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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

Sylvia Kusemererwa ,[1] Eugene Ruzagira,[1,2] Martin Onyango,[1] Anita Kabarambi,[3] Andrew Abaasa [2,4]

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For numbered affiliations see end of article.

Correspondence to
Dr Sylvia Kusemererwa; Sylvia.Kusemererwa@mrcuganda.org

ABSTRACT

Objectives We assessed associations between intravaginal practices (IVPs) and 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.

Methods This was a retrospective secondary analysis of data collected from women at risk of HIV infection recruited into the Ring Study. The latter evaluated the safety and efficacy of the DVR between 2013 and 2016. At baseline, a behavioural questionnaire was administered to obtain information on sexual activity and IVP (exposure) defined as; insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Each participant self-inserted the DVR/placebo and replaced it every 4 weeks for 2 years. Outcomes were diagnosis of STIs, that is, *Chlamydia trachomatis*, *Neisseria gonorrhoea*, *Trichomonas vaginalis* (TV), HIV and BV. The incidence rate of STI/BV was estimated, overall, by IVP and trial arm in single-event-per-participant and multiple-event-per-participant analyses.

Results Of the 197 women enrolled, 66 (33.5%) were <25 years of age. Overall, 93 (47.2%) practised at least one form of IVP. During the follow-up, 172 (87.3%) women were diagnosed with an STI/BV at least once. The majority had TV (73.6%, n=145). Overall rate of STI/BV was 51.9/100 person-years, 95% CI 44.7 to 60.3 (IVP: yes, 51.0 (40.8–63.8) vs no, 52.6 (43.0–64.4)). IVPs were not statistically significantly associated with rate of individual STIs/BV. Similar results were observed when the analyses were conducted separately for each trial arm.

Conclusions IVP was not associated with risk of STIs/BV in the Ring Study.

Trial registration number NCT01539226.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study was able to investigate multiple sexually transmitted infections (STIs) and bacterial vaginosis (BV) in the same population.
⇒ The longitudinal nature of the study allowed for both single-event-per-participant and multiple-event-per-participant analyses.
⇒ Extensive analysis was to some extent limited by a small sample size.
⇒ Confirmatory tests were not done to confirm absence of an STI/BV prior to dapivirine vaginal ring insertion.

INTRODUCTION

Sexually transmitted infections (STIs) remain a health challenge globally. In 2018, WHO estimated that nearly one million people become infected every day with a curable STI caused by *Chlamydia trachomatis* (CT), *Neisseria gonorrhoea* (NG), Syphilis or *Trichomonas vaginalis* (TV).[1] STIs disproportionately affect low-income and middle-income countries, with 90% of the new infections occurring in these countries.[2] Women in sub-Saharan Africa have a high prevalence of STIs, particularly those at high risk of HIV infection also having a high STI burden.[3 4] Bacterial vaginosis (BV), a common vaginal condition has also been associated with increased risk of STIs and HIV infection.[5] High rates (37%–68%) of BV have been reported among women at high risk of HIV acquisition in Southern and East Africa.[6]

BV results from variation in normal vaginal flora attributed to reduction in the prevalence of lactobacilli (dominant species in healthy vaginal environment) and an increase in the concentration of pathogenic organisms:

Gardnerella vaginalis, *Bacteroides (Prevotella)* species, *Mobiluncus* species and *Mycoplasma hominis*.[7 8] Increasing evidence shows that vaginal microbiota may play a role in mediating susceptibility to STIs. Vaginal lactobacilli use a number of actions to protect against colonisation by genital pathogens.[9] This can be through production of lactic acid that supports the maintenance of a lower vaginal pH, which may prevent pathogen growth,[10] exposure to hydrogen peroxide that has also been shown to decrease activity of BV and other genital tract organisms.[11 12]

