PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Associations between intravaginal practices and incidence of sexually transmitted infections and bacterial vaginosis among women enrolled in the Dapivirine vaginal ring trial (The Ring Study) in southwestern Uganda, a retrospective secondary analysis
AUTHORS	Kusemererwa, Sylvia; Ruzagira, Eugene; Onyango, Martin; Kabarambi, Anita; Abaasa, Andrew

VERSION 1 – REVIEW

REVIEWER	Sadiq, S. Tariq St George's University of London, Institute for Infection and Immunity Research Institute
REVIEW RETURNED	02-Oct-2023

GENERAL COMMENTS	This manuscript describes a secondary analysis from a randomised placebo controlled Dapivirine vaginal ring trial, in which the authors investigate whether any intravaginal practices (IVP) influence the incidence of STIs/BV over a two-year follow-up, overall and in intervention and control arms. This is a reasonably well written manuscript with data that might be of interest to readers. There needs to be a more detailed discussion Some comments on content and format are listed below. Introduction 1. Line 7 (page 4): Syphilis should also be included as a cause of the million new infections acquired per day figure from WHO. 2. Line 11 (page 4): 'Low-income' and 'Middle-income' can be merged to 'low-and-middle-income'. 3. Line 12 (page 4): authors should consider rewording the phrase 'majorly affected by STIs….' , for example to 'women in sub-Saharan Africa have a high prevalence of STIs, particularly those at high risk of HIV….' 4. L22 (page 4): The general description of the IVPs that are in use is perhaps insufficient; the authors should enlarge on the type of products being used for cleaning and genital hygiene, with examples (para2 introduction); very briefly give example cases of how these products might effect changes that are described in para2; some of these products are later discussed in the results, this is another reason for describing them generally here. Methods The individuals included in this study are those at high-risk of acquiring HIV. Although the authors reference another manuscript providing details of recruitment, it would be beneficial to the reader to have a sentence or two briefly describing the characteristics of these individuals, for example how many were sex workers, or in a

	discordant HIV relationship, and so forth. This does not need to be overly detailed. 1. Line 43 (p6): What was the HIV RNA PCR? Was this an in-house test, or has it been described elsewhere? Further detail required. 2. Line 49: STATA does not need to be capitalised; it is not an acronym. 3. Line 6 (p7): explain single and multiple event analyses more clearly as to what is being compared. 4. Line 17 (p7): There is a spelling mistake for 'Poison regression'. 5. Line 51 (p7): Abbreviation missing for community advisory board (CAB) as it is used further on in the text. Results 1. line 15 (p8): the brackets containing "67% on the DVR…)" is poorly placed and the sentence should be re-rewritten for clarity. 2. Line 21 (p9): What is detergent in this context? Washing detergent? How does this differ from soap? 3. Line 34 (p9): does the 87.3% refer to STI or STI/BV? Please check. Discussion It is not sufficient in a discussion to simply summarise the overall findings and give a statement of strengths and limitations. The authors should discuss their findings in the wider context of HIV and STI prevention in sub-Saharan Africa, including the broader literature. 1. Line 7 (p12): Data on IVP among those recruited appears to be in line with national data regarding IVP; the authors suggest however that those at risk of HIV infection might have higher infection? This supposed difference should be discussed. 2. Line 28: Although no association between IVP and any STIs/BV were found, there was a very high background prevalence of T. vaginalis infection, which is not discussed, and should be. TV is associated strongly with BV and the high background prevalence may be an explanatory in this lack of association of developing BV in this population? Similarly high background rates of STIs in general would make any additional increment in STI from IVP less detectable. 3. Line 54: The absence of symptoms at enrolment may not be a good enough proxy for absence of STIs at enrolment given the time gap between screening and enrolment, especially with the high rate of STIs found in the study. This should be emphasised in the limitations sections.

REVIEWER	Chimoyi, Lucy The Aurum Institute, Implementation Research
REVIEW RETURNED	20-Oct-2023

GENERAL COMMENTS	General comments - Use of participant rather than subject is recommended - In the introduction, the authors seem to focus on STIs such as TV, NG, and CT. however, HIV is introduced in the methods and results. If the intention is to also include HIV as part of the STIs, my suggestion is for the authors to revise the introduction to include this. - Out of curiosity, why was syphilis not included as part of the STIs under investigation? - What is the public health significance of the findings from this study? This is lacking from this manuscript. The discussion is

	lacking in detail and depth. The significance of the findings is not clearly articulated Abstract - Methods: What was the authors' study investigating? The authors have described the Ring study in their methods. Introduction - It is unclear why STIs/BV have been considered as one outcome. If BV is associated with increased risk of STI acquisition, should this be considered as one of the covariates? - In the last paragraph, is the risk reduction supposed to be risk reduction counselling or could the authors have other strategies in mind? Main Body Methods - Are the authors reporting the study procedures for their current study or the parent study? What was the eligibility criteria for this current study? Is it over and above the normal pelvic exam and a HIV negative test? This should be made clear. - Measurement of outcome: If the outcome of the study was BV/STIs, why is the HIV diagnosis and confirmatory tests included in this section? - Study design: The parent study followed a trial design, what is the design for this study? - The authors indicate that this was a secondary analysis of Trial data. I am assuming the trial was longitudinal. Is this a retrospective analysis? - The definition of multiple-event-per subject is unclear Statistical analysis - Were the characteristics summarized those collected at baseline? Make this clear at this stage and maintain consistency even as you progress to the results - Did you include in your analysis women with previous IVP history? - Authors have included HIV in their methods section but this variable is missing in the statistical analysis and results Results - The socio-demographic characteristics are baseline, right? - The title for Table 1 seems incomplete. Here the authors are emphasizing the ring study instead of the IVP status. The year is also missing in the title. - Is the title after Table 1 "IVP" referring to the proportion reporting IVP? Its vague and should be comprehensive but concise. It is also good practise to write out the full name especially if this term is a heading or sub-heading - How come the known factors such as contraception (spermicide), condom use, sexual activities (frequency of condomless sex acts or even multiple partnerships etc) were not considered for inclusion in the adjusted model? Yet, in the methods under procedures, authors mention that this information was collected at baseline - Some of these variables would be considered as confounders or effect modifiers for STI acquisition. - A suggestion would be to describe the population of women diagnosed with a single vs multiple STIs. This is because the authors have chosen to present the regression analysis of the STI/BV stratified by these two categories. - For Table 2 and 3; does the multiple-event-per subject mean that for each STI listed, the study participant acquired it more than once during the study?

