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INTRODUCTION

This report summarizes key Rakai Health Sciences Program (RHSP) research and service activities between 2007 and 2010. It provides an overview of the main scientific findings in that period, as well as planned new research directions.

The Rakai Health Sciences Program

The Rakai Health Sciences Program (RHSP) was established in 1987 as a collaboration between researchers at Makerere and Columbia Universities, and the Uganda Virus Research Institute. Subsequently, the collaboration extended to Johns Hopkins University, and in 2002 to the Division of Intramural Research (DIR) at the National Institute of Allergy and Infectious Diseases, US National Institutes of Health (NIH). In that year, the RHSP was designated by the DIR as one of only three International Centers for Excellence in Research (ICER).

The RHSP’s operational structure is summarized in the following diagram.

In Uganda, the RHSP is registered as a non-profit (“Company Limited by Guarantee, and Not Having a Share Capital”). For official and scientific purposes, RHSP is under the oversight of the Uganda Virus Research Institute (UVRI) of the Ministry of Health. The Scientific and Ethics Committee of UVRI provides the Institutional Review Board approval and monitoring of all Rakai research.
The Rakai Community Cohort Study (RCCS)

The core of RHSP research is the Rakai Community Cohort Study (RCCS) which, since 1994, has conducted annual surveys with all consenting adults aged 15-49 years (approximately 15,000 persons annually) resident in 50 Rakai communities. Households are mapped by GPS and are censused annually, to list all household residents regardless of age, and to record all births, deaths, changes in marital status, and in and out migration. Household socioeconomic status is also noted. Following the census, adults in the target age range of 15-49 are interviewed in private and provide extensive, detailed information on sociodemographic characteristics, sexual risk behaviors, sexual networks, health status, health care seeking behaviors, and knowledge, attitudes and practices related to HIV prevention. Venous blood samples and genital swabs are collected for HIV/STI assays, and duplicate aliquots of all samples are archived at the RHSP and at Johns Hopkins.

RCCS in the field:
1. GPS mapping
2. Blood sample
3. Interview
4. Mobile coordination “hub”
Key RHSP studies and services covered in this report are summarized in the following diagram.

**RHSP Funding**

Since its inception in 1988, the RHSP has acquired multiple research grants from the US NIH (including RO1 and UO1 grants from the National Institute of Allergy and Infectious Diseases, National Institute of Child Health and Development, National Institute of Mental Health, National Cancer Institute) foundations, including The Fogarty International Foundation, The Bill and Melinda Gates Foundation, the Doris Duke Foundation, and the Rockefeller Foundation, and from international and governmental institutions (WHO, the World Bank, US Centers for Disease Control and Prevention, Walter Reed Army Institute of Research). Funding for HIV treatment and care services has been made available through the President’s Emergency Program for AIDS Relief (PEPFAR). Current grants are listed at the end of this report.

**International Center for Excellence in Research (ICER) in Uganda**

The International Center for Excellence in Research (ICER) program was launched in 2002 by the Intramural Research Division of the National Institute of Allergy and Infectious Diseases to develop and sustain research programs in low-income countries through partnerships between NIH intramural and in-country local scientists. The Rakai ICER focuses on clinical research in infectious diseases. ICER has developed a core program of studies, supported the improvement of laboratory, data and clinical infrastructure and the enhancement of information technology capability.
MAJOR RESEARCH ACTIVITIES

THE RAKAI TRIALS OF MALE CIRCUMCISION (MC)

Between 2004 and 2009, the RHSP conducted two randomized controlled trials to assess the efficacy of male circumcision (MC) for HIV/sexually transmitted infection (STI) prevention in men and in women. One trial, supported by NIH, enrolled 4996 HIV-negative uncircumcised men aged 15-49 years who were randomized to receive either immediate circumcision (intervention arm) or MC delayed for 24 months (control arm). The objective was to determine the effects of MC on the acquisition of HIV and sexually transmitted infections (STI) in men.

A second trial, supported by the Gates Foundation, enrolled 922 HIV-infected men who were similarly randomized to immediate or delayed MC, as well as the spouses of the HIV-infected men and the HIV-negative men described above. The trial had two main goals. The first was to assess the safety of MC in HIV-infected men and the effects of circumcision on male STI acquisition. The second was to assess whether MC would reduce the risk of HIV and STI transmission to female partners. All men and enrolled women partners were followed up at 6, 12 and 24 months to determine HIV and STD acquisition, and to assess sexual risk behaviors.

The Gates funded trial also allowed us to follow the general population of the 50 RCCS villages to assess MC acceptability, and the effects of MC provision on community-level HIV/STI incidence and HIV risk behaviors.

1. Trial Male Circumcision and HIV/STD Acquisition in HIV-negative Men

MC and HIV acquisition

In 2007 RHSP reported that circumcision reduced male HIV acquisition by 60% over two years. This paper was selected among the 10 most important reports in *Lancet* in 2007. The findings are summarized in Figure 1.