The high prevalence and increased risk of acquisition of STIs/BV and HIV has been associated with vaginal practices.[13] These practices include various behaviours used to maintain health, wellness and enhance sexual pleasure.[14–16] The WHO has suggested seven classifications for vaginal practices: external washing, intravaginal cleansing, external application, intravaginal insertion, oral ingestion, vaginal streaming or smoking and anatomical modification.[17] On the other hand, intravaginal practices (IVPs) refer to both intravaginal cleansing (cleaning or washing inside the vagina with fingers or substances like soap to remove fluids), and intravaginal insertion (placing something inside the vagina, such as powders, creams, herbs or tissue).[18 19]

Soaps, detergents and antiseptics used to cleanse inside the vagina can cause chemical damage and increase vaginal pH resulting in overgrowth of BV-related organisms, which has also been associated with increased risk of HIV acquisition.[19 20] Other products have also been reported to cause genital lesions, and swellings, creating favourable conditions for the transmission of STIs, including HIV.[17] Items such as cloth commonly used in some communities to clean the vagina repeatedly might also act as fomites, carrying TV organisms.[21] TV has also been associated with increased risk of HIV acquisition.[22 23] These practices which increase a woman's susceptibility to HIV could reduce the effectiveness of vaginal microbicides.[24 25]

The monthly dapivirine vaginal ring (DVR) microbicide, a female controlled HIV prevention tool, was found to reduce the risk of HIV acquisition by approximately 30% in two phase 3 trials.[26 27] With continued and consistent use, the risk of HIV acquisition was even lower (62%) in an open-label extension trial.[28] However, data are limited on the effect of IVPs on vaginal flora and risk of STIs/BV among women using the DVR. We assessed associations between IVPs and the incidence of STIs including HIV, and BV among women using the DVR or placebo vaginal ring in southwestern Uganda.

METHODS

Study design

This was a retrospective secondary analysis using data collected in the Ring Study, a phase 3 microbicide trial.[26]

Study setting and population

Details of the Ring Study (registration number: NCT01539226) have been described elsewhere.[26] Briefly, the Ring Study was a multicentre microbicide trial, which evaluated the safety and efficacy of the DVR between 2013 and 2016 in Uganda and South Africa. The study was sponsored by the International Partnership for Microbicides. In Uganda, the study was conducted by the Medical Research Council/Uganda Virus Research Institute and London School of Hygiene and Tropical Medicine Uganda Research Unit in Masaka, southwestern Uganda. The site recruited women at high risk of HIV infection from towns along the trans-African highway and the shores of Lake Victoria. Details of the recruitment procedures and study population have been described elsewhere.[29] Briefly, the research site enrolled 197 women (18–45 years of age) at high risk of HIV infection. Women were identified from sex work hotspots (bars, restaurants, hair salons, small shops and other small-scale businesses). High risk was defined by the presence of any two of the following: (1) history of STIs in the past 3 months; (2) self-reported condom less sex with multiple sex partners or a new partner in the past 3 months and (3) use of recreational drugs (marijuana, alcohol) in the past 3 months. Of the women enrolled, two in five were working in bars and restaurants and nearly a third had small scale businesses. A woman was included in the main study if they were not pregnant, not breast feeding, asymptomatic for genital infections and tested HIV negative at the time of enrolment. Those diagnosed with any clinically significant curable STI were initiated on treatment for at least a week prior and enrolled after completing a full course of treatment.

Study procedures

Participants consented for screening and enrolment on two separate occasions. At the first visit (screening), data on demographics, inclusion and exclusion criteria were obtained. A physical and genital examination was done. All potential participants were provided with HIV/STI risk-reduction counselling and HIV pretest and post-test counselling. At the second visit (enrolment), women who met all inclusion criteria and no exclusion criteria, had a normal pelvic examination and negative HIV rapid tests were enrolled into the trial. Eligible women were randomised in a 2:1 ratio to either the DVR arm or placebo arm. At the enrolment visit, an interviewer-administered behavioural questionnaire was used to obtain baseline information on sexual activity and vaginal practices. At 4 weeks post-enrolment and every 24 weeks thereafter for the next 2 years, follow-up data on vaginal practices were collected using an interviewer-administered behavioural questionnaire. The latter included questions to which participants replied by selecting from a variety of prespecified responses and provided open-ended responses about their IVP. Participants self-inserted a vaginal ring every 4 weeks for up to 104 weeks.

