Original article

HIV-1 and other sexually transmitted infections in a cohort of female sex workers in Chiang Rai, Thailand

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Objectives: To determine demographic and behavioural factors and sexually transmitted infections (STIs) associated with prevalent HIV-1 infection among brothel based and other female sex workers (FSWs) in Chiang Rai, northern Thailand.
Methods: Data were collected from questionnaires, physical examinations, and laboratory evaluations on Thai FSWs enrolled in a prospective cohort study in Chiang Rai, Thailand, from 1991 to the end of 1994.
Results: HIV-1 seroprevalence was 32% among 500 women: 47% for 280 brothel workers and 13% for 220 other FSWs ($p<0.001$); 96% of infections were due to HIV-1 subtype E. At enrolment, other STIs were common: chlamydia, 20%; gonorrhoea, 15%; active syphilis (serological diagnosis), 9%; genital ulcer, 12%; seroreactivity to *Haemophilus ducreyi*, 21%, and herpes simplex virus type 2 (HSV-2), 76%. On multiple logistic regression analysis, HIV-1 was associated with brothel work, birth in upper northern Thailand, initiation of commercial sex at <15 years of age, syphilis, HSV-2 seropositivity, and genital ulcer.
Conclusions: Young Thai FSWs working in brothels in northern Thailand in the early phase of the HIV epidemic have been at very high risk for HIV-1 infection and several other STIs. Programmes are needed to prevent girls and young women from entering the sex industry and to reduce the risk of infection with HIV-1 and other STIs.
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Keywords: HIV; Thailand; sex workers; STIs

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Introduction

Male patronage of female sex workers (FSWs) has played a central role in the explosive epidemic of heterosexual HIV-1 transmission that has occurred in Thailand since 1989 and has been most severe in the upper northern region of the country.[1–8] To better understand the epidemic in northern Thailand, we report here the results of a comprehensive evaluation of a cohort of FSWs in Chiang Rai designed to examine demographic, behavioural, and other sexually transmitted infections (STIs) associated with HIV-1 infection. Chiang Rai is Thailand's northernmost province, bordering Laos and Burma (Myanmar) in an area known as the "Golden Triangle". In June 1991, a national sentinel surveillance survey conducted by the Thai Ministry of Public Health documented the highest rate of HIV-1 infection among brothel based FSWs in Chiang Rai (62.9%; 283 of 450) (Division of Epidemiology, Ministry of Public Health, Thailand). In 1991, an estimated 700 FSWs (70% Thai and 30% foreign) were working in 68 commercial sex establishments in the municipal district of Chiang Rai (Chiang Rai Provincial Health Office, 1992, unpublished data). Although prostitution is illegal in Thailand, FSWs are generally required by government officials to undergo regular (weekly, biweekly, or monthly) examinations for detection and treatment of sexually transmitted infections (STIs).[9] An epidemiological study of young men from Chiang Rai identified sex with FSWs as the principal mode of HIV-1 transmission and did not identify increased risk associated with sex with women who were not prostitutes, sex with men, injecting drug use, blood transfusion, or tattooing.[1 5]

Cross sectional studies of FSWs in Thailand have identified brothel based, or "direct," FSWs, who generally have more clients per day and receive less compensation for sexual services, to be at substantially higher risk for HIV-1 infection than women working as "indirect" FSWs in massage parlours, night clubs, bars, restaurants, etc.[2 10–12] To date, no study has examined in detail other STIs among brothel based and other FSWs in Thailand. These data are important to guide STI and HIV prevention efforts in Thailand and elsewhere in Asia.

Methods

STUDY PARTICIPANTS

The Chiang Rai Health Club study participants were women at least 16 years of age who had Thai national identification cards and who reported current employment as FSWs in Chiang Rai Province. Women were approached by study staff at the Chiang Rai provincial sexually transmitted disease (STD) clinic, other district STD clinics, and at their places of work. The women received written materials and were told about the study; those who gave written, informed, voluntary consent were enrolled for baseline evaluation and follow up every 3

Table 1 Characteristics of the 500 study participants and sexually transmitted infections identified at enrolment, by type of workplace (brothel or non-brothel). (Percentage of subjects, except where noted)