The findings from the Rakai trial, in conjunction with very similar results from trials in South Africa and Kenya, were instrumental in persuading WHO/UNAIDS to adopt a policy promoting MC as a proven effective strategy for HIV prevention. This has led to scale up of circumcision services in several East and Southern African countries where HIV rates are high and medical MC is uncommon.
MC effects on herpes, genital ulcer disease and syphilis in HIV-negative men

Further analysis of secondary trial end points showed that circumcision reduced the acquisition of herpes simplex virus type 2 (HSV-2) infections and genital ulcer disease in men.6 (Figure 2)

Genital ulcer disease (GUD) was significantly reduced by 47% (95%CI 36-57%) among circumcised men, whether or not they had serological evidence of HSV-2 infection, suggesting that the ulcers prevented by circumcision are likely to be due to traumatic lesions.7 The presence of both herpes and genital ulceration increased HIV acquisition in men, and reduction in these risk factors accounted for approximately 19% of the protection from HIV afforded by circumcision (GUD 11% and herpes 8%).7 Thus it is likely that most of the protection from HIV due to circumcision resulted from removal of vulnerable foreskin tissues per se (See section 3). However, MC did not affect the rates of syphilis infection in men (intervention 2.4%, controls 2.1%).

MC effects on Human Papillomavirus Virus (HPV) in HIV-negative men

Human papillomavirus (HR-HPV) infections are the cause of genital cancers in men and women, and Uganda has one of the highest rates of penile and cervical cancer in the world. HPV was assessed by Roche Linear Array which detects 47 HPV genotypes. After two years, the prevalence of penile HR-HPV infections were reduced in circumcised men (efficacy 35%, 95%CI 6-55%); circumcision also markedly reduced multiple HR-HPV infections (efficacy 65%, 95%CI 29-83%).6 Figure 3

MC reduced new HR-HPV infections in men, and this was observed for all 14 high risk viral genotypes (Figure 4). MC also increased the clearance of pre-existing HPV infections by 39% (95%CI 17-64%).8 Thus, it is likely that circumcision will reduce male-to-female HPV transmission, and analyses on women’s vaginal swab samples are currently being carried out.
2. Trial Male Circumcision safety and STI prevention in HIV-infected men

**MC safety and STIs in HIV-positivemen.**

MC programs cannot exclude HIV-infected men because this would be stigmatizing. Moreover, if MC was perceived by the population as a “marker” for HIV-negativity, it might induce HIV+ men to seek the procedure from unsafe sources in order to mask their serostatus, and could also lead to riskier sexual behaviors by circumcised HIV-negative men. Therefore, we assessed the safety of MC in HIV-infected men with CD4 counts >350 with WHO stage 1-2 disease, compared with HIV-negative men.

**Safety of MC in HIV-infected and uninfected men**

Rates of complications following MC in HIV+ men (3.1%) were comparable to those in HIV-negative men (3.5%), and did not differ by type of adverse event (e.g., infection 1.9% in HIV+ and 2.2% in HIV-negative). However, wound healing was slower in HIV+ men. At 30 days after surgery, complete wound healing was observed in 73.0% of HIV+ men and 83.2% of HIV-negative men (p < 0.001). At 6 weeks, 92.7% of HIV+ and 95.8% of HIV-negative men had completed healing (p = 0.007). All circumcised men and their partners were advised to refrain from intercourse until complete wound healing had been certified. However, 15.7% of HIV+ men resumed intercourse before wound healing was completed, compared to 10.7% of HIV-negative men (p= 0.003). Early resumption of intercourse occurred more frequently among married men (HIV+ 27.8% and HIV-negative men 29.1%), compared with single men (HIV+ 13.8% and HIV-negative men 6.3%).

**MC and genital ulcer disease in HIV-infected men**

HIV+ individuals experience frequent, recurrent and prolonged genital ulceration which increases the likelihood of HIV transmission. Circumcision significantly reduced GUD in both HIV-infected and uninfected men, despite the fact that GUD was three times more common in the HIV+ men (Table 1).

Table 1. Genital Ulcer Disease (GUD) prevalence by HIV-status and randomization arm.

<table>
<thead>
<tr>
<th></th>
<th>Circumcised GUD %</th>
<th>Control GUD %</th>
<th>Prevalence Rate Ratio (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-Negative</td>
<td>3.2</td>
<td>6.0</td>
<td>0.54 (0.5-0.6)</td>
</tr>
<tr>
<td>HIV-Positive</td>
<td>10.1</td>
<td>16.0</td>
<td>0.63 (0.5-0.8)</td>
</tr>
</tbody>
</table>

Figure 4. Incidence of all HR-HPV genotypes was reduced in HIV-negative circumcised men compared with uncircumcised men over 24 months.
**MC and HPV infection in HIV+ men**

Penile HPV infection was assessed in HIV+ men at enrollment and at 24 months follow up. At enrollment HR-HPV infection was common and comparable between study arms (Intervention 72.2%, control 76.6%). However, HR-HPV prevalent and incident infections were significantly reduced over 24 months, and this protective effect of circumcision was most marked for prevention of multiple HR-HPV infections (Table 2).