Measurement of exposure (IVP)

IVP was defined as insertion inside the vagina of any items aimed at cleaning the vagina for any reason before, during or after sex other than practices to manage menses. Items included materials such as paper, cloth or cotton wool; water only; water and soap; fingers to clean or insert something. Women were asked if they were inserting or using any of the aforementioned items inside the vagina to clean their vagina either as a general cleaning/hygiene practice before or after sex or to prepare the vagina for sex in the past, at baseline and every 24 weeks.

Measurement of outcome (diagnosis of STIs/BV)

Cervicovaginal swabs were collected at the first screening visit and every 12 weeks (3 months) for 2 years. The swabs were tested for TV (OSOM Trichomonas Rapid test-Sekisui Diagnostics, USA) and CT/NG (Cobas Amplicor CT/NG -PCR test, Roche Diagnostic Systems, Branchburg, New Jersey, USA). Vaginal fluid samples were also collected by trained clinicians using sterile swabs for assessment of vaginal flora (using Nugent's score) and vaginal fluid pH at the enrolment visit (prior to ring insertion) and every 12 weeks. Samples with a score of ≥7 were classified as BV present. Each slide was scored by trained laboratory technologists. For internal quality control, a single batch of slides were re-examined by an independent reader weekly. Discrepant results were resolved by expert consensus. External quality control was assured using College of American Pathologists Vaginitis screen, vaginal gram stain-VS2 as part of the site standard operating procedures.

Participants were tested for HIV at the screening and enrolment visit using whole blood samples collected by venipuncture. Serial rapid HIV antibody tests were done using Alere Determine HIV-1/2 (Alere, Medical co., Matsuhidai, Matsudo-shi, Chiba, Japan) followed by OraQuick- ADVANCE Rapid HIV-1/2 Test (OraQuick-OraSure Technologies, Pennsylvania, USA) to confirm a positive Determine result and Uni- Gold HIV (Trinity Biotech, Ireland) as the tie breaker. At the screening/enrolment visits, a participant was confirmed to have HIV infection if they tested positive on at least two rapid HIV antibody tests. Post-enrolment, HIV testing was done serially as described above. However, for participants who tested positive or discordant on two rapid HIV antibody tests, a confirmatory test on stored plasma was done using Western Blot (J. Mitra and Co., India) as previously described. Blood samples were collected and plasma stored every 4 weeks. Stored plasma samples for participants with confirmed HIV infection were retrospectively tested at a central laboratory in South Africa (Bio Analytical Research Corporation) for HIV RNA copies (viral load) using the PCR assay.[26]

Statistical analysis

Data analyses were performed in Stata V.15.0 (Stata). Participants' baseline characteristics were summarised using frequencies and percentages overall and by IVP status and compared between IVP users and none users using a χ^2 test. We determined the proportion of participants who were positive for a given STI/BV (event) as the number who tested positive for an STI/BV at least once during the study divided by the total number tested. We determined associations between IVP and incidence of each STI/BV, by estimating the rate of STI/BV overall and stratified by IVP status. 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. The incidence rate of a given STI/BV was estimated as the number of participants who tested positive for STI/BV divided by the person-time (years) at risks (pyr) expressed as per 100 pyr. PYRs were estimated as a sum of the time from enrolment into the trial (those negative at baseline) to the date of trial completion or censoring (trial end, end of ring use and their first event for a given outcome). Participants who tested positive for STI/BV at baseline were given treatment and started to contribute person-time after completing the course of treatment. Similar approach was followed for those that got infected during follow-up though the person time was segmented to allow for multiple entry and exit from the analysis following treatment. We further adjusted the effect of IVP on rate of STI/BV for age and baseline STI/BV status by fitting Poisson regression models. In the analysis, we used Poisson regression model with time-varying covariates, allowing for intergroup correlation (because women had multiple records) by using cluster robust standard errors. For HIV, we estimated the rate of HIV infection as number of HIV positive 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 was calculated as sum of the time from enrolment to the last HIV seronegative date (for those that remained negative throughout the trial) or to the estimated date of HIV infection. The HIV infection date was estimated as a multiple imputation random date between the last HIV seronegative and the first HIV+result date.[30]