	- Please confirm that the HIV incidence rate is the same for Tables 2 and 3. Is this coincidental? Discussion/conclusions - IVP increases susceptibility to HIV, STI and BV infections among women. Since your study did not find an association, what are the implications of your findings? What does this mean from a public health perspective? - The first paragraph summarizes the main finding of the study. Discussion that confirms or contrasts the findings should be made in the subsequent paragraphs. Please move the last statement from the first paragraph to a different paragraph. - Is a smaller sample size the main reason a link between DVR, IVP and incidence of the outcome was not found? The original trial was powered to detect a difference in HIV incident cases. I think a sufficiently powered study to detect the differences in the outcome of interest would be a better suggestion.

REVIEWER	Carter, Kayla University of Maryland School of Medicine, Institute for Genome Sciences
REVIEW RETURNED	27-Oct-2023

GENERAL COMMENTS	Kusemererwa and colleagues present the results of a secondary analysis of the associations between IVP and subsequent STI and BV acquisition among participants at the Uganda site of the Ring trial. This is an important area of research as IVP are a potentially modifiable risk behavior and are prevalent in some sub-Saharan African populations, as are BV, STI, and HIV. The authors generally observed no significant relationship between IVP and STI/BV, the exception being significantly lower chlamydia rate among those reporting IVP in unadjusted analysis. Overall, the manuscript can be improved with more thorough reporting of the analytic methods and exposure and outcome data. Major comments 1. The introduction doesn't discuss BV very thoroughly, and adding some more detail can help support the rationale for the study and provide context for interpreting the results. Please consider discussing Lactobacillus dominance vs. diverse anaerobes in BV, importance of vaginal pH, Nugent scoring, and associations with STI in addition to HIV. 2. Nugent scoring can be prone to inter-observer differences/bias and measurement error. In the methods, please discuss any training provided to those performing the Nugent scoring, efforts to limit inter-observer variation, and other QA and QC activities. 3. Currently, it is challenging to interpret the IVP data, particularly through follow-up, and the manuscript can benefit from more detailed reporting of how IVP were measured and operationalized. Specifically: a. If possible, please including the questionnaire items used to evaluate IVP as supplemental material or provide a link to access the questionnaire if it is already available online. b. Did the questionnaire include open-ended questions for reporting materials used for IVP and motivations for engaging in IVP? Or was it limited to the options listed in the Measurement of exposure [intravaginal practices (IVP)] section? If it was limited to the options listed, this is a limitation of the study. Prior work consistently reports that women in sub-Saharan African countries use additional materials to what is listed in the text (e.g. herbs, leaves, talc, alum, Coca Cola, antiseptics, vinegar, see https://doi.org/10.1177/1099800420940788) and for reasons other than what is listed in the text (e.g. vaginal drying, vaginal tightening, see https://doi.org/10.2147/IJWH.S180233). If the questionnaire was limited to the options listed in the Measurement of exposure [intravaginal practices (IVP)], exposure measurement error may be differential with respect to outcome status (e.g. if the

	questionnaire did not capture antiseptic use, it might be reasonable to expect a particularly harsh substance like antiseptic to increase STI/BV risk more than the substances listed in the text). Please clarify whether the questionnaire was limited to the options listed in the text and if so, discuss this as a limitation. (If there were open ended questions, then this isn't a concern.) c. How was IVP status operationalized for the analyses (e.g. any reported vs none reported, number of IVP reported)? d. Was follow-up IVP data used as a time-varying exposure in analyses? Or just baseline IVP data? If the current analyses only used baseline IVP data, please consider re-running the analyses using time-varying IVP exposure data. e. Please report IVP summary data through follow-up. A plot of the proportion of participants reporting IVP at each visit broken out by trial arm and BV/STI acquisition might be helpful. 4. The manuscript can be improved with more thorough reporting of how person-time was counted and how much person-time was accrued. Specifically: a. In the multiple-event analysis, it is unclear when participants restart accruing person-time after their first visit with BV/STI. Do they begin accruing after they complete treatment immediately following a study visit where STI/BV is detected (similar to those with STI/BV at baseline)? b. Please report the amount of person-time accrued in each of the subgroups analyzed. c. Consider including survival curves for each outcome. 5. Were any community advisory board members offered the opportunity to be a co-author on the manuscript? Were CAB members compensated in any way for their work? 6. Did you collect data on history of/current engagement in sex work/exchange sex? If yes, please report those data in Table 1 and consider adjusting for this in adjusted analyses. If no, please discuss this as a limitation due to uncontrolled confounding. 7. In Table 1, please include column percents instead of row percents so covariate distributions can be compared by IVP status. Please also report the baseline prevalence of BV and each STI separately. 8. The abstract and discussion state that no significant differences were observed in BV/STI rate by IVP; however, those reporting IVP had a significantly lower rate of CT in unadjusted multiple-event analysis. This result is worth some attention, and the statements in the abstract and discussion that no significant differences were observed should be revised accordingly. 9. The Results subsection Effect of IVP on rate of STIs/BV states "There was also no effect of IVP on STI/BV rates in the different trial arms" and refers to Table 3. However, Table 3 presents differences in STI/BV rate between DVR and placebo among those reporting IVP. These data would estimate the effect of DVR among those engaging in IVP and show that there is no effect of DVR on STI/BV among those engaging in IVP. Please either revise the text accordingly or present and analyze a stratified analysis that compares STI/BV rate by IVP status within the DVR arm and within the placebo arm. (This is why I replied 'No' to Do the results address the research question or objective?) Minor comments 10. The introduction states "High rates (37% to 68%) of BV have been reported among women in Southern and East Africa." This is true, but it is a bit misleading because BV prevalence doesn't tend to be this high in the general population. A recent meta-analysis estimated 25% BV prevalence in the general population of sub-Saharan Africa (https://journals.lww.com/stdjournal/fulltext/2019/05000/high_global_burden_and_costs_of_bacterial.5.aspx). Please revise accordingly. 11. Please provide the rationale for restricting the current study to the Uganda site of the Ring trial. 12. Please report any age-based inclusion/exclusion criteria and whether pregnant and breastfeeding individuals were eligible for the Ring study. 13. Please provide additional detail on the types of samples collected for each diagnostic assay.

VERSION 1 – AUTHOR RESPONSE

Comments from Reviewer 1 and responses
Prof. S. Tariq Sadiq, St George's University of London
Comments to the Author:
This manuscript describes a secondary analysis from a randomised placebo controlled Dapivirine vaginal ring trial, in which the authors investigate whether any intravaginal practices (IVP) influence the incidence of STIs/BV over a two-year follow-up, overall and in intervention and control arms. This is a reasonably well written manuscript with data that might be of interest to readers. There needs to be a more detailed discussion Some comments on content and format are listed below.
Response: We wish to thank Reviewer 1 for the compliment on our manuscript. Detailed responses are provided for each comment below.
Introduction
Comment 1. Line 7 (page 4): Syphilis should also be included as a cause of the million new infections acquired per day figure from WHO.
Response: We thank the reviewer for their suggestion. Syphilis has been added.

