

A randomized, community trial of intensive sexually transmitted disease control for AIDS prevention, Rakai, Uganda

Maria J. Wawer, Ronald H. Gray*, Nelson K. Sewankambo[†], David Serwadda[†], Lynn Paxton, Seth Berkley[‡], Denise McNairn*, Fred Wabwire-Mangen[†], Chuanjun Li*, Fred Nalugoda[§], Noah Kiwanuka[§], Thomas Lutalo[§], Ronald Brookmeyer*, Robert Kelly* and Thomas C. Quinn*^{||}

Objective: To describe the design and first-round survey results of a trial of intensive sexually transmitted disease (STD) control to reduce HIV-1 incidence.

Study design: Randomized, controlled, community-based trial in Rakai District, Uganda.

Methods: In this ongoing study, 56 communities were grouped into 10 clusters designed to encompass social/sexual networks; clusters within blocks were randomly assigned to the intervention or control arm. Every 10 months, all consenting resident adults aged 15–59 years are visited in the home for interview and sample collection (serological sample, urine, and, in the case of women, self-administered vaginal swabs). Sera are tested for HIV-1, syphilis, gonorrhea, chlamydia, trichomonas and bacterial vaginosis. Following interview, all consenting adults are offered directly observed, single oral dose treatment (STD treatment in the intervention arm, anthelmintic and iron-folate in the control arm). Treatment is administered irrespective of symptoms or laboratory testing (mass treatment strategy). Both arms receive identical health education, condom and serological counseling services.

Results: In the first home visit round, the study enrolled 5834 intervention and 5784 control arm subjects. Compliance with interview, sample collection and treatment was high in both arms (over 90%). Study arm populations were comparable with respect to sociodemographic and behavioral characteristics, and baseline HIV and STD rates. The latter were high: 16.9% of all subjects were HIV-positive, 10.0% had syphilis, and 23.8% of women had trichomonas and 50.9% had bacterial vaginosis.

Conclusions: Testing the effects of STD control on AIDS prevention is feasible in this Ugandan setting.

© 1998 Lippincott-Raven Publishers

AIDS 1998, 12:1211–1225

Keywords: Sexually transmitted disease (STD) control, AIDS prevention, randomized controlled trial, STD mass treatment, STD/HIV prevalence, Uganda

From Columbia University, New York, New York, *Johns Hopkins University, Baltimore, Maryland, USA, the [†]Makerere University, Kampala, Uganda, the [‡]Rockefeller Foundation, New York, New York, USA, the [§]Rakai Project, Uganda Virus Research Institute, Entebbe, Uganda, and the ^{||}National Institutes of Health, Bethesda, Maryland, USA.

Sponsorship: Funded by the National Institutes of Health, National Institute of Allergy and Infectious Diseases (RO1-AI 34826) and the National Institute of Child Health and Development (5P30-HD 06268); the Rockefeller Foundation; and the World Bank/Uganda STI Project. Drugs and laboratory tests provided by Pfizer, Bayer and Abbott.

Requests for reprints to: Dr Maria J. Wawer, Center for Population and Family Health, Columbia University School of Public Health, 60 Haven Avenue, B-3, New York NY 10032, USA.

Date of receipt: 28 July 1997; revised: 19 February 1998; accepted: 27 February 1998.

Introduction

There is substantial biological and epidemiological evidence to suggest that classical sexually transmitted diseases (STD) and reproductive tract infections increase HIV transmission and acquisition [1–7]. The control of classical STD and reproductive tract infections may thus offer a feasible means of attenuating the AIDS epidemic [8–10]. Much of the available information on HIV–STD associations, however, has been accrued through cross-sectional and non-randomized longitudinal studies. Given the limitations of such data (including potential behavioral confounding), Mertens *et al.* [11] argued that randomized trials represent the most direct approach to establish a causative association between STD and HIV incidence. Subsequently, the Mwanza community-based randomized trial reported the effects of health education and improved clinic-based STD management using the syndromic approach [12]. In the fully adjusted analysis, HIV-1 incidence in the intervention arm was significantly reduced by 38% compared with the control arm. However, the Mwanza study demonstrated only limited STD effects. At follow-up, the only significant difference noted between arms was a lower prevalence of active syphilis in intervention communities. No significant differences were seen between study arms in reported STD symptomatology, laboratory-diagnosed male urethritis, or in any STD in women using antenatal services [13]. Although the Mwanza HIV results are encouraging, additional studies are needed to replicate and confirm the findings, to study the impact of treating asymptomatic as well as symptomatic STD, and to test the effects of STD control on HIV transmission in populations at different stages of the AIDS epidemic.

In this study, we describe the design of the Rakai STD Control for AIDS Prevention Study, an ongoing trial to test the hypothesis that intensive control of STD will result in reduced HIV incidence. The trial represents a research collaboration between the Ugandan Ministry of Health/Uganda Virus Research Institute (Entebbe, Uganda), Columbia University (New York, USA), Johns Hopkins University (Baltimore, Maryland, USA), and researchers at Makerere University (Kampala, Uganda). Since 1988, the Rakai Project team has conducted a series of district-level HIV and STD epidemiological and behavioral studies that provided the data used in the design of the STD control trial described here [14–20]. Rakai District, in southwestern Uganda, borders Lake Victoria and northern Tanzania. The district has a population of 384 000 (Ugandan National Census of 1991) in an area of approximately 2500 km². An estimated 80% of the district population resides in small agrarian villages and the remaining 20% are divided between main road trading centers that service long distance traffic and secondary road trading villages that serve as hubs of local communications.

The district had limited health services, comprising two government rural hospitals and 39 small outpatient facilities, many of which experience chronic shortages of personnel and supplies, as well as some scattered private and traditional practitioners.

In 1990, the Rakai Project reported adult HIV rates of 35.0% in main road trading centers, 23.1% in trading villages situated on secondary roads, and 11.8% in rural agrarian villages [14]. Between 1989 and 1992, HIV incidence was 2 per 100 person years (95% confidence interval, 1.4–2.6) amongst adults aged 15–59 years [15,16], despite health education, serological counseling and condom promotion conducted by the project. The rate of syphilis was 11% in a 1992 random subsample of the adult population [17] and, at the time of annual survey visits, up to 10% of subjects reported having had an STD symptom in the previous year [18]. The data also suggested that the ongoing behavioral change efforts were having only a limited effect on sexual practices, and that Rakai District would therefore be an appropriate site for an STD intervention trial.

Field work in the STD Control for AIDS Prevention Study was initiated in December 1994, and is projected to end in mid-1998. In this paper we describe the project design and provide data on enrolment, compliance, subject characteristics and STD/HIV prevalence in the first-round baseline survey.

Methods

The Rakai STD Control for AIDS Prevention Study is a community-based, randomized, controlled, single-blinded trial. In this ongoing study, all consenting individuals aged 15–59 years who are permanent residents of study communities are eligible for full enrollment (regardless of HIV status), and are visited in the home at approximately 10-month intervals. During the home visit, subjects are administered extensive questionnaires, provide biological samples and are offered mass treatment (STD treatment in the intervention arm, anthelmintic and iron-folate in the control arm).

Sampling and randomization

Community clusters rather than individuals are the unit of randomization in this study, and all consenting adults in the community are offered treatment. The community-based approach was selected because randomization and treatment of individuals without concurrent treatment of their sexual contacts would result in rapid reinfection with STD [21]. Moreover, given that STD may affect both HIV transmission and acquisition, it is desirable to treat STD in both HIV-negative individuals (to reduce susceptibility) and in HIV-positive persons (to reduce infectiousness) in order to achieve

the maximum potential impact of STD control at the population level. The sampling and randomization strategy had four main objectives: (i) to achieve adequate study power to detect a plausible reduction in HIV incidence; (ii) to select communities with high HIV incidence; (iii) to prevent contamination via exposure of subjects to STD from outside study communities; and (iv) to avoid bias in treatment allocation by achieving comparability in population size and HIV risk in intervention and control arms.

Study power and sample size estimation

Estimates of study power and sample size requirements were based on assumptions regarding the magnitude of the decline in HIV incidence that might result from the STD control intervention, and on projections of the magnitude of the design effect (i.e., the degree to which the sample size should be increased to account for the cluster design). Data from prior Rakai Project HIV cohort studies provided empirical parameters for estimating the number of persons years of observation (PYO) and the number of community clusters needed [15,16,19,20].