Patient and public involvement

Communities where the study was conducted were involved from the inception of the study. Community gate keepers: local council leaders, political leaders, village health teams, community-based and faith-based organisations were informed of the study via various engagement meetings prior to study start. The site Community Advisory Board (CAB) members were engaged to review study documents, consent and other study literacy documents and confirm translations to the local language. The CAB was compensated for all the activities they were involved in. Volunteer recruitment and retention were supported by local leaders. Results were disseminated to study participants, CAB and community members through community meetings on study completion and presentations

Table 1 Baseline sociodemographic characteristics of 197 women enrolled in the Ring Study in southwestern Uganda between 2013 and 2016

		Intravaginal practices		
	All	No	Yes	
Variable	n (%)	n (%)	n (%)	P value
Overall	197	104 (52.8)	93 (47.2)	
Trial arm				0.835
Dapivirine vaginal ring	132 (67.0)	69 (66.4)	63 (67.7)	
Placebo	65 (33.0)	35 (33.6)	30 (32.3)	
Age (years)				0.275
18–24	66 (33.5)	30 (28.8)	36 (38.7)	
25–34	98 (49.8)	57 (54.8)	41 (44.1)	
35+	33 (16.7)	17 (16.4)	16 (17.2)	
Education level				0.276
Incomplete primary school	68 (34.5)	37 (35.6)	31 (33.3)	
Complete primary school	105 (53.3)	58 (55.8)	47 (50.5)	
Secondary school*	24 (12.2)	9 (8.6)	15 (16.1)	
Marital status				0.020
Single and never married	90 (45.7)	38 (36.5)	52 (55.9)	
Single but previously married	25 (12.7)	14 (13.5)	11 (11.8)	
Married	82 (41.6)	52 (50.0)	30 (32.3)	
Number of life time sex partners				0.002
Median (IQR)	6 (4–12)	5 (3–9)	8 (5–20)	
Has main partner				0.194
No	35 (17.8)	15 (14.4)	20 (21.5)	
Yes	162 (82.2)	89 (85.6)	73 (78.5)	
Duration lived with main partner (years)				0.690
<1	35 (21.6)	17 (19.1)	18 (24.7)	
1–2	34 (21.0)	19 (21.3)	15 (20.5)	
3+	93 (57.4)	53 (59.6)	40 (54.8)	
Lived with (main) partner in the past year				0.171
All the time	48 (37.8)	38 (42.7)	24 (32.9)	
Some of the time	14 (11.0)	7 (7.9)	12 (16.4)	
No	65 (51.2)	44 (49.4)	37 (50.7)	
Currently lives with main partner				0.376
No	87 (53.7)	45 (50.6)	42 (57.5)	
Yes	75 (46.3)	44 (49.4)	31 (42.5)	
Unprotected sex with multiple/new partner in the past 3 months				
No	38 (19.3)	18 (17.3)	20 (21.5)	0.456
Yes	159 (80.7)	86 (82.7)	73 (78.5)	
Drug/alcohol use in the past 3 months				
No	48 (24.4)	32 (30.8)	16 (17.2)	**0.027**
Yes	149 (75.6)	72 (69.2)	77 (82.8)	
Baseline STI/BV status				
TV				
Negative	121 (61.4)	61 (58.7)	60 (64.5)	0.399
Positive	76 (38.6)	43 (41.3)	33 (35.5)	