Comment 2. Line 11 (page 4): 'Low-income' and 'Middle-income' can be merged to 'low-and-middle-income'.
Response: We appreciate the reviewer for this suggestion. We have revised the line to read "low-and-middle-income countries"

Comment 3: Line 12 (page 4): authors should consider rewording the phrase 'majorly affected by STIs….' , for example to 'women in sub-Saharan Africa have a high prevalence of STIs, particularly those at high risk of HIV….'
Response: We thank the reviewer or this suggestion. The sentence has been revised as suggested.

Comment 4. L22 (page 4): The general description of the IVPs that are in use is perhaps insufficient; the authors should enlarge on the type of products being used for cleaning and genital hygiene, with examples (para2 introduction); very briefly give example cases of how these products might effect changes that are described in para2; some of these products are later discussed in the results, this is another reason for describing them generally here.
Response: We thank the reviewer for this suggestion. More detail has been included in the introduction with definitions and examples in the revised manuscript.

Methods
Comment 5: The individuals included in this study are those at high-risk of acquiring HIV. Although the authors reference another manuscript providing details of recruitment, it would be beneficial to the reader to have a sentence or two briefly describing the characteristics of these individuals, for example how many were sex workers, or in a discordant HIV relationship, and so forth. This does not need to be overly detailed.
Response: We appreciate the reviewer for their suggestion. More detail on recruitment has been included in the methods section under setting and population. We did not specifically ask women whether they were sex workers or not. The median number of sex partners was 6 in the past 3 months (Range 1-366), 191 (97%) of the women recruited had 2 or more sex partners. This data has been added to Table 1 (baseline characteristics)

Comment 6. Line 43 (p6): What was the HIV RNA PCR? Was this an in-house test, or has it been described elsewhere? Further detail required.
Response: A clarification has been made in the revised copy to include where the assays were done. The sentence now reads "Stored samples for participants with confirmed HIV infection were

retrospectively tested at a central laboratory in South Africa (Bioanalytical Research Corporation) for HIV ribonucleic acid (RNA) copies (viral load) using the polymerase-chain-reaction (PCR) assay"

Comment 7: Line 49: STATA does not need to be capitalised; it is not an acronym.
Response: We thank the reviewer for their correction. The word has been corrected accordingly.

Comment 8: Line 6 (p7): explain single and multiple event analyses more clearly as to what is being compared.
Response: Survival methods can have single-event-per-participant, in our case first STI/BV event treated as end of follow up or allowing for multiple-event-per participant, in our case allowing for two or more STI/BV events in the same participant since these are likely to reoccur. We wanted to see if the rates differ between the two methods of defining outcome. The statement below has been added to the manuscript to further explain. "We used two approaches for measuring the rate of STI/BV; (a) a single-event-per-participant (allowing for one event per participant-first STI/BV event) and (b) a multiple-event-per-participant (allowing for two or more STI/BV events for the same participant) since these are recurrent events".

Comment 8. Line 17 (p7): There is a spelling mistake for 'Poison regression'.
Response: We thank the reviewer for pointing this out. The spelling has been corrected accordingly.

Comment 9: Line 51 (p7): Abbreviation missing for community advisory board (CAB) as it is used further on in the text.
Response: The abbreviation has been added.

Results
Comment 10: line 15 (p8): the brackets containing "67% on the DVR…)" is poorly placed and the sentence should be re-rewritten for clarity.
Response: We appreciate the reviewer for their keen review. The bracket has been moved and the sentence re-written.

Comment 11: Line 21 (p9): What is detergent in this context? Washing detergent? How does this differ from soap?
Response: Detergent has been included as part of the soap category.

Comment 12: Line 34 (p9): does the 87.3% refer to STI or STI/BV? Please check.
Response: We have revised the line to include BV. The proportion 87.3% refers to STI/BV.

Discussion
Comment 13: It is not sufficient in a discussion to simply summarise the overall findings and give a statement of strengths and limitations. The authors should discuss their findings in the wider context of HIV and STI prevention in sub-Saharan Africa, including the broader literature.
Response: We appreciate the reviewer for their suggestion. We would like to clarify that a lot has been done around IVP and STIs/BV and HIV with literature showing that IVPs increase the risk of women acquiring STI/BV and HIV. However, minimal data exists on whether IVPs in the presence of a dapivirine vaginal ring microbicide would put women at risk of acquiring STIs/BV. This is the information that this article provides. We have expanded the discussion as suggested.

Comment 14. Line 7 (p12): Data on IVP among those recruited appears to be in line with national data regarding IVP; the authors suggest however that those at risk of HIV infection might have higher infection? This supposed difference should be discussed.

Response: We thank the reviewer for this suggestion. More information on IVP in this population has been added to the discussion section.

Comment 15. Line 28: Although no association between IVP and any STIs/BV were found, there was a very high background prevalence of T. vaginalis infection, which is not discussed, and should be. TV is associated strongly with BV and the high background prevalence may be an explanatory in this lack of association of developing BV in this population? Similarly high background rates of STIs in general would make any additional increment in STI from IVP less detectable.
Response: We appreciate the reviewer for their thoughts. More has been added to the discussion on STI/BV interplay as well as IVP/BV/STI interplay

Comment 16. Line 54: The absence of symptoms at enrolment may not be a good enough proxy for absence of STIs at enrolment given the time gap between screening and enrolment, especially with the high rate of STIs found in the study. This should be emphasised in the limitations sections.
Response: We appreciate the reviewer for this comment. We did acknowledge this as a limitation to our study. Participants were only enrolled into the study after completion of treatment and reporting no symptoms.

Comments from Reviewer 2 and responses
Dr. Lucy Chimoyi, The Aurum Institute
Comments to the Author:

General comments
Comment 1: Use of participant rather than subject is recommended
Response: We appreciate the reviewer for their suggestion. The wording has been revised to "participant" throughout the manuscript.

Comment 2: In the introduction, the authors seem to focus on STIs such as TV, NG, and CT. however, HIV is introduced in the methods and results. If the intention is to also include HIV as part of the STIs, my suggestion is for the authors to revise the introduction to include this.
Response: We appreciate the reviewer for their comment. The named STIs are the curable ones. We did include information on the fact that intravaginal practices have been known to increase the risk of acquiring HIV. More has been added to the introduction.

Comment 3: Out of curiosity, why was syphilis not included as part of the STIs under investigation?
Response: As part of the main study protocol, syphilis testing was only done as part of the screening visit and at the last product use visit (when the dapivirine vaginal ring use was stopped i.e. at 104 weeks). We do not have sufficient data similar to the rest of the STIs evaluated to measure incidence.