	All (n=500)	*Brothel workers (n=280)*	*Non-brothel workers (n=220)*	*p* Value*
Characteristic:				
Age (years) mean	23.6	22.2	25.4	<0.001
<20	37.6	45.7	27.3	<0.001
20–29	42.6	41.1	44.6	0.4
⩾30	19.8	13.2	28.2	<0.001
Education (number of years)				
0–4	38.1	35.8	40.9	0.3
5–6	51.5	61.3	39.1	0.001
⩾7	10.4	2.9	20.0	0.001
Ever legally married	20.2	14.6	27.3	<0.001
Currently have husband or steady partner	28.8	17.1	43.7	<0.001
Ever had a child	51.2	41.8	63.2	<0.001
Age at first commercial sex				
<15	13.8	21.4	4.1	<0.001
15–17	32.5	38.9	24.2	<0.001
18–20	20.8	18.6	23.7	0.2
⩾21	32.9	21.1	48.0	<0.001
Most recent day worked:				
No of sex partners, mean (range)	2.1 (0–12)	2.8 (0–12)	1.1 (0–5)	<0.001
Fee customer paid for sex (Thai baht†)				
mean	608	181	1178	<0.001
range	(0–8000)	(18–1000)	(0–8000)	
Sexually transmitted infections:				
HIV-1 seropositive	32.0	47.1	12.7	<0.001
C trachomatis	20.2	23.9	15.5	0.02
N gonorrhoeae	15.4	23.2	5.5	<0.001
TPHA positive and RPR ⩾4	8.5	12.7	3.2	<0.001
RPR positive	19.2	24.9	11.8	<0.001
TPHA positive	32.1	38.5	24.0	0.001
H ducreyi seropositive	20.8	25.8	14.6	0.002
HSV-2 seropositive	75.6	78.2	72.3	0.1
Genital ulcer	12.0	13.6	10.0	0.2
HSV-1 seropositive‡	91.0	92.9	88.6	0.1

TPHA = *Treponema pallidum* haemagglutination assay; RPR = rapid plasma reagin.
*p Values are for comparisons between brothel workers and non-brothel workers.
†$1 = 25 Thai baht.
‡HSV-1 is not considered a sexually transmitted infection, but is shown for comparison.

months. Each member received a Chiang Rai Health Club identification card with a unique study number. At enrolment, women received HIV counselling and education about avoiding acquisition and transmission of HIV and other STIs, including the use of condoms. Condoms were available to women free of charge. Women were categorised as working in brothels or in other establishments where sex services were available, such as massage parlours, music clubs, bars, restaurants, barbers' shops, etc. In general, brothels were establishments where the FSWs resided and where the clients paid the proprietor for sex with FSWs. At other establishments, the FSWs lived elsewhere; other non-sex services (for example, food, massage, singing, and dancing) were provided; and the cost of the sex service generally was negotiated between the clients and the FSWs and paid to the FSWs. Upper northern Thailand was defined as the following provinces: Chiang Rai, Payao, Chiang Mai, Lampang, Lamphun, Mae Hong Son, Nan, and Phrae. The study protocol was approved by the Committee on Ethical Review of Research on Human Subjects of the Thai Ministry of Public Health and an institutional review board of the US Centers for Disease Control and Prevention (CDC).

DATA COLLECTION

At enrolment, women were interviewed by trained Thai staff fluent in the northern Thai dialect who used a questionnaire that addressed demographics, sexual behaviour, injecting drug use, other HIV risk behaviours, history of commercial sex work, contraception, condom use, and medical history. A physical examination (including a genital and pelvic examination) was performed by a study nurse practitioner.