Continued

Table 1 Continued

Variable	All	Intravaginal practices		P value
		No	Yes	
	n (%)	n (%)	n (%)	
NG				
Negative	173 (87.8)	92 (88.5)	81 (87.1)	0.770
Positive	24 (12.2)	12 (11.5)	12 (12.9)	
CT				
Negative	177 (89.8)	97 (93.3)	80 (86.0)	0.093
Positive	20 (10.2)	7 (6.7)	13 (14.0)	
BV				
Negative	191 (96.9)	102 (53.4)	89 (46.6)	0.332
Positive	6 (3.1)	2 (33.3)	4 (66.7)	

*Includes one woman who had greater than secondary education.
BV, bacterial vaginosis; CT, *Chlamydia trachomatis*; NG, *Neisseria gonorrhoeae*; STI, sexually transmitted infection; TV, *Trichomonas vaginalis*.

at national and international meetings, seminars and conferences. All stakeholders were compensated for the time spent during engagement meetings.

RESULTS

Baseline sociodemographic characteristics

In total, 197 women enrolled, 67% on the DVR trial arm. Of those, 66 (33.5%) were less than 25 years of age, 82 (41.6%) were married and very few 24 (12.2%) had secondary school education. The majority, 162 (82.2%), reported having a main partner but only 75 (46.3%) lived with this partner (table 1). About a half, 100 (50.8%), tested positive for an STI/ BV at baseline. 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%), 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.

Proportion of women reporting IVP

93 (47.2%) women reported at least one form of IVP. The commonly used substances to clean the vagina included: soap (n=76, 81.7%), cloth (n=5, 5.4%) and others (honey, herbs, perfume n=12, 12.9%). Reported IVPs were more common among single and never married women compared with single but previously married or currently married women (p=0.020), among those with more sex partners (p=0.002) and those who used drugs/alcohol in the past 3 months (p=0.027) (table 1).

Proportion and rate of STIs/BV

A total of 172 (87.3%) women were diagnosed with an STI/BV at least once during follow-up, with an overall single-event-per participant incidence rate of 51.9 per 100 pyrs. The overall incidence rate for HIV was 5.8 per 100 pyrs. The most common STI was TV (73.6%, n=145/197 diagnosed at least once) with a rate of 92.7 per 100 pyrs in the single-event-per-participant analysis (table 2).

Associations between IVP and rate of STIs/BV

In the single-event-per-participant analysis, STI/BV and HIV rates were not associated with reported IVP (table 2). However, in the multiple-events-per-participant analysis, the rate of CT was statistically significantly lower among women who reported IVP vs those who did not (p=0.030). On stratification by trial arm, the rate of NG was higher in the DVR arm compared with the placebo arm in both the single and multiple-events-per-participant analysis among women not using IVP (p=0.024 and p=0.007, respectively) (table 3). After adjusting for participant baseline characteristics, overall, in the multiple-event per participant analyses, IVP was only associated with lower rates of CT among women in the placebo arm (adjusted rate ratio 0.33, 95% CI 0.14 to 0.78) (table 3).

DISCUSSION

Our study aimed to assess associations between IVP and incidence of STIs including HIV, and BV among women using the DVR or placebo. Overall, we found that nearly one in every two women practised at least one form of IVP. IVP is reported to be high in African women with proportions of between 30% and 50%, and even higher among women at high risk of acquiring HIV.[31 32] Generally, IVP in this population is driven by cultural and social norms as well as the need for personal hygiene in relation to sexual health and relationships.[33] Women recruited in our study were those engaging in transactional sex and are thus expected to present themselves to their male partners in a fresh vaginal state.[34 35] The frequency of IVP among women involved in transactional sex may be influenced by the need to remain clean/fresh coupled with

Table 2 Associations between intravaginal practices and rate of sexually transmitted infection/bacterial vaginosis among women in the Ring Study in southwestern Uganda