Comment 4: What is the public health significance of the findings from this study? This is lacking from this manuscript. The discussion is lacking in detail and depth. The significance of the findings is not clearly articulated.
Response: We appreciate the reviewer for their comments. The manuscript discussion has been revised accordingly to provide more depth.
The authors set out to find out if using intravaginal practices among women using a microbicide in this case the dapivirine vaginal ring would increase their risk of acquiring STIs/BV including HIV. The data from the Ugandan cohort suggests that the STI/BV rates are not different between those using a microbicide with or without dapivirine. Implying that women who practice intravaginal cleansing/insertion could continue using these practices in the presence of the microbicide. However, this conclusion cannot be made clearly with the small sample size.

Abstract
Comment 5: Methods: What was the authors' study investigating? The authors have described the Ring study in their methods.
Response: We wish to clarify that the authors were assessing the effect of intravaginal practices (IVP) on the incidence of sexually transmitted infections (STIs) and bacterial vaginosis (BV) among women using the dapivirine vaginal ring (DVR) or placebo vaginal ring in southwestern Uganda. The methods have been revised to include the study design being a retrospective analysis of data collected for The Ring Study.

Introduction
Comment 6: It is unclear why STIs/BV have been considered as one outcome. If BV is associated with increased risk of STI acquisition, should this be considered as one of the covariates?
Response: Though written as STIs/BV, these are considered separate outcomes. BV (vaginal condition) could not be grouped with STIs the reason it is written as STIs/BV.

Comment 7: In the last paragraph, is the risk reduction supposed to be risk reduction counselling or could the authors have other strategies in mind?
Response: We wish to clarify that risk reduction refers to the rate at which risk of HIV was reduced and does not refer to risk reduction counselling. The sentence has been updated to make this clarification "… the risk of HIV acquisition was even lower (62%) in an open-label extension trial"

Main Body
Methods
Comment 8: Are the authors reporting the study procedures for their current study or the parent study? What was the eligibility criteria for this current study? Is it over and above the normal pelvic exam and a HIV negative test? This should be made clear.
Response: We wish to clarify that this was a secondary analysis of data collected for the parent study (The Ring Study). Women eligible for The Ring Study were eligible for the secondary analysis. Details have been added to the methods section.

Comment 9: Measurement of outcome: If the outcome of the study was BV/STIs, why is the HIV diagnosis and confirmatory tests included in this section?
Response: We wish to clarify that one of the STIs included in the outcomes is HIV. Because other STIs investigated were likely to reoccur, in the write up, we wrapped up all the STIs into STIs and reported HIV separately because it is a one off life time infection. Under the section for statistical analysis, details for measurement of HIV incidence have been added to the revised manuscript.

Comment 10: Study design: The parent study followed a trial design, what is the design for this study?
Response: The study design is a retrospective secondary analysis of data from the parent trial. This has been included in the study title and also clarified in the methods section.

Comment 11: The authors indicate that this was a secondary analysis of Trial data. I am assuming the trial was longitudinal. Is this a retrospective analysis?
Response: We wish to confirm that this was a retrospective analysis. The term retrospective has been added to the study design section of the revised manuscript.

Comment 12: The definition of multiple-event-per subject is unclear
Response: Thank you for the comment, we have edited the statement in the statistical analysis to make this clearer. "(b) a multiple-event-per-participant (allowing for two or more STI/BV events for the same participant) since these are recurrent events." To further explain, STI/BV free participants are followed until they acquire an STI (end of first follow up). They're treated and followed up until the next

episode of STI/BV positive. The analysis allowing for this recurrent events is the multiple-event-per-participant survival analysis.

Statistical analysis
Comment 13: Were the characteristics summarized those collected at baseline? Make this clear at this stage and maintain consistency even as you progress to the results
Response: This has been revised to baseline characteristics and now reads… "Participants' baseline characteristics were summarized…."

Comment 14: Did you include in your analysis women with previous IVP history?
Response: Yes, at baseline information on IVP use in the past 3months was collected using a standardized questionnaire. The question was "In the past 3 months have you put anything inside your vagina for general cleaning/ hygiene, to clean before or after sex, to prepare the vagina for sex, to heal or treat the vagina, for any other reason?"

Comment 15: Authors have included HIV in their methods section but this variable is missing in the statistical analysis and results
Response: Thank you for identifying this inadvertent omission. Statistical analysis methods have been edited to include this. "For HIV, we estimated the rate of HIV infection as number of HIV+ cases divided by the total person years at risk expressed as per 100 person years at risk in a single-event-per-participant survival analysis. Person time at risk were calculated as sum of the time from enrolment to the last HIV seronegative date or to the estimated date of HIV infection. The HIV infection date was estimated as a random date between the last HIV seronegative and the first HIV+ result date in a multiple imputation". Also the results section updated to include HIV.

Results
Comment 16: The socio-demographic characteristics are baseline, right?
Response: We wish to confirm that these are baseline. The word baseline has been added. The subsection title now reads "Baseline socio-demographic characteristics"

Comment 17: The title for Table 1 seems incomplete. Here the authors are emphasizing the ring study instead of the IVP status. The year is also missing in the title.
Response: We wish to clarify that this is a secondary analysis of data collected for women in The Ring Study. The baseline characteristics are for the 197 women in The Ring Study. They are the same women whose data was analysed for the IVP study. We also thank the reviewer for their suggestion, the year 2013 to 2016 has been added to the title for Table 1 which now reads "Baseline socio-demographic characteristics of 197 women enrolled in The Ring Study in southwestern Uganda between 2013 and 2016"

Comment 18: Is the title after Table 1 "IVP" referring to the proportion reporting IVP? Its vague and should be comprehensive but concise. It is also good practice to write out the full name especially if this term is a heading or sub-heading
Response: We appreciate the reviewer for their comment. The title has been revised and written in full as "Proportion of women reporting intravaginal practice (IVP)"

Comment 19: How come the known factors such as contraception (spermicide), condom use, sexual activities (frequency of condomless sex acts or even multiple partnerships etc) were not considered for inclusion in the adjusted model? Yet, in the methods under procedures, authors mention that this information was collected at baseline. Some of these variables would be considered as confounders or effect modifiers for STI acquisition
Response: Thank you very much for this suggestion. Yes, these variables could confound or even modify the effect of IVP on STI acquisition. Due to the limited sample size, this kind of extensive

analysis could not be performed. We only adjusted for age, trial arm and status at baseline in the non-trial arm stratified analysis.