LABORATORY TESTING

At enrolment, endocervical swabs were collected and tested for the presence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by using a nucleic acid hybridisation test (Gen-Probe Pace 2 System, San Diego, CA, USA) according to the manufacturer's instructions. Serum specimens were collected and tested at the HIV/AIDS collaboration laboratory, Nonthaburi (near Bangkok), for antibodies to HIV by using an enzyme immunoassay (EIA; Genetic Systems HIV-1/HIV-2 EIA, Genetic Systems Corp, Redmond, WA, USA) and, if positive, a western blot (NovaPath HIV-1 Immunoblot, BioRad Diagnostics Group, Hercules, CA, USA); and for reactivity to the syphilis rapid plasma reagin (RPR) test (MacroVue RPR Card Test, Becton Dickinson Microbiology Systems, Cockeysville, MD, USA); and the *Treponema pallidum* haemagglutination assay (TPHA; TPHA Reagents, Fujirebio Inc, Tokyo, Japan). Serum specimens were frozen and shipped to CDC, Atlanta, where they were tested for antibodies to herpes simplex virus (HSV) types 1 and 2 by using specific immunoblots employing recombinant gG1 and gG2 antigens[13] and for antibody to *Haemophilus ducreyi*.[14] Genital ulcers were scraped, and smears were prepared, fixed, and examined by direct fluorescent antibody (DFA) staining for *T pallidum*[15] and herpes simplex virus (HSV) (Kallestad Diagnostics, Austin, TX, USA). HIV seropositive specimens were tested at the HIV/AIDS collaboration laboratory to determine the infecting HIV-1 envelope (env) subtype with highly specific HIV-1 V3-loop peptide EIAs for subtypes E and B' (Thai B).[16 17] Laboratory diagnoses of treatable infections were communicated to the staff of the Chiang Rai Health Club in order to arrange for appropriate treatment.

DATA ANALYSES

All epidemiological data were double entered by using EPI-INFO, version 5.01b (CDC, Atlanta, GA, USA), laboratory data were entered by using Paradox versions 4.0 and 5.0 (Borland, Inc, Scotts Valley, CA, USA), and SAS versions 6.02 and 6.11 (SAS Institute Inc, Cary, NC, USA) were used for data management and analyses, including forward and stepwise multiple logistic regression. Variables significantly associated ($p<0.05$) with HIV-1 seropositivity on univariate analyses were included as independent variable in multiple logistic regression models. For variables that were clearly collinear, a single variable was chosen for the final forward logistic regression model. Also included in the final model were selected variables representing important domains, even if these variables were not significant on univariate analyses.

Table 2 Factors and their associations with HIV-1 seropositivity at enrolment by univariate analysis (n=500). Numbers (%) of subjects HIV-1 seropositive

	HIV-1 seropositivity			
	Factor present	*Factor not present*	*PR (95% CI)*	*p Value*
Factor:				
Brothel worker	132/280 (47.1)	28/220 (12.7)	3.70 (2.56–5.35)	<0.001
Birth in upper north	157/463 (33.9)	3/37 (8.1)	4.18 (1.40–12.5)	0.001
Education <7 years	156/447 (34.9)	4/52 (7.7)	4.54 (1.75–11.7)	<0.001
First commercial sex at <15 years	40/69 (58.0)	120/430 (27.9)	2.08 (1.61–2.67)	<0.001
Worked ⩾2 years as a FSW	118/301 (39.2)	42/199 (21.1)	1.86 (1.37–2.52)	<0.001
Have husband or steady partner	34/144 (23.6)	126/356 (35.4)	0.67 (0.48–0.92)	0.01
Most recent day worked:				
Customer paid ⩽200 Thai baht†	105/215 (48.8)	52/262 (19.9)	2.46 (1.86–3.25)	<0.001
Had >1 customer	96/217 (44.2)	61/264 (23.1)	1.91 (1.47–2.50)	<0.001
During preceding 3 months:				
Vaginal sex during menstruation	51/109 (46.8)	106/372 (28.5)	1.64 (1.27–2.12)	<0.001
Worked ⩾60 days	106/270 (39.3)	51/212 (24.1)	1.63 (1.23–2.16)	<0.001
Contraceptive:				
DMPA	23/59 (39.0)	130/423 (30.7)	1.27 (0.89–1.80)	0.2
Oral pills	97/267 (36.3)	56/215 (26.1)	1.39 (1.06–1.84)	0.02
Condom use for vaginal sex:				
Every time	91/297 (30.6)	66/182 (36.2)	0.84 (0.65–1.09)	0.2
⩾50% of times	58/144 (40.3)			
<50% of times	6/18 (33.3)			
Never	2/20 (10.0)			
Sexually transmitted infections:				
C trachomatis	42/100 (42.0)	116/395 (29.4)	1.43 (1.08–1.89)	0.02
N gonorrhoeae	39/76 (51.3)	119/419 (28.4)	1.81 (1.38–2.36)	<0.001
TPHA and RPR ⩾4	26/42 (61.9)	132/450 (29.3)	2.11 (1.60–2.78)	<0.001
H ducreyi seropositive	48/160 (32.1)	56/339 (16.5)	1.82 (1.30–2.54)	<0.001
HSV-2 positive	135/374 (36.1)	24/122 (19.7)	1.53 (1.25–2.69)	0.001
Genital ulcer	26/60 (43.3)	133/437 (30.4)	1.42 (1.03–1.97)	0.05
HSV-1 positive*	141/451 (31.2)	18/45 (40.0)	0.78 (0.53–1.15)	0.2