Sexually transmitted infection/ condition	Overall	Intravaginal practices				
		Yes	No			
	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	P value*	uRR (95% CI)	aRR (95% CI)
Single-event-per-participant analysis						
HIV	5.8 (3.0 to 11.1)	7.0 (2.9 to 17.0)	4.7 (1.8 to 12.4)	0.278	1.51 (0.39 to 5.81)	1.29 (0.29 to 5.67
Trichomonas vaginalis	92.7 (78.7 to 109.0)	97.4 (76.9 to 123.3)	88.7 (70.9 to 111.1)	0.288	1.10 (0.79 to 1.52)	1.10 (0.67 to 1.63)
Neisseria gonorrhoea	23.4 (18.3 to 29.9)	22.8 (15.9 to 32.9)	23.8 (17.0 to 33.4)	0.435	0.96 (0.58 to 1.57)	0.99 (0.56 to 1.79)
Chlamydia trachomatis	28.4 (22.7 to 35.6)	27.9 (20.2 to 38.8)	28.9 (21.2 to 39.4)	0.446	0.97 (0.62 to 1.52)	0.83 (0.50 to 1.38)
Bacterial vaginosis	14.1 (10.6 to 18.6)	13.4 (8.9 to 20.4)	14.7 (10.1 to 21.4)	0.383	92 (0.52 to 1.61)	1.13 (0.53 to 2.37)
Multiple-events-per-participant analysis						
T. vaginalis	88.9 (79.6 to 99.5)	90.2 (76.8 to 106.0)	87.9 (75.3 to 102.5)	0.406	1.03 (0.77 to 1.37)	1.07 (0.80 to 1.43)
N. gonorrhoea	28.4 (23.3 to 34.6)	28.7 (21.5 to 38.1)	28.2 (21.5 to 37.0)	0.935	1.02 (0.61 to 1.69)	0.99 (0.59 to 1.67)
C. trachomatis	43.6 (37.2 to 51.1)	36.6 (28.4 to 47.1)	49.9 (40.7 to 61.2)	0.030	0.73 (0.45 to 1.20)	0.64 (0.38 to 1.05)
Bacterial vaginosis	18.8 (11.1 to 31.7)	12.1 (4.5 to 32.2)	24.1 (13.0 to 44.8)	0.124	0.51 (0.13 to 1.95)	0.48 (0.16 to 1.47)

Adjusted for age, trial arm, STI/BV at baseline.

*Unadjusted p value comparing the rate of each STI between IVP use and none use.
aRR, adjusted rate ratio; BV, bacterial vaginosis; IVP, intravaginal practice; PYR, person-years at risk.STI, sexually transmitted infection; uRR, unadjusted rate ratio;

Table 3 Associations between intravaginal practices and rate of sexually transmitted infection/Bacterial vaginosis stratified by trial arm among women in the Ring Study in southwestern Uganda