To project the expected reduction in HIV incidence that could be achieved as a consequence of STD mass treatment, an estimate was made of the probable contribution of STD to HIV transmission in this population, that is, the proportionate attributable risk of HIV infection associated with STD. The proportionate attributable risk was calculated from the estimated prevalence of STD in the population (derived from Rakai and other African data [17,18,22–25]) and the relative risk of HIV infection associated with each STD, based on published estimates of the association between HIV and specific STD or syndromes [1,3,6]. Projections of likely treatment efficacy were derived from reported cure rates with the drug regimens used [26–34]. The likely levels of treatment coverage in intervention communities were based on assumed compliance with treatment of 80%. The reduction of HIV incidence in the intervention communities as a consequence of STD control treatment was derived from the reduced attributable risk of HIV associated with the lower, post-treatment STD prevalence. On the basis of these calculations, it was estimated that the study could achieve a reduction of HIV incidence in the intervention communities relative to the control communities of at least 35%, a conservative projection compared with the empirical findings of the Mwanza study [12] and with model-based estimates [35].

Based on a simple random sample assumption ($\alpha = 0.05$, $1 - \beta = 0.80$), and an assumed relative risk of HIV seroconversion of 0.65 in the intervention arm, the trial required approximately 4375 PYO of HIV-negative individuals per study arm. However, the simple random sampling assumptions clearly cannot be

applied to a study in which the unit of randomization is a cluster of communities [36]. The methods of Katz *et al.* [37] and incident data from our prior cohort study were used to adjust for the assumed HIV heterogeneity between clusters; the design effect was estimated to be 1.8, based on previous Rakai data. Thus, the person-year requirement was increased by 80% from 4375 to 7875 PYO of HIV-negative individuals per study arm. Based on previous Rakai cohort data, it was estimated that the STD Control Study would achieve a follow up of 80%, that rates of in- and out-migration in this open cohort would be essentially balanced [16], and that the baseline community HIV seroprevalence would be approximately 20% [16,19,20]. The total number of HIV-negative individuals required per arm to achieve 7900 PYO over 3.5 years of follow-up was 4000, yielding a total enrolment of approximately 5000 per arm (HIV-positive and HIV-negative persons combined).

Community and subject selection, strategies to reduce STD reintroduction, and randomization

Community selection criteria included an expected HIV incidence of up to 2 per 100 PYO, year-round road access, and relatively stable populations needed to achieve long-term follow-up.

Previous Rakai Project studies [15,16] have indicated that villages with a baseline prevalence of 10% or more experience an adult seroincidence of approximately 2 per 100 PYO. Low prevalence communities (predominantly rural agrarian villages distant from main or secondary roads) were excluded from the present trial, because they were unlikely to provide sufficient incident cases and because it was difficult to maintain year-round access to these more peripheral areas. High prevalence (30% or more HIV-positive) trading centers were also excluded because of excessive population mobility, and because the limited numbers of seronegative individuals would reduce study efficiency.

Fifty-six intermediate trading villages on secondary roads were selected as meeting trial criteria of known or estimated seroprevalence of 10–25%, stable populations (previous annual follow up of 80%) and year-round road access. The relative homogeneity of these trading village communities also served to reduce the intercluster variation in HIV prevalence.

In order to minimize the risk of contamination due to reintroduction of STD into the intervention arm population, communities were grouped so as to be endogamous with respect to sexual contacts. The 56 communities were aggregated into 10 clusters of between four and seven communities each; the groupings (referred to as community clusters) were designed to encompass patterns of maximal internal social and thus, presumably, sexual interchange (i.e., sexual

networks). Communities were grouped on the basis of (i) distance to major highways and trading centers, (ii) quality of roads into the community, (iii) natural boundaries such as hills and swamps (which represent substantial barriers to mobility in Rakai), and (iv) community travel patterns for market attendance, work and social purposes (including travel to neighboring towns to frequent 'discos' and bars).

Community clusters were used as the units for randomization, using a stratified random allocation scheme. The 10 clusters were ranked into three blocks, based on projected HIV prevalence at baseline [19,20]. Block A comprised four clusters of trading villages with a projected HIV prevalence above the sample median; block B contained four clusters of trading villages with a projected HIV prevalence below the sample median, and block C was a 'periurban stratum' containing two community clusters in close proximity to two small trading centers not situated on the trans-African highway. Randomization was carried out within blocks. Intervention and control clusters are shown in Fig. 1.

In order to assess the adequacy of this number of units of randomization, methods described by Hayes *et al.* [38], based on the between-cluster coefficient of variation in HIV incidence or prevalence rates (k), were used. In the Rakai study population, the estimated k was approximately 0.15 (ignoring blocking), indicating a high degree of heterogeneity. In addition, the probability of detecting a consistently lower HIV incidence rate in all intervention clusters compared with control clusters, as was found in Mwanza [12], was estimated, using permutation methods [39]. Thus, with five clusters per arm, a blocked stratification design, and $k = 0.15$, the Rakai STD study has adequate power to detect a 35% lower HIV incidence in intervention than in control communities clusters.

Criteria for eligibility were permanent residence in study communities, age 15–59 years, and provision of informed consent. Permanent residents, defined as living in a study community for 6 months or more of the year, were fully enrolled into the study and were administered a full questionnaire, sample collection and treatment. Transient individuals (present in the community 0.5–6 months per year) were not enrolled into the cohort. However, in order not to lose data on this epidemiologically important group and to prevent spread of infection from transient to permanent residents, consenting transient individuals underwent a truncated interview and serological sample collection, and were offered mass treatment. Similarly, if an enrolled subject had a spouse who lived beyond the cluster boundary or who was outside the eligible age range, that spouse was offered a truncated questionnaire, serological sample collection and treatment, in order to cover sexual contacts and avoid stigmatization

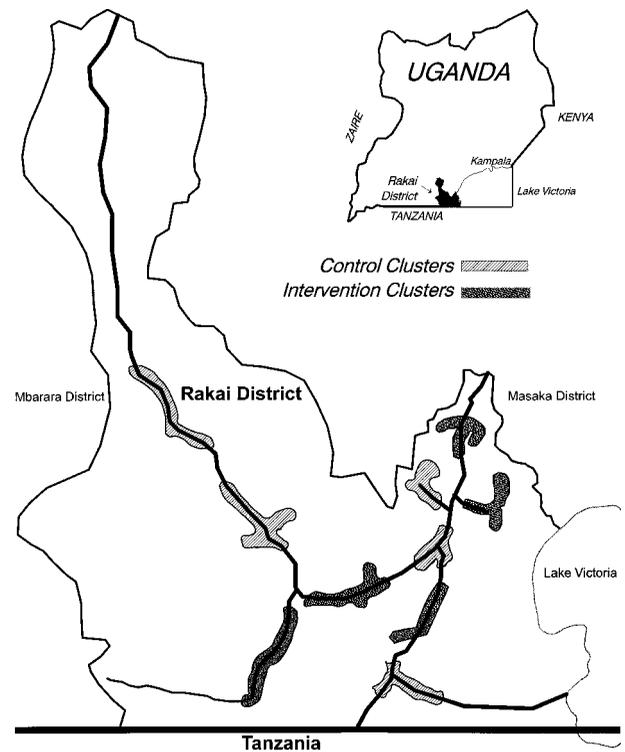


Fig. 1. Map of Rakai District. 1 cm = 8 km.

of one partner. Visitors (persons spending < 2 weeks in the community) were enumerated, but not surveyed or treated. In this open cohort, individuals who move into the study clusters, or who become age-eligible during the project, are enrolled in subsequent survey/treatment rounds.

In previous Rakai data, over 95% of all HIV prevalent and incident HIV cases were identified in the 15–59-year age-group. Epidemiological and cost considerations militated against including individuals outside this age range. However, younger or older individuals who report symptoms of STD are referred to project clinics for free treatment.

Blinding and consent procedures

Pre-project interviews indicated that compliance would be substantially reduced if subjects were randomized to inert placebo, and thus communities were randomly selected to receive STD or anthelmint/iron-folate mass treatment. The study was single blinded because (i) it would be ethically questionable to administer inert intramuscular injections in the control arm as a blinded alternative to the intramuscular penicillin provided in the home to intervention subjects with serological evidence of syphilis, and (ii) the STD antibiotics and the anthelmint/iron-folate capsules were not identical. Subjects were not informed of the study arm to which

their community was randomized, but project workers were unblinded. Intensive and repetitive personnel training and field supervision were carried out to ensure equivalent activities in both study arms. The number of contacts with study subjects, treatment schedules, and all other general health and preventive measures (mobile clinics, health education, condom promotion, access to HIV serological counseling) were identical in both arms.