Comment 20: A suggestion would be to describe the population of women diagnosed with a single vs multiple STIs. This is because the authors have chosen to present the regression analysis of the STI/BV stratified by these two categories.
Response: The description has been added under the results section as; “Compared with those who had one episode of STI/BV, participants with two or more episodes were likely to be single and never married (47.8% vs. 35.6%; p=0.127), not currently living with a main partner (56.5% vs.37.5%, p=0.033) and inconsistently living with the main partner in the past year (69.6% vs. 41.7%; p=0.001) but otherwise similar in regard to other participant characteristics.”

Comment 21: For Table 2 and 3; does the multiple-event-per subject mean that for each STI listed, the study participant acquired it more than once during the study?
Response: We wish to clarify that not all the participants but majority 57.4% got two or more episodes of STIs.

Comment 22: Please confirm that the HIV incidence rate is the same for Tables 2 and 3. Is this coincidental?
Response: Thank for identifying this error. This has been corrected to reflect the correct incidence in table 3.

Discussion/conclusions
Comment 23: IVP increases susceptibility to HIV, STI and BV infections among women. Since your study did not find an association, what are the implications of your findings? What does this mean from a public health perspective?
Response: As mentioned above, we set out to find out if using intravaginal practices among women using a microbicide in this case the dapivirine vaginal ring would increase their risk of acquiring STIs/BV including HIV. The data from the Ugandan cohort suggests that the STI/BV rates are not different between those using a microbicide with or without dapivirine. Implying that women who practice intravaginal cleansing/insertion could continue using these practices in the presence of the microbicide. However, this conclusion cannot be made clearly with the small sample size.

Comment 24: The first paragraph summarizes the main finding of the study. Discussion that confirms or contrasts the findings should be made in the subsequent paragraphs. Please move the last statement from the first paragraph to a different paragraph.
Response: We appreciate the reviewer for this suggestion. The discussion has been revised accordingly.

Comment 25: Is a smaller sample size the main reason a link between DVR, IVP and incidence of the outcome was not found? The original trial was powered to detect a difference in HIV incident cases. I think a sufficiently powered study to detect the differences in the outcome of interest would be a better suggestion.
Response: We appreciate the reviewer’s thoughts on this and concur that the small sample size could be one of the reasons for not detecting a difference. We included this as one of the study’s major limitations.

Comments from Reviewer 3 and responses
Dr. Kayla Carter, University of Maryland School of Medicine
Comments to the Author:

Kusemererwa and colleagues present the results of a secondary analysis of the associations between IVP and subsequent STI and BV acquisition among participants at the Uganda site of the Ring trial. This is an important area of research as IVP are a potentially modifiable risk behavior and are prevalent in some sub-Saharan African populations, as are BV, STI, and HIV. The authors generally observed no significant relationship between IVP and STI/BV, the exception being significantly lower chlamydia rate among those reporting IVP in unadjusted analysis. Overall, the manuscript can be improved with more thorough reporting of the analytic methods and exposure and outcome data.
Response: We appreciate the reviewer for their overall comments and provide point to point responses below. We have noted the low chlamydia rates among those reporting IVP. This finding has been included in the discussion.

Major comments
Comment 1: The introduction doesn't discuss BV very thoroughly, and adding some more detail can help support the rationale for the study and provide context for interpreting the results. Please consider discussing Lactobacillus dominance vs. diverse anaerobes in BV, importance of vaginal pH, Nugent scoring, and associations with STI in addition to HIV.
Response: We thank the reviewer for their observation and suggestion. The introduction has been modified accordingly, also to include BV targeted literature.

Comment 2. Nugent scoring can be prone to inter-observer differences/bias and measurement error. In the methods, please discuss any training provided to those performing the Nugent scoring, efforts to limit inter-observer variation, and other QA and QC activities.
Response: We thank the reviewer for their suggestion. Details on training, interpretation and quality assurance have been added to the methods section.

Comment 3: Currently, it is challenging to interpret the IVP data, particularly through follow-up, and the manuscript can benefit from more detailed reporting of how IVP were measured and operationalized. Specifically:
a. If possible, please including the questionnaire items used to evaluate IVP as supplemental material or provide a link to access the questionnaire if it is already available online.
Response: We appreciate the reviewer for this suggestion. Information of IVP was collected at the enrolment visit (baseline), at 4 weeks post enrolment, then every 24 weeks using a standardized behavioral questionnaire. Questions included "In the past 3 months have you put anything inside your vagina for general cleaning/ hygiene, to clean before or after sex, to prepare the vagina for sex, to heal or treat the vagina, for any other reason… to which a Yes/No answer was expected. If yes, information on what was used was collected: Materials such as paper, cloth or cotton wool? Water only? Water plus soap?; Fingers, to
clean or insert something? Other, specify.
Below are copies of the questionnaires used (baseline, at 4 weeks and follow up- every 24 weeks)

b. Did the questionnaire include open-ended questions for reporting materials used for IVP and motivations for engaging in IVP? Or was it limited to the options listed in the Measurement of exposure [intravaginal practices (IVP)] section? If it was limited to the options listed, this is a limitation of the study. Prior work consistently reports that women in sub-Saharan African countries use additional materials to what is listed in the text (e.g. herbs, leaves, talc, alum, Coca Cola, antiseptics, vinegar, see https://doi.org/10.1177/1099800420940788) and for reasons other than what is listed in the text (e.g. vaginal drying, vaginal tightening, see https://doi.org/10.2147/IJWH.S180233). If the questionnaire was limited to the options listed in the Measurement of exposure [intravaginal practices (IVP)], exposure measurement error may be differential with respect to outcome status (e.g. if the questionnaire did not capture antiseptic use, it might be reasonable to expect a particularly harsh substance like antiseptic to increase STI/BV risk more than the substances listed in the text). Please

clarify whether the questionnaire was limited to the options listed in the text and if so, discuss this as a limitation. (If there were open ended questions, then this isn't a concern.)
Response: We thank the reviewer for their comment and insight. The questionnaire provided for both list of items and other to be specified. For any item that was listed e.g soap, details were required on what type of soap.
Above are copies of the questionnaires.

c. How was IVP status operationalized for the analyses (e.g. any reported vs none reported, number of IVP reported)?
Response: IVP was operationalized as any reported vs none as the data was collected as such.

d. Was follow-up IVP data used as a time-varying exposure in analyses? Or just baseline IVP data? If the current analyses only used baseline IVP data, please consider re-running the analyses using time-varying IVP exposure data.
Response: Follow up IVP data were used as time varying exposure in the analysis. This was highlighted in the statistical methods of the manuscript

e. Please report IVP summary data through follow-up. A plot of the proportion of participants reporting IVP at each visit broken out by trial arm and BV/STI acquisition might be helpful.
Response: The proportions of IVP at each visit have been presented overall and broken out by trial arm and STIs infection, see the graph below. HIV and BV are not included because of small numbers to be stratified over time. Given that there was no statistically significant association between IVP and incidence of STIs/BV, we prefer not to include this graph in the manuscript as it does not add new information.