PR = prevalence ratio; DMPA = depot medroxyprogesterone acetate; TPHA = *Treponema pallidum* haemagglutination assay; RPR = rapid plasma reagin.
†$1 = 25 Thai baht.
*HSV-1 is not considered an STI, but is shown for comparison.

Results

COMPARISONS OF BROTHEL AND NON-BROTHEL SEX WORKERS

Demographics

From October 1991 to the end of December 1994, 500 FSWs were enrolled; 280 (56.0%) were brothel workers and 220 (44.0%) were non-brothel workers (table 1). Most of the women were young (mean age 23.6 years), and of Thai ethnicity (98.6%), and born in upper northern Thailand (92.6%); 79.3% were from Chiang Rai Province, and only seven (1.4%) were members of hill tribes. Only 10.4% of women had more than primary school (6 years) education, 20.2% had ever been legally married, 28.8% had a husband or steady partner at enrolment, and 51.2% had ever had a child. Nearly half (46.2%) began commercial sex work before 18 years of age, including 13.8% who began before age 15. Compared with the others, brothel workers were younger (45.7% were less than 20 years old), had less education, and were less likely to be married or have a regular partner. Brothel workers had started commercial sex work earlier in life: 21.4% began sex work before age 15 (table 1).

Commercial sex activity

For the most recent day worked, brothel workers reported a mean of 2.8 (range 0–12) clients, compared with 1.1 (range 0–5) for non-brothel workers. The amount paid by the customer for sex was more than six times higher in non-brothel settings (table 1). Almost all (98.9%) sex acts with all FSWs were penile-vaginal intercourse.

HIV-1 infection

At enrolment, 160 (32.0%) of 500 women were HIV-1 seropositive, including 47.1% of brothel workers and 12.7% of non-brothel workers (table 1). According to V3 loop peptide EIA testing, 157 (98.1%) HIV-1 seropositive specimens were subtypable; two were non-reactive, and one was dually reactive and could not be typed. Of the 157 specimens that could be typed, 154 (98.1%) were reactive to HIV-1 subtype E and three (1.9%) were reactive to subtype B' (Thai B).

Other STIs

Other STIs were common in this population. Brothel workers were more likely than other FSWs to have cervical infection with *C trachomatis* and *N gonorrhoeae,* to have active syphilis (TPHA positive and RPR ⩾4), and to have a positive RPR or TPHA. They were also more likely to be seroreactive to *H ducreyi*, the agent of chancroid. Similar proportions of brothel workers and other FSWs were seropositive for HSV-1 and HSV-2 and had a genital ulcer on examination. Of 60 genital ulcers identified at enrolment, DFA test results indicated that 41 (68.3%) were positive for HSV and that two (3.3%) were positive for *T pallidum*. Of 38 ulcers in brothel workers, 25 (65.8%) were positive for HSV, and one (2.6%) was positive for *T pallidum*; of 22 ulcers in other FSWs, 16 (72.7%) were positive for HSV, and one (4.5%) was positive for *T pallidum*.

Table 3 Factors and their associations with HIV-1 seropositivity at enrolment, by forward logistic regession analysis

Factor	*OR (95% CI)*	*p Value*
Brothel worker	3.4 (1.6–7.0)	0.001
Syphilis (TPHA positive and RPR≥4)	3.7 (1.6–8.5)	0.002
HSV-2 seropositive	2.4 (1.3–4.6)	0.006
Birth in upper northern Thailand	5.9 (1.2–28.1)	0.02
First commercial sex <15 years old	2.0 (1.1–3.9)	0.03
Genital ulcer	2.0 (1.0–4.1)	0.05
Education <7 years	3.4 (0.9–12.4)	0.06
C trachomatis cervicitis	1.7 (0.9–3.2)	0.09
Customer paid ≤200 Thai baht†	1.6 (0.8–3.2)	0.2
N gonorrhoeae cervicitis	1.5 (0.8–2.8)	0.2
Usually >1 customer per day in preceding 3 months	0.7 (0.4–1.4)	0.3
Vaginal sex during menstruation in preceding 3 months	1.3 (0.7–2.3)	0.4
Reported using condoms every time for vaginal sex in preceding 3 months	0.8 (0.5–1.3)	0.4
Use of oral contraceptives	1.3 (0.7–2.2)	0.5
Use of DMPA	1.3 (0.6–2.9)	0.5
Have a husband or steady partner	1.2 (0.7–2.2)	0.6
H ducreyi seropositive	0.9 (0.5–1.5)	0.7
Age <18 years	1.1 (0.6–2.1)	0.8