The project followed a three-tier consent procedure. In the first stage, communities consented to inclusion via civic and traditional community leaders. (Only one religious subcommunity declined to participate.) In the second stage, the head of each household was asked permission to contact other household members, according to standard operating procedure in this rural traditional population. Only 2% of household heads did not consent to participation by family members. (Adult residents of such excluded households could still obtain free treatment in Rakai Project mobile clinics but were not included in the study.) Finally, each person was read a standard, individual consent form that detailed study goals, randomization procedures, blinding, and potential STD and anthelmintic/iron-folate treatment risks and benefits. Each individual was informed of the right to decline all or part of the study activities, without loss of benefits such as access to Rakai Project clinics. All consenting subjects signed or fingerprinted the form. The consent procedures were repeated at every survey round. Subjects were not paid to participate, but all households, whether they consented to enrollment or not, were offered a free bar of soap (worth approximately US\$ 0.50) to ‘promote handwashing’ and to indicate project appreciation of community involvement.

The study and consent procedures were reviewed and approved by the AIDS Research Subcommittee of the Uganda National Council for Science and Technology, the Columbia University Institutional Review Board, the Johns Hopkins Committee on Human Research, and the National Institutes of Health Office for Protection from Research Risk. Study safety was assessed by an independent Data Safety and Monitoring Board (composed of US-based and Ugandan partici-

pants) established by, but independent from, the US National Institutes of Health.

Home visit: enumeration, interview, sample collection and treatment

Table 1 summarizes project activities. To determine the study catchment population, a household map was prepared for each study village at baseline. A census was conducted in every home: the head of the household or a proxy was administered a detailed enumeration questionnaire regarding all permanent, transient and visiting persons in the household, whether they were present at the time of the enumeration or not. All adults and children were listed without age-truncation. The age, sex and relationship to the head of the household was noted for each person, as were reasons for any absence. House construction materials, sanitary facilities and possessions were recorded. Enumeration data were updated at the beginning of each follow-up round, and included information on all in- and out-migration, marriages, births and deaths.

Home-based surveys (baseline and four follow-up rounds) were conducted immediately after the household enumeration, at approximately 10-month intervals. The Rakai Project survey team (midwives, nurses and other experienced interviewers) conducted all procedures (interview, sample collection, treatment) in strict privacy in the local languages (primarily Luganda). Women were visited exclusively by female staff, and men by male staff. Approximately 4 days were required to cover a village, and each community cluster was covered in 3–4 weeks.

Lists of potential subjects were developed from the household census. At the time of the survey/treatment visit, all persons aged 13–63 years were contacted to verify age, and those aged 15–59 years were enrolled and provided with a photo identification card. A copy of the card was labeled with a study identification number, and was retained in secure files by the survey team to confirm subject identity in subsequent follow-up rounds. The permanent study identification number consisted of a unique, computer-generated alphanumeric check sequence to prevent data entry errors. In addition, each subject had an individual, household

Table 1. Sexually transmitted disease (STD) control project design.

Intervention arm	Control arm
28 communities in 5 community clusters	28 communities in 5 community clusters
Home visit every 9–10 months	Home visit every 9–10 months
Household enumeration	Household enumeration
Home-based survey	Home-based survey
Sociodemographic, behavioral, health	Sociodemographic, behavioral, health
Samples: sera, urine, vaginal swabs	Samples: sera, urine, vaginal swabs
Home-based mass STD treatment	Home-based mass anthelmintic/iron-folate
Referral: genital ulcer swabs, STD symptoms, general health	Referral: ulcer swabs, STD symptoms, syphilis, general health
Preventive and health program	Preventive and health program
Health education, condoms, clinics, HIV serocounseling	Health education, condoms, clinics, HIV serocounseling
Repeat every 3 years	Repeat every 3 years

and community locator number. Preprinted labels with the identification number were computer-generated for each subject prior to the survey visit to avoid mislabeling of forms and samples.

In each survey round, consenting subjects were administered a detailed sociodemographic, behavioral, sexual networks/practices and health interview in the home. Information collected included age, marital status, occupation, education, length of residence, travel and mobility, alcohol use, detailed pregnancy history, breastfeeding, contraceptive and condom use, male circumcision, vaginal/genital practices including douching, number and characteristics of sexual partners, commercial sex, sexual practices, sex during menses, non-consensual sex, AIDS and STD-related knowledge, detailed AIDS and STD symptomatology, STD treatment seeking for self and for partners, source of treatment, and general health and morbidity status. A sexual networks component collected detailed information on the last four partners, including relationship to the partner, partner's place of residence and sociodemographic characteristics, types of sexual activities undertaken (including forced sex), use of alcohol or condoms within this partnership, duration of the relationship, and frequency of intercourse. Names of marital partners were recorded to permit spousal linkages, but in order to increase response rates, names of non-marital partners were not collected. Transient persons complete a truncated interview (sociodemographic characteristics and mobility).

Biological sample collection and testing

To assess whether STD control results in reduced HIV incidence, it was necessary to determine the comparability of the intervention and control arms with respect to key STD at baseline, and to demonstrate reductions in STD rates over time. All samples were collected in the home immediately following the interview, to reduce the self-selection that could occur with clinic referral. Collection methods were selected so as to be feasible in the home and to yield specimens that are stable under field conditions and amenable to processing in a small field laboratory. Consenting subjects in both study arms, whether symptomatic or not, were asked to provide all samples during every survey round. The project maintains a field laboratory in Rakai District and additional laboratory facilities situated at the Uganda Virus Research Institute in Entebbe.

For serological samples, 10 ml venous blood were collected, stored in cool boxes in the field, and centrifuged within 10 h in the field laboratory. Double enzyme immunoassay testing for HIV-1 was carried out in Entebbe using two different assays (Vironostika HIV-1, Organon Teknika, Charlotte, North Carolina, USA, and Cambridge Biotech, Worcester, Massachusetts, USA), with Western blot confirmation of all discordant

tests and of all HIV seroconverters (HIV-1 WB Bio-Merieux-Vitek, St Louis, Missouri, USA). Syphilis screening was conducted using the toluidine red unheated serum test (TRUST, New Horizons, Columbia, Maryland, USA) non-treponemal test. Positive samples were subjected to confirmation by *Treponema pallidum* hemagglutination assay (TPHA; TPHA Sera-Tek, Fujirebio, Tokyo, Japan). TRUST testing was carried out in the Rakai District field laboratory, with TPHA confirmation in Entebbe. A random selection of sera was sent to Johns Hopkins University for HIV and syphilis (Fluorescent Treponemal Antibody Absorption Test) quality control.

For urine samples, subjects were asked to provide 10 ml or more of first-catch urine. Samples were centrifuged and processed in the Rakai field laboratory and were subsequently assayed for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* at the Johns Hopkins University Chlamydia Reference Laboratory (ligase chain reaction, Abbott Laboratories, Abbott Park, Illinois, USA). Urine-based ligase chain reaction testing for gonorrhea and chlamydia has been shown to have sensitivity and specificity comparable to or higher than culture in both symptomatic and asymptomatic men and women [40-43]. To identify pregnant women in order to modify treatment regimens in both study arms (see below), urine samples were tested for human chorionic gonadotropin (hCG; CARDS Brand OS, Pacific Biotech, Inc., San Diego, California, USA).

All women were asked to provide two self-administered vaginal swabs during the home visit. Subjects collected each swab by squatting, inserting a 20 cm dacron or cotton-tipped swab high in the vagina, and swirling it around the vaginal vault [44]. One vaginal swab was immediately inoculated into an InPouch TV *Trichomonas vaginalis* culture kit (BioMed Diagnostics, San Jose, California, USA), which was subsequently read in the field laboratory after 24 and 48 h of incubation. The InPouch equals or surpasses the sensitivity and specificity of standard culture techniques [45,46]. At the time of the home visit, the interviewer rolled the second vaginal swab onto a glass slide, which was air-dried. The slide was then Gram-stained and read for bacterial vaginosis using a quantitative, morphologic scoring system based on the relative predominance of *Lactobacillus* morphotypes compared with Gram-negative organisms (i.e., *Gardnerella*, *Bacteroides* and *Mobiluncus* morphotypes) [47]. The scoring system has been shown to have high sensitivity and specificity compared with clinical diagnosis [47,48]. Slides were processed and read in the project field laboratory, with a subsample reread for quality control purposes at Dr S. Hillier's laboratory (Pittsburgh, Pennsylvania, USA). The correlation between scores assigned by technicians in Uganda and Pittsburgh has been high ($r = 0.75$).

For ulcer swabs, all men who reported a genital ulcer were asked to permit the survey worker to collect ulcer swabs for assessment of herpes simplex virus via HERP CHEK Direct Herpes Simplex Virus Antigen Test (E.I. Dupont De Nemours and Co. Medical Products, Charlotte, North Carolina, USA), and for Multiplex PCR (Roche Molecular Systems, Alameda, California, USA). Multiplex PCR distinguishes between syphilitic ulcers and those caused by herpes simplex virus type 2 and chancroid [49]. Female genital examination was not feasible in the home, and women with a reported ulcer were referred for swab collection to the Rakai Project mobile clinics that accompanied the survey team. (Ulcer swabs represent the one sample collection that depends on self-reporting and, in women, acceptance of referral.) HERPCHEK and multiplex PCR testing is conducted at Johns Hopkins University.