Comment 4. The manuscript can be improved with more thorough reporting of how person-time was counted and how much person-time was accrued. Specifically:
a. In the multiple-event analysis, it is unclear when participants restart accruing person-time after their first visit with BV/STI. Do they begin accruing after they complete treatment immediately following a study visit where STI/BV is detected (similar to those with STI/BV at baseline)?
Response: Participants started accruing person-time the next visit following treatment visit. Of course some individuals may not have fully healed as there was no confirmatory test and this approach was highlighted in the discussion section as a likely source of bias in the estimation of person time in the multiple-event-per-participant survival analysis.

b. Please report the amount of person-time accrued in each of the subgroups analyzed.
c. Consider including survival curves for each outcome.
Response: b&c above, while we acknowledge this provides data visualization, it makes tables and figures rather large and unwieldy as there are two analysis approaches (single-event-per-participant & multiple-event-per-participant) for five STIs provided overall and stratified by IVP status and trial arm. We preferred presenting the most minimal data but covering the important information to make results interpretable.

Comment 5: Were any community advisory board members offered the opportunity to be a co-author on the manuscript? Were CAB members compensated in any way for their work?
Response: We appreciate the reviewer for this comment. The CAB were not offered a chance to be part of the co-authors. However, they were involved in the review of study documents, participant recruitment and follow-up of study participants. Yes, they were compensated for their work. This has been included in the public engagement section of the revised manuscript.

Comment 6. Did you collect data on history of/current engagement in sex work/exchange sex? If yes, please report those data in Table 1 and consider adjusting for this in adjusted analyses. If no, please discuss this as a limitation due to uncontrolled confounding.
Response: We did collect information on sexual behavior using the same questionnaire. Yes, these variables could confound or even modify the effect of IVP on STI acquisition. Due to the limited sample size, this kind of analysis could not be performed. We only adjusted for age, trial arm and status at baseline in the non-trial arm stratified analysis.

Comment 7. In Table 1, please include column percents instead of row percents so covariate distributions can be compared by IVP status. Please also report the baseline prevalence of BV and each STI separately.
Response: We wish to clarify that we included column percentages (under All n (%) for all the baseline characteristics. It is the proportions for IVP use per characteristic that were provided as row percentages. This was aimed at displaying the prevalence of IVP by the different categories of the baseline characteristics. We have provided the baseline prevalence of each STI and BV.

Comment 8. The abstract and discussion state that no significant differences were observed in BV/STI rate by IVP; however, those reporting IVP had a significantly lower rate of CT in unadjusted multiple-event analysis. This result is worth some attention, and the statements in the abstract and discussion that no significant differences were observed should be revised accordingly.
Response: We thank the reviewer for their observation. We have taken note that CT was lower and discussed it accordingly.

Comment 9: The Results subsection Effect of IVP on rate of STIs/BV states “There was also no effect of IVP on STI/BV rates in the different trial arms” and refers to Table 3. However, Table 3 presents differences in STI/BV rate between DVR and placebo among those reporting IVP. These data would estimate the effect of DVR among those engaging in IVP and show that there is no effect of DVR on STI/BV among those engaging in IVP. Please either revise the text accordingly or present and analyze a stratified analysis that compares STI/BV rate by IVP status within the DVR arm and within the placebo arm. (This is why I replied ‘No’ to Do the results address the research question or objective?)
Response: We appreciate the reviewer for their observation. The results text has been revised as suggested. The sentence now reads….” There was no statistically significant effect of IVP use on the rates of STIs/BV among women using the DVR compared to placebo”.

Minor comments
Comment 10. The introduction states “High rates (37% to 68%) of BV have been reported among women in Southern and East Africa.” This is true, but it is a bit misleading because BV prevalence doesn’t tend to be this high in the general population. A recent meta-analysis estimated 25% BV prevalence in the general population of sub-Saharan Africa (https://journals.lww.com/stdjournal/fulltext/2019/05000/high_global_burden_and_costs_of_bacterial.5.aspx). Please revise accordingly.
Response: We appreciate the reviewer for this reference. However, our study focuses on women who engage in transactional sex (at high risk of HIV infection). These have been reported to have higher rates of BV compared to women in the general population. The suggested reference excluded studies involving this group of women.

Comment 11. Please provide the rationale for restricting the current study to the Uganda site of the Ring trial.

Response: The authors only have access to data from Uganda, which was the only site outside South Africa that had 5 sites.

Comment 12. Please report any age-based inclusion/exclusion criteria and whether pregnant and breastfeeding individuals were eligible for the Ring study.
Response: Women 18 to 45 years were the ones recruited in the study. Those who were pregnant or breastfeeding were excluded. We have included details of the inclusion criteria in the methods section.

Comment 13. Please provide additional detail on the types of samples collected for each diagnostic assay.
Response: We thank the reviewer for this suggestion. Details have been added to the methods section.

Signed: Kayla A. Carte

VERSION 2 – REVIEW

REVIEWER	Chimoyi, Lucy The Aurum Institute, Implementation Research
REVIEW RETURNED	13-Dec-2023

GENERAL COMMENTS	The responses are satisfactorily.

REVIEWER	Carter, Kayla University of Maryland School of Medicine, Institute for Genome Sciences
REVIEW RETURNED	02-Jan-2024

GENERAL COMMENTS	Kusemererwa and colleagues present the results of a secondary analysis of the associations between IVP and subsequent STI and BV acquisition among participants at the Uganda site of the Ring trial. This is an important area of research as IVP are a potentially modifiable risk behavior and are prevalent in some sub-Saharan African populations, as are BV, STI, and HIV. The authors generally observed no significant relationship between IVP and STI/BV, the exception being significantly lower chlamydia rate among those reporting IVP in unadjusted analysis. The authors addressed most of my prior comments appropriately in their response and revised manuscript. There are a few exceptions where I would like additional clarification/detail in the text (included below). Given the substantial revisions they made since the prior version, I have a few additional comments as well. Major comments 1. Thank you for sharing the survey questions used to evaluate IVP in your response. I still think it is important to include this information in the manuscript itself, or at a minimum to state that participants could select from a variety of pre-specified responses as well as provide open-ended responses about their IVP. 2. It is my understanding that cluster robust standard errors can be used to account for repeat measures, but not 'typical' robust standard errors. Did the authors use cluster robust standard

