4, the denominator seems to vary from symptom to symptom. Please be clear for the denominator in every table either in the overall description or at each variable.

**Thank you for pointing this out. We have added now the “n” (number of respondents) for every variable within Table 1, 2 and 4, to clearly state the denominator. In addition to that, we have also included the number of participants eligible for each variable. Hence, the difference between the number of eligible participants for a given question and those who actually responded (n) gives the number of missing data for that question. Eligibility for the different syndromic variables was dominantly dependent on age. For example, for girls younger than 14, questions linked to sexual health were avoided. In the methods section as well, we have explained this accordingly (on lines 150-163, 173-182, and 197-200).

--lines 270, 272, 273, 280, 295 (authors check for elsewhere), just like FGS is the name of the disease and not the infection, UGS is the name of the disease and not the infection. Thus, constructs like "UGS infection" and "infection with UGS" are incorrect.

**Sir, we have now corrected and changed this everywhere in the manuscript, where FGS/UGS were referred to as an infection (lines 107, 311, 318, 320, 341, 348, 481, 417, 418, 440, 441, 444, 447, 454, 456). Thank you.

--Tables 2 and 4--as mentioned above, the number of respondents seems to be different for each symptom (lower abdominal pain, coital pain, etc.) --why is this? How are the authors clearly differentiating between a non-response from a "no" response?

**Sir, as mentioned above, the difference between the number of eligible participants for a given question (such as lower abdominal pain and coital pain) and those who actually responded (n) was dominantly dependent on age. For example, for girls younger than 14, questions linked to sexual health were avoided, and non-sexual related questions such as lower abdominal pain, vaginal itches or discharge, were administered to all participants irrespective of age, except for very young girls whose parent/guardant was not available to respond for them after urine collection. In the methods section, we have explained this for more clarity (lines on line 150-163, 173-182, and 197-200). Also, we have also included the number of participants eligible for each variable, to bring out the difference between the number of eligible participants for a given question and those who actually responded (n), giving the number of missing data for that question.

--Line 377--presumably the authors mean "menstrual health" rather than "mental health"?

**Thank you for highlighting this. Yes, this is “menstrual” health, as confirmed with the references sited on this line. We have now corrected this on line 394 (previously 377).

--Line 385-386--the authors suggest an analysis of proximity to the lake and disease manifestation (which disease? UGS or FGS) in Table 5 but Table 5 does not include this information.

**The disease referred to here is FGS. Also, the table this line refers to is table 4 rather than table 5. We have now specified here the analysis on lake proximity and FGS manifestation and have redirected this analysis to Table 4. Both can be seen on line 403. Thank you.

VERSION 3 – REVIEW

REVIEWER	W Secor Centers for Disease Control and Prevention
REVIEW RETURNED	06-Dec-2022

GENERAL COMMENTS	Thanks to the authors for making the requested revisions. The manuscript is much clearer now although there are still a couple of minor things that could be improved. line 60--should there be a "Limitations" heading here? lines 213-215--the definitions for how sub fertility and infertility are not very clear. Please revise. line 258--all 304 participants were assessed for UGS but not all participants were diagnosed with FGS Tables--please check formatting. Many brackets are backwards and some hyphens are missing that can cause confusion. E.g., line 58 is written as [26] rather than [2-6] for age of last child.

VERSION 3 – AUTHOR RESPONSE

Reviewer 3:

Thanks to the authors for making the requested revisions. The manuscript is much clearer now although there are still a couple of minor things that could be improved.

Sir, thank you very much for your time, recommendations and corrections all through this review process which has greatly improved the quality of this manuscript. We have now taken into consideration your comments and proposals for this minor review.

line 60--should there be a "Limitations" heading here?

Thank you, we have now added the heading “Limitations” to this paragraph. Line 60.

lines 213-215--the definitions for how sub fertility and infertility are not very clear. Please revise.

Thank you for highlighting this. We defined subfertility here as being within reproductive age, but have not achieved pregnancy after two years of having regular unprotected sex. Infertility was defined as not having been pregnant after more than 5 years of being married or having regular unprotected sexual intercourse. However, given that this variable was not further considered in subsequent analyses, we resorted to excluding it from the analysis as its definition still remains debatable (Gnoth et al, 2005. DOI: 10.1093/humrep/deh870. PMID: 15802321). Line 210, 286, Table 1.

line 258--all 304 participants were assessed for UGS but not all participants were diagnosed with FGS

Thank you for this Sir, we have added this line to the text, and it reads clearer. Line 262-263.

Tables--please check formatting. Many brackets are backwards and some hyphens are missing that can cause confusion. E.g., line 58 is written as [26] rather than [2-6] for age of last child.

Thank you for highlighting this, we have relooked at the tables and adjusted the formatting and figures accordingly. Sir, the backward brackets on the right of some intervals were used to indicate that the maximum value in that range is not included. For example, an age range of [14-20[indicates that 20 is not included in the range. We have now modified intervals like [14-20[to [14-19].