STD mass treatment in the intervention arm

STD mass treatment (intervention arm) and anthelmintic/iron-folate (control arm) were offered to all consenting permanent subjects and transients, whether they were symptomatic or not, and independent of laboratory testing. (Syphilis represents the one exception with respect to laboratory testing, as described below.) Mass treatment was offered in the home immediately after sample collection. All drugs were taken in the presence of the health worker to increase compliance and assess treatment coverage. The worker recorded drugs taken and date of administration.

Drugs and drug combinations were selected to meet the following criteria: (i) single oral dose administration to increase acceptability, ensure administration of curative doses, and allow direct observed treatment; (ii) safety, including safety in pregnancy; (iii) minimal side-effects to increase tolerance; and (iv) minimization of drug resistance. To ensure safety in pregnancy, the study provided US Food and Drug Administration (FDA) category A or B drugs to pregnant women, and avoided administration of category C drugs (defined as those in which potential fetal risk cannot be ruled out, because human studies are lacking and animal tests are lacking or positive for risk [50].)

STD mass treatment consisted of the following:

- (1) Azithromycin 1000 mg. Single oral dose azithromycin is highly active against *Haemophilus ducreyi* (chancroid) [26], *Chlamydia trachomatis* [27] and many strains of *N. gonorrhoeae* [28]. Azithromycin is also active against *T. pallidum* [51], is likely to abort incubating syphilis and may cure some cases of active syphilis.
- (2) Ciprofloxacin 250 mg. Single oral dose ciprofloxacin is effective against *N. gonorrhoeae* [29] and *H. ducreyi* [30]. Ciprofloxacin is an FDA category C drug and is not administered to pregnant women.

- (3) Cefixime 400 mg (FDA category B) is used instead of ciprofloxacin in pregnant women. Cefixime is effective in a single oral dose against *N. gonorrhoeae* [31], and is also likely to be active against *H. ducreyi* based on *in vitro* data [32] and the activity of other cephalosporins; however, clinical efficacy against chancroid has not been studied.
- (4) Metronidazole 2.0 g. A single, oral dose of metronidazole is the recommended regimen for trichomoniasis. Cure approaches 95% if both partners are treated simultaneously (as occurs in the majority of partnerships in the mass treatment strategy) [33]. Single oral dose metronidazole also provides short-term control (as measured between 1 week and 1 month) in 70–85% of bacterial vaginosis cases [34]. Multidose courses of metronidazole and clindamycin are only modestly more effective against bacterial vaginosis [34], and given the added complexity of administration and monitoring, the Rakai project selected single dose treatment. Metronidazole is an FDA category B drug and a recent meta-analysis confirmed its safety in pregnancy [52].
- (5) Benzathine penicillin 2.4×10^6 IU intramuscularly was administered in the home within 24 h of sample collection to intervention arm subjects with a positive TRUST test. As yet, there is no accepted oral single dose regimen for syphilis.

The selected single dose drugs are thus effective against gonorrhoea, chlamydia, chancroid, syphilis and trichomoniasis, and provide short-term control of bacterial vaginosis. Treatment strategies were designed to minimize the potential for the emergence of drug resistance in the STD target pathogens. *N. gonorrhoeae* and *H. ducreyi* were targeted with combination therapy (i.e., azithromycin and ciprofloxacin) and the directly observed, single dose treatment strategy ensured that subjects received a curative dose. The project conducted *N. gonorrhoeae* culture and antibiotic sensitivity testing on all discharge samples collected in Rakai project mobile and fixed clinics. E Test (AB Biodisk, Solna, Sweden) [53] assessment was conducted in Uganda; *N. gonorrhoeae* isolates were retested at Johns Hopkins for azithromycin, ciprofloxacin and cefixime resistance using the E Test and agar dilution [54].

Azithromycin and ciprofloxacin are generally well tolerated. Azithromycin side-effects include gastrointestinal symptoms, notably transient cramping or diarrhea [28]. Since metronidazole may also cause gastrointestinal intolerance, an operational decision was made to stagger the treatment regimen over 2 days. Thus, metronidazole was offered during a second, supervised home visit conducted 1 day after azithromycin–ciprofloxacin treatment. This second visit also provided a non-stigmatizing setting for intramus-

cular penicillin treatment of intervention arm subjects with serological evidence of syphilis.

Screening questions were used to rule out potential drug allergy and pregnancy. Women who were unsure of their pregnancy status (i.e., no menses in the past month) were offered immediate urinary hCG pregnancy testing. If they declined the test, they were treated as if pregnant (i.e., ciprofloxacin was replaced with cefixime). Subjects were also advised to avoid alcohol use following metronidazole.

In addition to STD mass treatment, all intervention arm community residents (whether they were study subjects or not) were offered free treatment of general health conditions (such as malaria) via the mobile clinics that accompanied the survey teams.

Anthelmintic/iron-folate mass treatment in the control arm

Control arm subjects were offered 2-day treatment with mebendazole, an effective anthelmintic, as well as iron-folate supplementation (2-day regimen). Since mebendazole is an FDA category C drug, pregnant women were screened through interview and urinary hCG testing, identical to the screening conducted in the intervention arm.

For comparability with the intervention arm, all control arm residents received free general health care at Rakai Project mobile clinics. For ethical reasons, control arm subjects who reported symptoms of STD or who had serological evidence of syphilis were referred for free treatment to Rakai Project mobile clinics or government health posts; trained counselors revisited communities to provide the latter results. The Rakai Project also helped stock government facilities with penicillin to ensure adequate supplies. Drugs used for treatment of control arm subjects were in accordance with Ugandan Ministry of Health syndromic treatment recommendations, which are based on guidelines developed by the Centers for Disease Control and Prevention [55].

Community mobilization, health outreach, preventive services

Prior to each census and survey round, communities in both arms were visited by a health education/motivation team that organized community meetings to encourage study participation and to provide health education. The project promotes and makes available condoms, through the health education and survey teams, and through Rakai Project community health workers in each study village. The Rakai Project has established a system of trained serological counselors to provide confidential HIV results and counseling, and all subjects were strongly urged to receive their results. In keeping with Ugandan Ministry of Health guidelines, acceptance of serological results was voluntary. All out-

reach activities were equivalent in both study arms. Both arms received the services of mobile clinics that accompanied each survey team and had access to the fixed Rakai clinic in Kalisizo.

Data management and analysis plans

All data (census, questionnaire, tracking forms for samples and treatment, field laboratory results) were entered daily at the Rakai Project field station, using FoxPro (Microsoft Corp., Rosell, Illinois, USA) and the Household Registration System (Population Council, New York, New York, USA). Data files and survey collection instruments were transferred to project headquarters in Entebbe for quality control (including double data entry), multiple back-up, and secure long-term storage. A copy of all data files was electronically transmitted to Johns Hopkins and Columbia by file transfer protocol, to permit collaborative analysis.

Analysis plans included (i) comparison of HIV incidence in intervention and control communities with adjustment for cluster randomization, (ii) monitoring of trends and differentials in STD incidence and prevalence in the two study arms, and linkage to changes in HIV incidence, and (iii) monitoring of changes in behaviors, study compliance or other factors that might potentially confound the comparisons of HIV or STD rates in the two study arms.

HIV incidence rates

The analysis of incidence in intervention compared with control communities is to be based on intent-to-treat, irrespective of whether an individual received treatment for STD. Tests of statistical significance for differentials in incidence between study arms will be based on comparisons of clusters within the three stratification blocks used for randomization. The community-based design mandates an analysis that takes into account community-level variation. Two approaches are available: an analysis of variance [56] and the permutation test [39]. If any characteristics suspected of being associated with STD and HIV risk show imbalance between study arms, adjustment for possible confounding will be conducted using multivariate methods such as Poisson regression or proportional hazards models (depending on the magnitude of the incidence rates). Analyses will incorporate terms for each community cluster, and apply appropriate adjustment for correlated data [57]. Since HIV prevalence is linked to HIV incidence, we will include adjustment for baseline HIV prevalence in the regression models [58].

STD prevalence and incidence

In order to establish the plausibility of an impact of STD treatment on HIV incidence, it is important to demonstrate declines in STD rates in the intervention arm compared with the control arm, consistent in mag-

nitude and in timing with observed HIV effects. The monitoring of differentials in STD rates between study arms will employ similar statistical methods to those described for HIV incidence. For each STD, we will measure serial prevalence at each follow-up round, and estimate approximate STD incidence rates per 100 PYO from new cases of infection in previously uninfected individuals.