	errors? If yes, can you revise the statistical analysis section accordingly? If no, the analysis should be re-run using cluster robust standard errors or another methods that accounts for repeat measures. 3. Why was HIV acquisition date estimated through multiple imputation, but not CT, NG, TV, or BV acquisition date? Additionally, the statistical analysis section states 'Person time at risk were calculated as sum of the time from enrollment to the last HIV seronegative date or to the estimated date of HIV infection.' Were there multiple HIV analyses that used these different person-time calculations? Or were the different person-time calculations used for different participants in the main HIV analyses presented in the text? It would be useful to clarify these points in the text and the rationale for defining person-time at risk differently for the different outcomes. 4. The methods and/or results need to state what test was used to generate p values presented in Table 1 and the results subsection titled 'Proportion of women reporting IVP'. Related to my prior comment about row vs. column percents in Table 1, if these tests compared covariate prevalences between those reporting IVP vs. no IVP, column percents should be presented because these are the values that were compared. 5. The methods and results need to state what test was used to generate p values presented in table 3 for the stratified analysis, without knowing how the data were compared it's very hard to interpret the p value and effect esttimates. Can the authors also present the outcome rates among those reporting no IVP in table 3? I recognize that this will make the table large, but it would maintain consistency with how the results of the main analysis are presented and improve interpretability. 6. Throughout the manuscript, the authors refer to the 'effect' of IVP on HIV, STI, and BV. Given that this secondary analysis does not preserve the benefits of randomization from the parent trial, it is in effect observational, subject to epidemiologic biases, and cannot be used to establish causality. Terms like 'effect' imply a causal relationship, please revise throughout to refer to 'associations' between IVP and HIV, STI, and BV instead of 'effects'. 7. Thank you for adding a more thorough discussion of the significant CT result to the discussion. Currently, this text is a bit hard to follow. I think it can be further improved by focusing a bit more on differences between your CT result and prior studies, and what might drive different results between studies (e.g. population, follow-up duration, etc). I think it's also important to note that the significant CT results was only in the unadjusted multiple-event analysis, so this result is not conclusive, but the direction of association is consistent across the estimates presented in table 2. For the points about vaginal bacteria, the production of molecules like tryptophan by BV-associated bacteria, and associations between IVP and BV would be consistent with IVP being associated with increased CT risk. Since this isn't what you observed, I would consider not including it. I would also consider not including the comment about glycogen, since that's not directly related to the current study. 8. One other important limitation is that measuring and evaluating any vs. no IVP is a very heterogeneous exposure. It is possible that subtypes/characteristics of IVP (certain materials, timing relative to sex, frequency, etc) do significantly impact HIV, STI, and BV risk, but this and prior studies weren't always able to measure or evaluate these differences.

	Minor comments 1. In your response, thank you for clarifying that you are including BV prevalence estimates from high-risk populations in the introduction. Can you also specify this in the introduction? 2. In the statistical analysis section, please clarify that in single-event analyses, participants were censored at trial end, end of ring use, AND their first event for a given outcome. 3. In your response, thank you for clarifying that for multiple-event analyses, person-time begins re-accruing at the post-treatment visit. Can you clarify this in the statistical analysis section as well? 4. Can the authors also present data on the HIV risk behaviors used as enrollment criteria in Table 1 (STI in past 3 months, self-reported condomless sex with multiple partners or a new partner in past 3 months, recreational drug use in past three months)? Can the authors also clarify whether participants were simply recruited from sex work hotspots or whether a recent history/any history of sex work was an enrollment requirement? 5. Is the incidence rate included in the first sentence of the results subsection titled 'Proportion and rate if STIs/BV' for the single or multiple event analysis? 6. Table 2 should specify whether p values are from unadjusted or adjusted models. Also for table 2, the HIV RR estimates are missing, there appears to be a typo in the aRR value for CT in the single event analysis (083 should be 0.83), and there may be a typo in the estimates for CT in the multiple-event analysis as p=0.03 but both CIs include 1. 7. There may be some errors in the in-text references to tables 2 and 3, please check and revise as needed. 8. I agree that the multiple event analysis is a strength of the study! The authors might want to further comment that this is a strength not just for statistical power but also because it is more clinically/real-world relevant because STI re-infection and BV recurrence are common.

VERSION 2 – AUTHOR RESPONSE

Comments from Reviewer 3 and responses

Kusemererwa and colleagues present the results of a secondary analysis of the associations between IVP and subsequent STI and BV acquisition among participants at the Uganda site of the Ring trial. This is an important area of research as IVP are a potentially modifiable risk behavior and are prevalent in some sub-Saharan African populations, as are BV, STI, and HIV. The authors generally observed no significant relationship between IVP and STI/BV, the exception being significantly lower chlamydia rate among those reporting IVP in unadjusted analysis. The authors addressed most of my prior comments appropriately in their response and revised manuscript. There are a few exceptions where I would like additional clarification/detail in the text (included below). Given the substantial revisions they made since the prior version, I have a few additional comments as well.

Response: We wish to thank the reviewer for their valuable insight and interest in the topic at hand.

Major comments

Comment 1. Thank you for sharing the survey questions used to evaluate IVP in your response. I still think it is important to include this information in the manuscript itself, or at a minimum to state that participants could select from a variety of pre-specified responses as well as provide open-ended responses about their IVP.

Response: We appreciate the reviewer for their suggestion. A statement has been included to indicate that participants could select from a variety of pre-specified responses and provided open-ended response about their IVP.

Comment 2. It is my understanding that cluster robust standard errors can be used to account for repeat measures, but not 'typical' robust standard errors. Did the authors use cluster robust standard errors? If yes, can you revise the statistical analysis section accordingly? If no, the analysis should be re-run using cluster robust standard errors or another method that accounts for repeat measures.

Response: We have clarified that cluster robust standard errors were used to account for repeat measurements.

Comment 3. Why was HIV acquisition date estimated through multiple imputation, but not CT, NG, TV, or BV acquisition date? Additionally, the statistical analysis section states 'Person time at risk were calculated as sum of the time from enrollment to the last HIV seronegative date or to the estimated date of HIV infection.' Were there multiple HIV analyses that used these different person-time calculations? Or were the different person-time calculations used for different participants in the main HIV analyses presented in the text? It would be useful to clarify these points in the text and the rationale for defining person-time at risk differently for the different outcomes.

Response: Kasamba et al 2019* have shown that multiple imputation of a random HIV seroconversion date between the last HIV negative test date and first positive test date provides better estimate of HIV incidence. We did not come about similar evidence for other STIs. Also unlike other STIs which can re-occur after treatment, HIV infection happens once. This partly explains why HIV infection was treated differently from other STIs.

As explained above, person time for HIV infected participants was estimated as time from enrolment date to a randomly imputed date between the last HIV negative date and the first positive date. While those remaining negative throughout the trial it was estimated as the time from enrolment to the last date seen in the trial.

For the other STIs, person time was estimated as time from enrollment (entry date) to STI infection or last date seen for those remaining negative. For those with recurring STIs, person time was segmented to allow for multiple entry and exit following treatment.