		Trial arm				
	Overall	DVR	Placebo			
Sexually transmitted infection/condition	IVP use	Rate/100 PYR (95% CI)	Rate/100 PYR (95% CI)	P value*	aRR (DVR) (95% CI)	aRR (Placebo) (95% CI)
Single-event-per-participant analysis						
HIV	No	3.3 (0.8 to 12.9)	8.4 (2.1 to 33.8)	0.374	to	to
	Yes	8.8 (3.3 to 23.5)	3.9 (0.6 to 27.9)	0.518	to	to
Trichomonas vaginalis	No	77.5 (58.4 to 102.8)	118.3 (81.6 to 171.3)	0.081	1	1
	Yes	105.8 (79.7 to 140.4)	82.4 (53.7 to 126.4)	0.171	1.34 (0.78 to 2.31)	0.59 (0.28 to 1.26)
Neisseria gonorrhoea	No	30.5 (21.1 to 44.2)	11.8 (5.3 to 26.2)	0.024	1	1
	Yes	20.6 (13.0 to 32.6)	28.0 (15.5 to 50.5)	0.211	0.73 (0.35 to 1.53)	1.88 (0.49 to 7.20)
Chlamydia trachomatis	No	28.5 (19.4 to 41.9)	29.5 (17.5 to 49.8)	0.911	1	1
	Yes	29.7 (20.2 to 43.6)	24.3 (13.1 to 45.2)	0.305	1.09 (0.60 to 2.00)	0.37 (0.11 to 1.36)
Bacterial vaginosis	No	13.7 (8.6 to 22.1)	16.5 (8.9 to 30.7)	0.636	1	1
	Yes	12.6 (7.5 to 21.3)	15.2 (7.6 to 30.3)	0.332	0.74 (0.26 to 2.09)	1.51 (0.35 to 6.52)
Multiple-events-per-participant analysis						
T. vaginalis	No	87.2 (72.2 to 105.3)	89.2 (68.3 to 116.5)	0.558	1	1
	Yes	94.4 (77.9 to 114.2)	81.5 (60.5 to 109.9)	0.213	1.12 (0.80 to 1.56)	0.76 (0.44 to 1.30)
N. gonorrhoea	No	34.7 (25.8 to 46.8)	14.9 (7.7 to 28.6)	0.007	1	1
	Yes	25.2 (17.4 to 36.4)	36.0 (23.0 to 56.5)	0.116	0.74 (0.42 to 1.30)	2.24 (0.61 to 8.31)
C. trachomatis	No	56.5 (44.7 to 71.5)	36.3 (24.0 to 55.2)	0.065	1	1
	Yes	40.4 (30.2 to 54.2)	28.4 (17.1 to 47.2)	0.126	0.75 (0.43 to 1.29)	0.33 (0.14 to 0.78)
Bacterial vaginosis	No	20.5 (9.2 to 45.7)	32.7 (12.3 to 87.0)	0.482	1	1
	Yes	12.5 (4.0 to 38.7)	11.1 (1.6 to 78.7)	0.491	0.64 (0.11 to 3.71)	0.39 (0.10 to 2.72)

Adjusted for age and STI/BV at baseline.
*Compares rates for IVP (yes or no) between DVR and placebo.
aRR, adjusted rate ratio; BV, bacterial vaginosis; DVR, dapivirine vaginal ring; IVP, intravaginal practice; PYR, person-years at risk; STI, sexually transmitted infection;

worries about HIV infection. Prior studies in Uganda and Tanzania showed that a higher frequency of sex was associated with more frequent engagement with IVP.[25 36 37]

The prevalence of STIs/BV in our study population was high confirming the fact that the women were at very high risk of HIV infection. More events were reported among single women and those who did not live with a main partner and those with more sex partners. Women at high risk of HIV infection engage in transactional sex and have multiple partners, that puts them at higher risk of acquiring STIs/BV. Those who were single also engaged more in IVP. Earlier studies showed that women engaging in high frequency of sex are required to present themselves as clean to their male partners.[14 34] IVP is generally practised for hygiene purposes and sexuality.[15 16] IVP has been associated with changes in vaginal flora and resulting BV. The latter is associated with increased susceptibility to STIs including HIV.[17] The interaction between BV and STIs including HIV has been well documented with each causing genital inflammation.[38] It is reported that organisms associated with BV may overgrow as a result of increased vaginal pH brought about by substances that may be used for IVP like soaps which most of the women in our study used. With increased pH, the protective lactobacilli species are replaced by pathogenic organisms resulting in BV.[19 20] The products used may cause genital lesions enabling the transmission of STIs.[17]

We observed a high incidence of STIs in this population, with the the most common STI being TV. TV is reported to be prevalent among women engaging in transactional sex with high proportions reported globally (16%) and the African region contributing even higher proportions (23%).[39] TV has also been associated with BV, especially among women that use cloth for IVP as these act as fomites.[21] Although the incidence of STIs was high, we did not see any significant difference in the rise in incidence of STIs/BV among women using IVP compared with those who did not, except for CT that was lower. It is not clear and has not been documented that IVP reduces the risk of acquiring STIs/BV, though the reverse has been reported. IVP causes changes to vaginal flora resulting in increased risk of STIs/BV including HIV. A recent systematic review found that IVP increased the risk of vaginal infections (BV, TV and vulvovaginal candidiasis),[40] two others found no association between IVP and TV[15] or BV.[41] One study in South Africa found that IVP was associated with increased risk of HIV infection but not other STIs.[42] The