Additional evidence of a plausible association between STD control and HIV infection will be derived from nested case-control analyses, whereby incident HIV cases will be compared with concurrent HIV-negative controls with respect to STD infection at the start of the preceding follow-up interval. This will potentially allow estimation of the attributable risk of incident HIV associated with reduced rates of specific STD. The units of analysis will include individuals and couples.

Trends in potential confounding behaviors such as sexual activity, use of condoms, STD treatment seeking, travel to urban areas and alcohol use will be monitored in both study arms at each survey round, in order to ensure comparability of study arm populations and to adjust for differentials, if needed.

Results

Compliance and coverage

The baseline census enumerated 18 039 adults aged 15–59 years in the study clusters (*de jure* population). Of these, 16 450 individuals met the permanent residency eligibility criterion and 1589 were transients (Table 2). Of all permanent residents enumerated, 13 704 (83.3%) were present in the community at the time of the survey (*de facto* population). Amongst those

absent, just over one-half (52.4%) were away due to work, 13.4% were enrolled in educational institutions outside the community, 9.3% were visiting distant family members, 19.3% were absent for other reasons, and no data were available on 5.5%. Of the *de facto* population, 12 827 (93.6%) consented to enrolment.

As expected, a higher proportion (30.0%) of the 1589 enumerated transients were absent at the time of the survey. Amongst the 1112 transients present, the refusal rate was low and 1030 (92.6%) consented to study participation. Finally, a total of 449 non-resident or age-ineligible spouses were identified (211 in the control and 238 in the intervention arm), of whom 92.7% consented to enrolment (questionnaire, serological sample and treatment).

Table 2 shows the number of permanent and transient adults enumerated, present and enrolled at baseline, and compliance rates with sample collection and treatment, by study arm. Ninety-two per cent of the 6926 permanent adults present in the intervention arm consented to enrolment, as did 95.4% of the 6778 adults present in the control arm. Amongst those consenting to participate, compliance with sample collection was high and equivalent in both arms for all biological specimens, including urine collection (over 94%) and self-administered vaginal swabs (over 92%). In total, 11 618 (90.6%) of the enrolled subjects provided a serological sample: it is this group that was considered as the core cohort for HIV analyses.

In the intervention arm, 94.0% of enrolled subjects accepted treatment, compared with 96.2% in the controls. Overall, the study provided STD treatment to 86.2% of all intervention arm permanent residents present at the time of the survey, as well as to 84.0% of all intervention arm transients present in the clusters.

Table 2. Eligible adults by treatment arm, consenting to enrolment, samples and treatment.

	n (%)*		
	Treatment arm	Control arm	Total
Permanent residents			
Age/residence-eligible subjects enumerated (<i>de jure</i> population)	8398	8052	16450
Age/residence-eligible subjects present in community	6926	6778	13704
Consented to enrolment	6355 (100)	6472 (100)	12827 (100)
Provided blood sample	5834 (91.8)	5784 (89.4)	11618 (90.6)
Provided urine	6027 (94.8)	6085 (94.0)	12112 (94.4)
Provided vaginal swab (women only)	3347 (94.5)	3297 (92.7)	6644 (93.6)
Accepted treatment	5971 (94.0)	6226 (96.2)	12197 (95.1)
Transients			
Enumerated	745	844	1589
Present in community	505	607	1112
Consented to enrolment	450 (100)	580 (100)	1030 (100)
Provided blood sample	411 (91.3)	520 (89.7)	931 (90.4)
Accepted treatment	424 (94.2)	561 (96.7)	985 (95.6)

*All percentages are calculated on the basis of persons consenting to enrolment.

Baseline sociodemographic characteristics and comparability between study arms

Table 3 shows selected sociodemographic and behavioral characteristics for both men and women in each study arm. Data were reported for the 11 618 permanent residents who consented to enrollment and provided a serological sample. Given the large sample sizes and multiple comparisons between arms in

Table 3, *P* values accepted as being statistically significant were adjusted using the Bonferroni procedure [59]. Overall, the study achieved a high degree of comparability in sociodemographic, behavioral and fertility-related characteristics between arms, and these characteristics were very similar to those reported for secondary road villages in previous Rakai Project studies [16,19,20].

Table 3. Selected baseline sociodemographic and behavioral characteristics by sex, intervention and control arm, permanent residents fully enrolled* in study cohort (n = 11 618).

	n (%)			
	Men (n = 5195)		Women (n = 6423)	
	Intervention (n = 2587)	Control (n = 2608)	Intervention (n = 3247)	Control (n = 3176)
Mean age (years)	28.1	28.6	28.8	28.5
Education				
Nil	152 (5.9)	217 (8.3)	387 (11.9)	576 (18.1)**
Primary only	1705 (65.9)	1729 (66.3)	2178 (67.1)	2062 (64.9)
Secondary only	552 (21.3)	514 (19.7)	616 (19.0)	467 (14.7)**
Technical/university	178 (6.9)	147 (5.6)	63 (1.9)	68 (2.1)
Occupation				
Agricultural/housework	1049 (40.5)	1173 (44.9)	2322 (71.5)	2424 (76.3)**
Clerical	194 (7.5)	147 (5.6)	156 (4.8)	116 (3.7)
Student	216 (8.4)	232 (8.9)	191 (5.9)	126 (4.0)
Shop/trading/vending	444 (17.2)	382 (14.6)	225 (6.9)	193 (6.1)
Bar work	9 (0.3)	3 (0.1)	55 (1.7)	61 (1.9)
Military/truck driver	60 (2.3)	67 (2.6)	5 (0.2)	9 (0.3)
Other [†]	615 (23.8)	604 (23.2)	293 (9.0)	247 (7.8)
Religion				
Catholic	1671 (64.6)	1570 (60.2)**	2078 (64.0)	1892 (59.6)**
Protestant	626 (24.2)	605 (23.2)	747 (23.0)	696 (21.9)
Muslim	261 (10.1)	376 (14.4)**	390 (12.0)	537 (16.9)**
Other/none	28 (1.1)	55 (2.1)	32 (1.0)	51 (1.6)
Resident in community < 1 year	96 (3.7)	116 (4.4)	299 (9.2)	290 (9.1)
Travel in past year				
Outside community	2509 (97.0)	2523 (96.4)	2909 (89.6)	2811 (88.5)
Kampala	824 (32.2)	813 (31.2)	454 (12.6)	386 (12.6)
Any large market	833 (32.2)	944 (36.2)	431 (13.3)	476 (15.0)
Current marital status				
Married/consensual				
Monogamous	1271 (49.1)	1292 (49.5)	1387 (42.7)	1421 (44.7)
Polygamous [‡]	251 (9.7)	271 (10.4)	587 (18.1) [‡]	648 (20.4) [‡]
Widowed	85 (3.3)	94 (3.6)	282 (8.7)	297 (9.4)
Divorced	31 (1.2)	23 (0.9)	33 (1.0)	41 (1.3)
Pregnancy				
Ever pregnant	–	–	2678 (82.5)	2671 (84.1)
Now pregnant	–	–	425 (13.1)	499 (15.7)
Ever subfertile [§]	–	–	281 (8.7)	307 (9.7)
Contraception [¶]				
Modern, ever use	–	–	432 (13.3)	394 (12.4)
No. partners, previous year				
0	557 (21.5)	582 (22.3)	807 (24.9)	682 (21.5)
1	1266 (48.9)	1300 (49.8)	2314 (71.3)	2378 (74.9)
2	527 (20.4)	464 (17.8)	112 (3.4)	107 (3.4)
≥ 3	237 (9.1)	262 (10.0)	14 (0.7)	9 (0.3)
Reported that all partners in past year lived ≤ 1 h walk from respondent's home	2277 (88.1)	2305 (88.4)	2997 (92.3)	2963 (93.6)
Sex for gift or money (ever)	674 (26.0)	799 (30.6)**	479 (14.8)	526 (16.6)
Condoms (ever use)	756 (29.2)	642 (24.6)**	419 (12.9)	334 (10.5)
Alcohol use in past month	1636 (63.2)	1510 (57.9)**	1463 (45.1)	1320 (41.6)**
Circumcised				
Under age 12 years	252 (9.7)	358 (13.7)**	–	–
Age ≥ 12 years	66 (2.6)	118 (4.5)**	–	–

*Full enrolment is defined as consenting to enter the study and provision of a serological sample. [†]Other occupational categories include fishing, unemployed, home-based beer brewing. [‡]For women, polygamy refers to husband's status. [§]Attempted to become pregnant for 24 months without success. [¶]Intrauterine device, oral contraceptives, injectable contraceptives, spermicide. ***P* < 0.05, using Bonferroni correction for multiple comparisons.