OR = odds ratio; CI = confidence interval; DMPA = depot medroxyprogesterone acetate; TPHA = *Treponema pallidum* haemagglutination assay; RPR = rapid plasma reagin.
†$1 = 25 Thai baht.

FACTORS ASSOCIATED WITH HIV-1 INFECTION

Demographics and commercial sex activity

On univariate analysis, HIV-1 infection was associated with brothel work, birth in the upper north of Thailand, fewer than 7 years education, initiation of commercial sex before 15 years of age, and not having a husband or steady partner (table 2). HIV-1 infection was associated with having more clients per day and with having customers who paid less that 200 baht ($US8) for sex. Vaginal sex during menstruation and working more than 60 days during the preceding 3 months were also associated with HIV-1 infection.

Contraception and condom use

On univariate analysis, use of oral contraceptives at enrolment was associated with HIV-1 infection (table 2). HIV-1 prevalence was 30.6% for women who reported having used condoms "every time" during vaginal sex with clients during the preceding 3 months, compared with 36.2% for women who reported that they had not consistently use condoms. There was no significant association between HIV-1 seropositivity and using condoms "every time" for brothel workers (PR, 1.01; 95% CI, 0.78–1.30) or non-brothel workers (PR, 0.73; 95% CI, 0.35–1.51).

Other STIs

All other STIs evaluated were associated with HIV-1 infection (table 2). HSV-1, although not considered primarily an STI, is shown for comparison.

MULTIPLE LOGISTIC REGRESSION ANALYSES OF FACTORS ASSOCIATED WITH HIV-1 INFECTION

Work in a brothel remained strongly associated with HIV-1 infection when other demographic, behavioural, and STI factors were controlled in a model (table 3). Birth in the upper north of Thailand and the initiation of commercial sex at less than 15 years of age were independently associated with HIV-1; having less than 7 years of education approached statistical significance. Three other STIs, syphilis, HSV-2 seropositivity, and genital ulcer were also associated with HIV-1 infection; chlamydia cervicitis approached statistical significance.

Discussion

Young Thai women employed as sex workers in brothels in northern Thailand in the early phase of the HIV/AIDS epidemic were at remarkably high risk for HIV-1 subtype E infection as well as for several other STIs. At particularly high risk were the FSWs originally from upper northern Thailand who had limited education and who began commercial sex work when less than 15 years old.

Brothel sex work in Thailand has consistently been found to carry more HIV-1 risk than work in other commercial sex venues.[2 3 10 12] In Chiang Rai, among Thai FSWs, more of the brothel FSWs than non-brothel FSWs were young, single, had less education, used oral contraceptives and injectable DMPA, had other STIs, and had more clients per day, who paid a lower fee for sex. When these factors were controlled in multivariate analyses, brothel work remained highly associated with HIV-1 infection. This association is likely to be due to the higher HIV-1 seroprevalence for brothel clients, compared with that for the higher paying clients of FSWs at other sex establishments, such as massage parlours and music clubs.[3]

The finding that women who began their commercial sex work at a very young age were at increased risk for HIV-1 infection at enrolment is troubling. This suggests a particularly high risk for HIV infection at the initiation of commercial sex work, perhaps related to a lack of control or power to negotiate condom use or other means of self protection. It is also possible that girls and young women have increased biological susceptibility to HIV-1 and other STIs because of traumatic lesions resulting from the initiation of vaginal sex. It is widely reported that young women are often offered as virgins to older clients in Thailand who are unlikely to use condoms because of the presumed lack of risk (for the men) of HIV-1 or other STIs.[18 19] This practice would place the young women at very high risk for infection with HIV-1 or other STIs.