fact that IVP results in BV, organisms related with BV have been associated with the production of metabolites that are used by STIs like CT as growth factors enabling their multiplication.[43] Incidence of CT has been reported to be high among women (5 per 100 pyrs), especially among younger women (27.6 per 100 pyrs) in Kenya, but not associated with vaginal washing.[44]

We also found that women not using IVP, but using the DVR had higher rates of NG compared with those in the placebo arm. Women in this study generally had high rates of STIs. We have previously reported that rates of STIs decreased over time in the same cohort of women.[45] Apart from lower rates of CT among women using the placebo vaginal ring, there was no statistically significant association between the DVR microbicide and STI/BV including HIV rates among those using IVP. The DVR has been associated with minimal changes in the vaginal microbiota that were likely not clinically significant.[46] It has generally been found to be well tolerated in adult as well as adolescent girls and young women.[47 48] Various studies on microbicides have reported that their use may result in little or no difference in the risk of acquiring STIs like CT, NG or TV.[49] In the Buffer Gel microbicide study, for example, no significant changes in colonisation with Lactobacillus species was reported.[50]

The strengths of this study included the opportunity to investigate multiple STIs and BV in the same population and the ability to collect recurrent data on genital conditions which allowed for conduct of both single-event-per-participant and multiple-event-per-participant analyses. The multiple-event-per-participant analysis models the total rate of events over the entire follow-up period and has more power for detecting associations compared with the single-event-per-participant analysis.[51] This analysis is also more clinically relevant because STI reinfection and BV recurrence are common.

A limitation of the study was the small sample size which may have impacted the study power and consequently the ability to detect any differences in STI/BV rates between women who reported IVP and those who did not. Additionally, no confirmatory tests were done to confirm absence of an STI/BV prior to DVR insertion. However, duration of treatment and the absence of symptoms and signs were used as a proxy for lack of an STI/BV. Furthermore, it was not possible to measure and evaluate any vs no IVP. This is challenging to do as different types of IVP and materials used affect the risk of HIV, STI and BV risk. This has also not been possible to measure in previous studies.

In conclusion, we found a high prevalence of IVP and incidence of STIs/BV among women enrolled in the Ring Study in Uganda. IVP did not statistically significantly increase STI/BV rates. Implying that women who practice intravaginal cleansing/insertion could continue using these practices in the presence of the microbicide. However, our results should be interpreted with caution because of a limitation in sample size that could generate a hypothesis and not conclusively test it. An analysis with a bigger sample size could be helpful to better understand whether there is a link between use of the DVR, IVP, and incidence of STIs/BV.

Author affiliations
[1]Viral Pathogens Epidemiology and Interventions, MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda
[2]Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, UK
[3]International Centre for Child Health and Development, Masaka, Uganda
[4]Statistics & Data Science, MRC/UVRI and LSHTM Uganda Research Unit, Entebbe, Uganda

X Sylvia Kusemererwa @Kushylvia

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Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and the approval was obtained from the Uganda Virus Research Institute Research Ethics Committee (Ref#-GC/127/13/03/33), the Uganda National Council of Science and Technology (Ref#- HS1362) and the National Drug Authority (Ref #-166/ESR/NDA/DID-07/2013). Written informed consent was obtained from each woman before any study procedures were performed. Women who tested HIV-positive were referred to an HIV care provider of their choice. Treatment was provided to those who tested positive for STIs/BV according to the Centers for Disease Control and Prevention (CDC) STD Treatment guidelines 2010.

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ORCID iDs
Sylvia Kusemererwa http://orcid.org/0000-0002-1390-109X
Andrew Abaasa http://orcid.org/0000-0002-6770-5588

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