Agriculture was the most common occupation. Trades previously found to be associated with very high risk of HIV infection (particularly bar work for women and trucking for men) were reported by fewer than 2% of respondents, with similar rates in the intervention and control arms. The proportion of Muslims was slightly higher in the control arm than in the intervention population (Bonferroni $P_i < 0.05$ for both sexes). The population was relatively stable, with only 6.9% overall reporting having lived in the community for less than 1 year. Similarly, although virtually all men and almost 90% of women reported some travel outside the immediate community within the previous year, only one-third of the men and less than 15% of the women had visited the capital city of Kampala or any large mobile markets; such travel has previously been found to be associated with increased risk of HIV [19].

Overall, 1490 men (28.7%) reported two or more sexual partners in the previous year (one-third of these men were in polygamous marital unions), compared with 3.8% of women, rates that are very similar to those reported previously [19,60]. Eighty-eight per cent of men and 92.8% of women reported that none of sexual their partners in the preceding year lived more than a 1 h walk (5 km) of the respondent's place of residence, a distance encompassed by the study clusters in the great majority of cases.

A significantly higher proportion of control arm men reported ever having had sex for gifts or money than intervention arm men (30.6 and 26.0%, respectively; Bonferroni $P_i < 0.05$; Table 3). Among women, the rates of reported sex for money or gifts were 16.6 and 14.8% in the control and intervention arms, respectively. Such relationships included long-term non-marital relationships in which monetary or material gifts were common, as well as commercial contacts. Control arm subjects also reported lower rates of ever use of condoms (24.6% among control men, compared with 29.2% in the intervention arm; Bonferroni $P_i < 0.05$). Rates of condom use reported by women

were less than half those reported by men, at 10.5 and 12.9% in the control and treatment arms, respectively. In keeping with the distribution of Muslims, a significantly higher proportion of control arm men had been circumcised in infancy or childhood (13.7 versus 9.7%; Bonferroni $P_i < 0.05$). Circumcision was previously found to be associated with lower HIV risk in this population [19]. Control arm subjects also reported lower rates of alcohol use (Bonferroni $P_i < 0.05$). Ever use of modern contraceptives (intrauterine device, oral or injectable contraceptives, or spermicide) was low in both arms, with 13.3% of intervention arm women reporting ever use, compared with 12.4% in the control group.

Reported rates of potential STD symptoms in the past year were very similar in both arms. Twenty-two per cent of intervention and 23.0% of control arm men reported at least one symptom (genital ulcer, discharge or dysuria); in women, the proportions reporting at least one symptom (genital ulcer, discharge, dysuria, dyspareunia or lower abdominal pain) were 39.5 and 39.4%, respectively.

Table 4 reports HIV, STD and reproductive tract infections prevalence at baseline. The overall prevalence of HIV was 16.9%, and 10.0% of subjects had serological evidence of syphilis (positive TRUST, with TPHA confirmation). Over 50% of women in both arms had bacterial vaginosis on Gram stain, and almost one-quarter had positive culture for *T. vaginalis*. Rates of HIV, syphilis, trichomonas and bacterial vaginosis were comparable between study arms. Due to resource constraints, only a subsample of urines was tested for gonorrhoea and chlamydia (Table 4). The gonorrhoea and chlamydia rates in the control population were lower than in the intervention group, but the number of infected subjects was small and differences were not statistically significant. Preliminary testing of genital ulcer swabs by HERPCHECK indicated that approximately one-third of genital ulcers were of herpetic etiology (not shown).

Table 4. Baseline HIV and sexually transmitted disease rates, men and women aged 15–59 years, for intervention and control arm.

	n (% positive)			
	Men		Women	
	Intervention	Control	Intervention	Control
HIV*	2551 (14.7)	2589 (13.6)	3196 (19.6)	3158 (18.5)
Syphilis [†]	2560 (10.7)	2603 (9.1)	3213 (10.5)	3170 (9.7)
Chlamydia [‡]	1087 (2.3)	823 (1.7)	1387 (3.1)	1079 (1.6)
Gonorrhoea [‡]	1090 (1.2)	825 (0.6)	1388 (1.7)	1080 (1.0)
Bacterial vaginosis [‡]	–	–	2438 (50.4)	2399 (51.4)
Trichomoniasis	–	–	3138 (23.7)	3045 (23.9)

*HIV results are reported on 11 494 of the 11 618 individuals providing a serological sample; of the remaining 126 samples, 42 (0.4% of total) were enzyme immunoassay-discordant/Western blot-indeterminate (22 in control arm, 20 in intervention arm); 47 (0.4%) samples collected on filter paper await testing; and 35 (0.3%) provided insufficient blood volume for testing. [†]Syphilis results are reported on 11 549 individuals; the remaining 69 individuals (0.6% of all who provided blood) provided an insufficient blood volume for testing. [‡]Subsample of specimens tested.

Reported treatment side-effects and adverse experiences

In both arms, a 5% subsample of treated subjects were revisited at 1 and 8 days following treatment. In the intervention arm, 25.5% reported some gastrointestinal side-effects in the 24 h after receiving the azithromycin-ciprofloxacin treatment (including nausea or diarrhea), compared with 3.4% in the control arm ($P < 0.001$). By the third day (a time period that also encompasses metronidazole administration), the rates were 4.1 and 1.1%, respectively ($P < 0.05$). The reported gastrointestinal side-effects were mild and no individuals required medical treatment. Out of 6395 persons (5971 permanent and 424 transient) treated for STD at baseline, two individuals (0.03%) experienced possible symptoms of allergic reaction following azithromycin-ciprofloxacin, as did one individual (0.2%) following intramuscular penicillin treatment for syphilis. The three cases were successfully managed in the field with 0.3 mg intramuscular epinephrine (EpiPen, Center Laboratories, EM Industries, Port Washington, New York, USA) with no persisting adverse sequelae, and no subject required hospitalization.

Discussion

At baseline, the Rakai STD Control for AIDS Prevention Study met key operational and research goals. Enrollment of HIV-negative subjects exceeded expectation (4765 HIV-negative subjects enrolled in the intervention arm and 4833 in the control arm, compared with the goal of 4000 per arm), treatment coverage was high (86.2% of all adults aged 15–59 years present in intervention arm communities were treated), and compliance with survey and sample collection (blood, urine and self-administered vaginal swabs) was over 90%. The safety, acceptability and feasibility of the mass treatment regimen were indicated by the minor and transient nature of reported gastrointestinal side-effects, the infrequency of adverse experiences, and by consistently high compliance with treatment regimens.

The block randomization achieved a high degree of sociodemographic and behavioral comparability between arms, and target STD prevalence rates were also comparable. Given the large sample sizes and number of variables explored, it is not unexpected that statistically significant differences in the prevalence of some risk factors were noted between arms. However, absolute differences were small. Control arm men reported significantly higher rates of circumcision and lower alcohol use, factors that might diminish HIV risk. Conversely, control subjects reported higher rates of sex for gifts or money and less use of condoms. Such differences will be adjusted for in future analyses. No

significant differences were noted in reported STD symptomatology, or most importantly, in STD rates. Nonetheless, given that the prevalence of several infections were slightly higher in one or the other arm, appropriate adjustment will be made for baseline prevalence in analyses of treatment effects.

The recent availability of STD screening technologies that can be adapted to home-based, self-administered sample collection (notably urine and vaginal swabs), without the need for pelvic or genital examination, greatly facilitates the ability to gather representative, community-level STD data and to monitor population trends in incidence and prevalence. Detailed STD assessment is not only crucial to proving that any observed HIV effects are indeed related to STD control, but such an assessment may also provide useful data as to which STD confer the greatest population attributable risk for HIV transmission and acquisition, permitting targeted control efforts in the future.

The Rakai Study selected a mass STD treatment strategy because this approach permits comprehensive community coverage, treats asymptomatic as well as symptomatic persons, provides treatment to large populations despite the paucity of clinical services, and encompasses sexual networks (both official and unofficial partners). The mass treatment strategy can be expected to reduce rates of new (incident) STD, since uninfected individuals will progressively have lower exposure to both symptomatic and asymptomatic infected partners. Rapid and intensive STD control maximizes the potential to observe effects on HIV dynamics. Historically, mass treatment strategies have been effective in controlling treponemal infections, such as yaws and syphilis [61,62], thus providing a precedent for this approach.