These clarifications have been added to the manuscript.

*Kasamba I, Nash S, Seeley J, Weiss HA. HIV incidence among women at high risk of HIV infection attending a dedicated clinic in Kampala, Uganda: 2008-2017. Sexually transmitted diseases. 2019 Jun 1;46(6):407-15.

Comment 4. The methods and/or results need to state what test was used to generate p values presented in Table 1 and the results subsection titled 'Proportion of women reporting IVP'. Related to my prior comment about row vs. column percents in Table 1, if these tests compared covariate

prevalences between those reporting IVP vs. no IVP, column percents should be presented because these are the values that were compared.

Response: The test used has been included in the statistics section of the methods. Also, column percentages have been presented instead of row percentages as the reviewer suggested.

Comment 5. The methods and results need to state what test was used to generate p values presented in table 3 for the stratified analysis, without knowing how the data were compared it's very hard to interpret the p value and effect estimates. Can the authors also present the outcome rates among those reporting no IVP in table 3? I recognize that this will make the table large, but it would maintain consistency with how the results of the main analysis are presented and improve interpretability.

Response: The statistical test used is included in the statistics analysis section of the methods, p-values presented in table 3 compare the rates between DVR and placebo arms for IVP users and for none users. An asterisk has been added and explained in the footnote. Additionally, rates for No IVP have been included as the reviewer suggested.

Comment 6. Throughout the manuscript, the authors refer to the 'effect' of IVP on HIV, STI, and BV. Given that this secondary analysis does not preserve the benefits of randomization from the parent trial, it is in effect observational, subject to epidemiologic biases, and cannot be used to establish causality. Terms like 'effect' imply a causal relationship, please revise throughout to refer to 'associations' between IVP and HIV, STI, and BV instead of 'effects'.

Response: We thank the reviewer for their suggestion. The wording has been revised to "association" throughout the revised manuscript.

Comment 7. Thank you for adding a more thorough discussion of the significant CT result to the discussion. Currently, this text is a bit hard to follow. I think it can be further improved by focusing a bit more on differences between your CT result and prior studies, and what might drive different results between studies (e.g. population, follow-up duration, etc.). I think it's also important to note that the significant CT results was only in the unadjusted multiple-event analysis, so this result is not conclusive, but the direction of association is consistent across the estimates presented in table 2. For the points about vaginal bacteria, the production of molecules like tryptophan by BV-associated bacteria, and associations between IVP and BV would be consistent with IVP being associated with increased CT risk. Since this isn't what you observed, I would consider not including it. I would also consider not including the comment about glycogen, since that's not directly related to the current study.

Response: The discussion has been revised as suggested.

Comment 8. One other important limitation is that measuring and evaluating any vs. no IVP is a very heterogeneous exposure. It is possible that subtypes/characteristics of IVP (certain materials, timing relative to sex, frequency, etc.) do significantly impact HIV, STI, and BV risk, but this and prior studies weren't always able to measure or evaluate these differences.

Response: We thank the review for this suggestion. We have included the limitation in the revised manuscript.

Minor comments

Comment 9. In your response, thank you for clarifying that you are including BV prevalence estimates from high-risk populations in the introduction. Can you also specify this in the introduction?

Response: We thank the reviewer for their suggestion. Reference to women at high-risk of HIV acquisition has been included in the introduction.

Comment 10. In the statistical analysis section, please clarify that in single-event analyses, participants were censored at trial end, end of ring use, AND their first event for a given outcome.

Response: Thank you for this suggestion. This has been added to the statistical analysis section of the manuscript.

Comment 11. In your response, thank you for clarifying that for multiple-event analyses, person-time begins re-accruing at the post-treatment visit. Can you clarify this in the statistical analysis section as well?

Response: Thank you for this suggestion as well. We have added the explanation in the statistical analysis section of the manuscript.

Comment 12. Can the authors also present data on the HIV risk behaviors used as enrollment criteria in Table 1 (STI in past 3 months, self-reported condom less sex with multiple partners or a new partner in past 3 months, recreational drug use in past three months)? Can the authors also clarify whether participants were simply recruited from sex work hotspots or whether a recent history/any history of sex work was an enrollment requirement?

Response: We have added the risk behavior data in table 1 as suggested by the reviewer.

Participants were not simply taken from sex work hotspots but had to be at high risk of HIV acquisition based on the criteria defined in the manuscript.

Comment 13. Is the incidence rate included in the first sentence of the results subsection titled ‘Proportion and rate if STIs/BV’ for the single or multiple event analysis?

Response: Thank you for this observation, this has been clarified to single-event-per-participant and the rest left in table 2.

Comment 14. Table 2 should specify whether p values are from unadjusted or adjusted models. Also for table 2, the HIV RR estimates are missing, there appears to be a typo in the aRR value for CT in the single event analysis (083 should be 0.83), and there may be a typo in the estimates for CT in the multiple-event analysis as p=0.03 but both CIs include 1.

Response: We have indicated in the table footnote that p-values are from unadjusted analysis and compare STI rates between IVP and no-IVP. Secondly, the HIV RR estimates have also been added.

Thank you for identifying the typo, this has been corrected to 0.83. For the CT in the multiple-event analysis, the p=0.03 is correct. It compares the rate of CT between IVP (36.6) and no-IVP (49.9) and not the unadjusted rate ratio (uRR) to refer to the CI.

Comment 15. There may be some errors in the in-text references to tables 2 and 3, please check and revise as needed.

Response: We appreciate the reviewer for pointing these out. In-text references to table 2 and 3 have been revised.

Comment 16. I agree that the multiple event analysis is a strength of the study! The authors might want to further comment that this is a strength not just for statistical power but also because it is more clinically/real-world relevant because STI re-infection and BV recurrence are common.

Response: We are grateful for this addition and have included it in the revised manuscript.

VERSION 3 – REVIEW

REVIEWER	Carter, Kayla University of Maryland School of Medicine, Institute for Genome Sciences
REVIEW RETURNED	28-Feb-2024

GENERAL COMMENTS	Thank you for your thoughtful responses to my prior comments! My only remaining comment is on Table 3 - the aRR for no IVP strata are all 1.00. Please revise to include the correct values. Otherwise, I have no further comments.

VERSION 3 – AUTHOR RESPONSE

Comment from Reviewer 3 and response
Thank you for your thoughtful responses to my prior comments! My only remaining comment is on Table 3 - the aRR for no IVP strata are all 1.00. Please revise to include the correct values. Otherwise, I have no further comments.
Response: We appreciate the reviewer for this comments and agree with the review. We have revised the aRR for no IVP (the reference group) from 1.00 to 1, throughout the table.