At enrolment, high prevalence rates of several other STIs were found, especially among brothel workers, and all STIs were associated with HIV-1 infection. The high prevalence of several STIs in FSWs and their male clients[5 8] has probably been an important factor in the explosive epidemic of HIV-1 in northern Thailand. STIs increase an individual's susceptibility to HIV-1 and, for HIV infected people, increase their infectiousness due to increased viral shedding.[20]

We found that brothel workers were more likely than other FSWs to use oral contraceptives and injectable DMPA; however, neither contraceptive was independently associated, in multivariate analysis, with HIV-1 infection at study enrolment. In a cross sectional study in northeastern Thailand, Rehle *et al* [10] found in multivariate analyses that the use of injectable contraceptives (such as DMPA) was associated with HIV-1 infection and suggested that this

association may have been due to the reuse of needles. In a prospective study in the same setting,[21] this group also found that injectable contraceptives were associated with HIV-1 seroconversion (RR, 3.4; 95% CI, 1.2–13.2). Recent findings that progesterone implants enhance simian immunodeficiency virus (SIV) transmission in macaques[22] highlight the need to further explore, in human populations, the possible association between progestin use and HIV-1 transmission.

The findings of this study contribute to an explanation for the higher rates of HIV-1 infection observed in people with sexual risk behaviours in upper northern Thailand compared with other regions. Nearly all (93%) the FSWs in this study were born in the upper north, and a disproportionately large number of FSWs in all regions of Thailand are from the upper northern region.[3 12 17 23] Data from a 1991–3 cohort study of 21 year old male military conscripts indicate that, compared with men from other provinces, young men from the upper north were more likely to have initiated sex before age 16, to have had more frequent sex with FSWs, to have paid less than 50 baht ($2) for sex, and to have had an STI; they were also less likely to have used condoms with FSWs.[1 8] Other studies of Thai military conscripts have confirmed higher rates of risky behaviours among young men from the upper north.[6 24] These findings suggest that sex between men and FSWs, especially in brothels, was more prevalent in upper northern Thailand than in other regions. This greater frequency of brothel commercial sex, coupled with lower condom use, apparently led to higher STI rates for men[8] and FSWs and provided a setting for more efficient HIV-1 transmission.

At study enrolment, only 62% of the women reported using condoms "every time" for vaginal sex, and reported condom use for vaginal sex was not clearly associated with HIV-1 risk. However, cross sectional studies are very limited in their ability to evaluate the protective effect of condom use. More recent data suggest that the Thai "100% condom programme"[4 9] targeted at commercial sex has had a dramatic impact on rates of other STIs[25] and HIV-1 in young men,[26] especially in northern Thailand.[7] The decline in HIV-1 and STI rates in the 1990s for young men in northern Thailand has been attributed to a sharp increase in condom use with FSWs and a decrease in the frequency of FSW contact.[26]

Although these declines in HIV-1 rates among men are encouraging, commercial sex remains common in Thailand, and FSWs continue to be at substantial risk for infection with HIV-1 and other STIs. Other studies suggest that there will continue to be barriers, notably alcohol use,[27 28] to universal, consistent condom use between FSWs and clients. The findings of our study can be used by public health officials in other Asian countries to mobilise efforts to prevent girls and young women from entering the commercial sex trade and to limit disease transmission among those that do. Although progress has been made by offering other economic opportunities for young Thai women,[29] it appears that disadvantaged young women from other countries, notably Burma,[19] but also Laos and China,[30] continue to travel to Thailand to work in brothels. Such women work in Thailand illegally, often do not speak Thai, and are probably less able to negotiate condom use and protect themselves from HIV and STI risk. For the women who continue to work in the sex trade, renewed efforts are needed to promote condom use, prevent and treat other STIs, and offer other alternatives for disease prevention. The development of safe and effective vaginal microbicidal products would offer a much needed female controlled modality for disease prevention.

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Contributors: KL and TDM were responsible for study design and supervision, data analysis, and preparation of the manuscript. SS, WU, and SK were responsible for subject recruitment and evaluations at the study site. JK was responsible for data management and statistical analyses. NLY, SAM, and DSS were the laboratory based scientists who, in addition to study design and manuscript preparation, were responsible for laboratory testing. BGW was also responsible for study design and, in addition to PN, was responsible for analysis and manuscript preparation.

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