The current STD control study was an efficacy trial designed to test the hypothesis that reducing STD will have beneficial effects on HIV incidence, and to provide the scientific basis for policy. It was not intended as an effectiveness study to test an operational strategy. Maximization of treatment coverage and compliance is standard procedure for clinical trials, as it would be scientifically and ethically questionable to miss a true intervention effect due to suboptimal treatment administration or coverage [63]. Questions have also been raised regarding the costs, sustainability and replicability of the mass treatment strategy [64]. Many of the drugs used in the Rakai project (ciprofloxacin, penicillin, metronidazole) are inexpensive, readily available in Uganda, and are already included in the Ugandan Ministry of Health list of first-line STD drugs. The cost of azithromycin is falling rapidly, and the current price in the United States is already comparable to other standard regimens for chlamydia, gonorrhoea and chancroid. (It is noteworthy that mass treatment with

azithromycin is being evaluated for the prevention of *Mycobacterium avium* complex in HIV-infected persons in the United States [65] and for prevention of trachoma internationally [66].) A mass treatment program requires resources such as transport and dedicated workers: however, all service delivery strategies require some level of such inputs, and the ultimate cost-effectiveness of mass treatment, clinic-based services, or combined strategies will depend in part on their success in reducing target infections. For example, rapid reductions in STD prevalence can be expected to reduce STD transmission, thus improving cost-effectiveness. As has been pointed out by the Mwanza research team, given the high proportion of STD that are asymptomatic and the lack of appropriate screening tests, mass treatment may offer the only feasible mechanism to reduce STD rates rapidly in developing country settings [13]. The Rakai Project is collaborating in modeling and cost studies to compare different delivery approaches. If the project is successful in controlling STD and HIV, the accrued data will guide the development of operations research to test different STD control strategies that may include the integration of targeted mass treatment with improved surveillance and clinical services.

Selection of drug-resistant organisms is a concern for all STD programs, whether they depend on subject compliance with a prescribed treatment regimen, or single dose mass treatment. The STD Control Study targets organisms with the greatest potential for resistance (*N. gonorrhoeae*, *H. ducreyi*) with multiple effective drugs, provides all medication under strict supervision (direct observed treatment), and conducts ongoing monitoring for drug resistance to *N. gonorrhoeae*.

For ethical reasons and to minimize the possibility of differential behavioral change, the project provided equivalent health education and HIV/STD prevention in both study arms. Control arm subjects who report symptoms of STD on interview, or who have serological evidence of syphilis, are referred for free treatment. Thus, project services provided in the control arm exceed the existing standard of care in Rakai and may dilute differences between study arms in STD prevalence/incidence, and potentially in HIV incidence. The project closely monitors treatment seeking and use of mobile, government and private clinics, drug shops and local markets (through review of service statistics and the collection of detailed survey information) in both study arms. These data will be incorporated into future analyses.

Given the inability to provide placebo intramuscular injections as a means of blinding the syphilis component of the intervention, the study was single blinded. Extensive efforts were made to ensure equivalent contact and health messages in both arms. Since some

degree of subject unblinding is possible, the project closely monitors compliance, follow-up and behaviors in both arms, in order to adjust for any differential trends that may arise as a result of potential unblinding.

In conclusion, at baseline, the Rakai Study surpassed the original sample size goal, demonstrated high compliance and coverage, and recruited populations with comparable sociodemographic, behavioral and STD profiles in both study arms. A community-based trial of intensive STD control on HIV transmission is thus feasible in the Rakai setting.

Acknowledgements

The authors acknowledge and thank the following individuals and institutions for their invaluable support and assistance: S. Sempala (Director, Uganda Virus Research Institute), G. Bagambisa and the Rakai District Medical Office, L. Tapert (Project Coordinator, Columbia University), S. Musgrave, without whom we would never have realized this study was possible, M. Musagara, H. Handsfield, C. Gaydos, S. Hillier, J. McGregor, and S. Morse.

References

1. Wasserheit JN: **Epidemiologic synergy. Interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases.** *Sex Transm Dis* 1992, **9**:2565–2569.
2. Sewankambo NK, Gray RH, Wawer MJ, *et al.*: **HIV-1 infection associated with abnormal vaginal flora morphology and bacterial vaginosis.** *Lancet* 1997, **350**:546–550.
3. Plummer FA, Simonson JN, Cameron DW, *et al.*: **Co-factors in male-female transmission of human immunodeficiency virus type 1.** *J Infect Dis* 1991, **163**:233–239.
4. Kreiss JK, Willerford DM, Hensel M, *et al.*: **Association between cervical inflammation and cervical shedding of human immunodeficiency virus DNA.** *J Infect Dis* 1994, **170**:1597–1601.
5. Moss GB, Overbaugh J, Welch M, *et al.*: **Human immunodeficiency virus DNA in urethral secretions in men: association with gonococcal urethritis and CD4 depletion.** *J Infect Dis* 1995, **172**:1469–1474.
6. Cameron DW, Simonson JN, D'Costa LJ, *et al.*: **Female to male transmission of human immunodeficiency virus type 1: risk factors for seroconversion in men.** *Lancet* 1989, **ii**:403–407.
7. Laga M, Manoka A, Kivuvu M, *et al.*: **Non ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study.** *AIDS* 1993, **7**:95–102.
8. Hoffman I, Maida M, Royce R, *et al.*: **Effects of urethritis therapy on the concentration of HIV-1 in seminal plasma.** *XI International Conference on AIDS.* Vancouver, July 1996 [abstract MoC903].
9. Cohen MS, Hoffman IF, Royce RA, *et al.*: **Reduction of concentration of HIV-1 in semen after treatment of urethritis: implications for prevention of sexual transmission of HIV-1.** *Lancet* 1997, **349**:1868–1873.
10. Laga M, Alary M, Nzila N, *et al.*: **Condom promotion, sexually transmitted diseases treatment, and declining incidence of HIV-1 infection in female Zairian sex workers.** *Lancet* 1994, **344**:246–248.

11. Mertens TE, Hayes RJ, Smith PG: **Epidemiological methods to study the interaction between HIV infection and other sexually transmitted diseases.** *AIDS* 1990, **4**:57-65.
12. Grosskurth H, Mosha F, Todd J, et al.: **Impact of improved treatment of sexually transmitted diseases on HIV infection in Tanzania: results of a randomized, controlled trial.** *Lancet* 1995, **346**:530-536.
13. Mayaud P, Mosha F, Todd J, et al.: **Improved treatment services significantly reduce the prevalence of sexually transmitted diseases in rural Tanzania: results of a randomized controlled trial.** *AIDS* 1997, **11**:1873-1880.
14. Sewankambo NK, Wawer MJ, Gray RH, et al.: **Demographic impact of HIV infection in rural Rakai District, Uganda: results of a population-based cohort study.** *AIDS* 1994, **8**:1707-1713.
15. Wawer MJ, Sewankambo NK, Berkley S, et al.: **Incidence of HIV-1 infection in a rural region of Uganda.** *BMJ* 1994, **308**:171-173.
16. Wawer MJ, Serwadda D, Gray RH, et al.: **Trends in HIV-1 prevalence may not reflect trends in incidence in mature epidemics: data from the Rakai population-based cohort, Uganda.** *AIDS* 1997, **11**:1023-1031.
17. Lutalo T, Musgrave S, Kigongo D, et al.: **HIV-1 and syphilis in Rakai District, Uganda [abstract].** Presented at the VI International Conference on AIDS in Africa. Yaounde, December 1992.
18. Serwadda DS, Wawer MJ, Musgrave SD, Stallings R, Sewankambo NK: **Concurrent partners and the results of the Uganda Rakai project [letter].** *AIDS* 1993, **7**:287-288.
19. Serwadda D, Wawer MJ, Musgrave SD, Sewankambo NK, Kaplan JE, Gray RH: **HIV risk factors in three geographic strata of rural Rakai District, Uganda.** *AIDS* 1992, **6**:983-989.
20. Wawer MJ, Serwadda D, Musgrave SD, Konde-Lule JK, Musagara M, Sewankambo NK: **Dynamics of spread of HIV-1 infection in a rural district of Uganda.** *BMJ* 1991, **303**:1303-1306.
21. Hayes R, Wawer M, Gray R, Whitworth J, Grosskurth H, Mabey D, and the HIV/STD Trials Working Group: **Randomized trials of STD treatment for HIV prevention: report of an international workshop.** *Genitourin Med* 1997, **73**:432-443.
22. Wasserheit JN: **The significance and scope of reproductive tract infections among third world women.** *Int J Gyn Obstet Suppl* 1989, **3**:145-168.
23. Laga M: **Epidemiology and control of sexually transmitted diseases in developing countries.** *Sex Trans Dis Suppl* 1994, **21** (suppl 2):45-50.
24. Wagner HV, Van Dyck E, Roggen E, et al.: **Seroprevalence and incidence of sexually transmitted disease in a rural Ugandan population.** *Int J STD AIDS* 1994, **5**:332-337.
25. Berkley SF: **Public health significance of STDs for HIV infection in Africa.** In *AIDS and Women's Reproductive Health*. Edited by Chen LC, Sepulveda J, Amor J, Segal SJ. New York: Plenum Press; 1991.
26. Ballard R, Morse S: **Chancroid.** In *Atlas of Sexually Transmitted Diseases and AIDS, Second Edition*. Edited by Morse SA, Moreland AA, Holmes KK. London: Mosby-Wolfe; 1996.
27. Martin DH, Mroczkowski TF, Dalu AA, et al.: **A controlled trial of single dose azithromycin for the treatment of chlamydial urethritis and cervicitis.** *N Engl J Med* 1992, **327**:921-925.
28. Handsfield HH, Dalu ZA, Martin DH, Douglas JD, McCarty JM, Schlossberg D: **Azithromycin Gonorrhoea Study Group. Multicenter trial of single-dose azithromycin vs. ceftriaxone in the treatment of uncomplicated gonorrhoea.** *Sex Transm Dis* 1994, **21**:107-111.
29. Echols RM, Heyd A, O'Keefe BJ, Schacht P: **Single-dose ciprofloxacin for the treatment of uncomplicated gonorrhoea: a worldwide summary.** *Sex Trans Dis* 1994, **21**:345-352.
30. Ballard R, Radebe F, Mampuru M, Dangor Y, Fehler HG: **A comparison of ofloxacin and ciprofloxacin in the treatment of chancroid [abstract].** *Sex Trans Dis* 1994, **21** (suppl 2):S189.
31. Handsfield HH, McCormack WM, Hook EW, et al.: **A comparison of single dose cefixime with ceftriaxone as treatment of uncomplicated gonorrhoea.** *N Engl J Med* 1991, **325**:1337-1341.
32. Knapp JS, Back AF, Babst AF, Taylor D, Rice RJ: **In vitro susceptibilities of isolates of *Haemophilus ducreyi* from Thailand and the United States to currently recommended and newer agents for treatment of chancroid.** *Antimicrob Agents Chemother* 1993, **37**:1552-1555.
33. Rein MF, Muller M: ***Trichomonas vaginalis* and trichomoniasis.** In *Sexually Transmitted Diseases, 2nd Edn*. Edited by Holmes KK, Mardh P-A, Sparling PF, Wiesner PJ. New York: McGraw-Hill; 1990:481-492.
34. Hillier S, Holmes KK: **Bacterial vaginosis.** In *Sexually Transmitted Diseases, 2nd Edn*. Edited by Holmes KK, Mardh P-A, Sparling PF, Wiesner PJ. New York: McGraw-Hill; 1990:547-559.
35. Robinson NJ, Mulder DW, Auver TB, Hayes RH: **Modeling the impact of alternative HIV intervention strategies in rural Uganda.** *AIDS* 1995, **9**:1263-1270.
36. Donner A, Klar N: **Methods of comparing event rates in intervention studies when the unit of allocation is a cluster.** *Am J Epidemiol* 1994, **140**:279-289.
37. Katz J, Carey VJ, Zeger SL, Sommer A: **Estimation of design effects and diarrhea clustering within households and villages.** *Am J Epidemiol* 1993, **138**:994-1006.
38. Hayes R, Mosha F, Nicoll A, et al.: **A community trial of the impact of improved sexually transmitted disease treatment on the HIV epidemic in rural Tanzania: I. Design.** *AIDS* 1995, **9**:919-926.
39. Gail H, Byar DP, Pechacek TF, Corle DK for the COMMIT Study Group: **Aspects of statistical design for the Community Intervention Trial for Smoking Cessation.** *Control Clin Trials* 1992, **13**:6-21.
40. Buimer M, Van Doornum GJ, Ching S, et al.: **Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by ligase chain reaction-based assays with clinical specimens from various sites: Implications for diagnostic testing and screening.** *J Clin Microbiol* 1996, **34**:2395-2400.
41. Lee HH, Chernesky MA, Schachter J, et al.: **Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain reaction assay of urine.** *Lancet* 1995, **345**:213-216.
42. Chernesky MA, Lee H, Schachter J, et al.: **Diagnosis of *Chlamydia trachomatis* urethral infection in symptomatic and asymptomatic men by testing first-void urine in a ligase chain reaction assay.** *J Infect Dis* 1994, **170**:1308-1311.
43. Birkenmeyer L, Armstrong AS: **Preliminary evaluation of the ligase chain reaction for specific detection of *Neisseria gonorrhoeae*.** *J Clin Microbiol* 1992, **30**:3089-3094.
44. Wawer MJ, McNairn D, Wabwire-Mangen F, Paxton L, Gray RH, Kiwanuka N: **Self-administered vaginal swabs for population-based assessment of *Trichomonas vaginalis* prevalence [letter].** *Lancet* 1995, **345**:131-132.
45. Borchardt KA, Smith RF: **An evaluation of an InPouch TV™ culture method for diagnosing *Trichomonas vaginalis* infection.** *Genitourin Med* 1991, **67**:149-152.
46. Draper D, Parker R, Patterson E, et al.: **Detection of *Trichomonas vaginalis* in pregnant women with the InPouch TV Culture System.** *J Clin Microbiol* 1993, **31**:1016-1018.
47. Nugent RP, Krohn MA, Hillier SL: **Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation.** *J Clin Microbiol* 1991, **29**:297-301.
48. Galask RP, Bowdler NC, Petzold CR: **Gram stain correlation with clinical diagnosis of bacterial vaginosis from multicenter trials [abstract].** Presented at the 1992 American College of Obstetrics and Gynecology Annual Meeting. Las Vegas, April 1992.
49. Orle KA, Gates CA, Martin DH, Body BA, Weiss JB: **Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum* and herpes simplex virus types 1 and 2 from genital ulcers.** *J Clin Microbiol* 1996, **34**:49-54.
50. Teratology Society Public Affairs Committee: **FDA classification of drugs for teratogenic risk.** *Teratology* 1994, **49**:446-447.
51. Verdon MS, Handsfield HH, Johnson RB: **Pilot study of azithromycin for treatment of primary and secondary syphilis.** *Clin Infect Dis* 1994, **19**:486-488.
52. Burtin P, Taddio A, Ariburnu O, Einarson TR, Koren G: **Safety of metronidazole in pregnancy: a meta-analysis.** *Am J Obstet Gynecol* 1995, **172**:525-529.
53. Van Dyck D, Smet H, Piot P: **Comparison of E test with agar dilution for antimicrobial susceptibility testing of *Neisseria gonorrhoeae*.** *J Clin Microbiol* 1994, **32**:1586-1588.
54. National Committee for Clinical Laboratory Standards: *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically, Second Edition; Approved Standard.* NCCLS Document M7-A2. Villanova: National Committee for Clinical Laboratory Standards; 1990.

55. Centers for Disease Control and Prevention: **CDC sexually transmitted disease treatment guidelines**. *MMWR* 1993, **43** (RR-14):1–102.
56. Donner A, Klar N: **Statistical considerations in the design and analysis of community intervention trials**. *J Clin Epidemiol* 1996, **49**:435–439.
57. Liang KY, Zeger SL: **Regression analysis for correlated data**. *Annu Rev Public Health* 1993, **14**:43–68.
58. Habbema JDF, de Vlass SJ: **Impact of improved treatment of STDs on HIV transmission**. *Lancet* 1995, **346**:1157–1158.
59. Abt K: **Problems of repeated significance tests**. *Control Clin Trials* 1981, **1**:377–381.
60. Morris M, Sewankambo N, Wawer M, Serwadda D, Lainjo B: **Concurrent partnerships and HIV transmission in Rakai District, Uganda**. *IX International Conference on AIDS and STD in Africa*. Kampala, December 1995 [abstract TuC105].
61. Wilcox RR: **Mass treatment campaigns against the endemic trepanematoms**. *Rev Infect Dis* 1985, **7**:S278–S283.
62. Zahra A: **Yaws eradication campaign in Nsukka Division, Eastern Nigeria**. *Bull World Health Organ* 1956, **15**:911–935.
63. Wawer MJ, Gray RH: **The Rakai Project STD Intervention [letter]**. *Science* 1995, **270**:564–565.
64. Novak R: **Testing AIDS interventions: when is the price too high?** *Science* 1995, **269**:1334.
65. Havlir DV, Dube MP, Sattler FR, et al.: **Prophylaxis against disseminated *Mycobacterium avium* complex with weekly azithromycin, daily rifambutin, or both**. *N Engl J Med* 1996, **335**:392–398.
66. West S, Munoz B, Bobo L, et al.: **Nonocular *Chlamydia* infection and risk of ocular reinfection after mass treatment in a trachoma hyperendemic area**. *Invest Ophthalmol Visual Sci* 1993, **34**:3194–3198.