Table 2. Prevalence and Incidence of HR-HPV in HIV+ men, over 24 months follow up

<table>
<thead>
<tr>
<th>Follow up over 24 months</th>
<th>Intervention</th>
<th>Control</th>
<th>Risk Ratio (95%CI)</th>
</tr>
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<tbody>
<tr>
<td>Prevalence (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any HR-HPV</td>
<td>55.3</td>
<td>71.7</td>
<td>0.77 (0.62-0.97)</td>
</tr>
<tr>
<td>Multiple HR-HPV infections</td>
<td>22.2</td>
<td>42.5</td>
<td>0.53 (0.33-0.83)</td>
</tr>
<tr>
<td>Incidence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any new HR-HPV infection</td>
<td>42.0</td>
<td>57.0</td>
<td>0.74 (0.54-1.01)</td>
</tr>
<tr>
<td>Multiple new HR-HPV infections</td>
<td>9.9</td>
<td>24.7</td>
<td>0.40 (0.19-0.84)</td>
</tr>
</tbody>
</table>

Circumcision reduced the acquisition of 12 out of 14 HR-HPV genotypes in HIV+ men (Fig. 5)

3. **Male Circumcision and HIV/STIs in Female Partners**

Married men enrolled in the male circumcision trial were asked to invite their wives to enroll into a parallel follow-up study of women.

**MC in HIV-infected men and HIV transmission to female partners**

We identified 163 couples in which the man was HIV-infected and the wife was initially uninfected. Among these couples, 93 men had been randomized to the intervention arm and 70 to the control arm. The rates of male-to-female transmission were higher among couples with circumcised men, although the differences were not statistically significant (Figure 6).
This finding was unexpected and contrary to a previous study in which MC performed in childhood was associated with a reduction of 59% in female HIV infection; furthermore, in a previous observational study, we had observed no transmissions from circumcised men with viral loads under 50,000 cps/mL. Therefore, we conducted additional analyses and found that among couples in which the HIV+ man was circumcised and intercourse was resumed prior to certified healing, 27.8% transmitted within the first 6 months. Among couples in which the circumcised man delayed intercourse until after the surgical wound was healed the transmission rate over six months was 9.5% and comparable to that observed among couples with uncircumcised control men. Additionally, we assessed the effects of circumcision on the viral load in HIV-infected men and found a 0.25 log increased viremia following surgery, which may have increased infectivity.

In summary, circumcision in childhood may be protective against HIV transmission, but adult MC may not reduce female infections in the short-term. However, ultimately women will benefit because as MC progressively reduces male infections in the population, fewer women will be exposed to HIV.

**Male Circumcision and STIs in Female Partners**

*Bacterial Vaginosis BV and trichomonas Infections in women partners of HIV-negative men*

From the trial populations, we identified couples in whom both the men and their female partners were HIV-negative; in 825 couples the man was circumcised and in 725 the man was uncircumcised. Wives of circumcised men had reduced rates of vaginal infection due to trichomonas (efficacy 48%, 95%CI 2-95%) and reduced prevalence of BV (efficacy 40%, 95%CI 6-62%). The protection was most marked against severe BV (efficacy 61%, 95%CI 36-76%). MC also reduced GUD in female partners (efficacy 22%, 95%CI 3-37%).

**MC and human papillomavirus (HPV) infections in women partners**

We assessed HPV infections in 648 wives of intervention arm and 597 wives of control arm trial participants. At 24 months follow up, HR-HPV prevalence was 27.8% in the intervention arm compared to 38.7% in the control arm (efficacy 28%, 95%CI 15-40%) Figure 7.a.). The incidence of new HR-HPV was reduced among wives of circumcised men (efficacy 23%, 95%CI 7-37%), and was observed for most HR-HPV genotypes tested (Figure 7.b). Clearance of pre-existing infections was increased by 14% (95%CI 2-28%). Thus, male circumcision reduced female HR-HPV infection and potentially could protect women from cervical cancer.
4. MC and Sexual Behaviors and Sexual Satisfaction

**Sexual behaviors**

There is a concern that if men or their partners believe circumcision protects them from HIV or STDs, they may become complacent and increase their sexual risk behaviors, a phenomenon termed “risk compensation” or “behavioral disinhibition.” However, we observed no differences in sexual risk behaviors reported by circumcised and uncircumcised men during the trial or during post-trial surveillance (Figures 8 and 9).

![Figure 7a. Female HPV prevalence after 24 months by male partner’s circumcision status](image1)

![Figure 7b. Incidence of 13 out of 14 HR-HPV genotypes was lower in wives of circumcised men compared to wives of uncircumcised control men](image2)

![Figure 8. Number of sex partners during the trial (years 1-2) and during post-trial surveillance (years 3-4), by circumcision status](image3)
Sexual satisfaction

Men were asked about their sexual satisfaction and sexual dysfunction. There were no differences in between circumcised men and uncircumcised controls in reported sexual satisfaction (98.6% and 99.4%, respectively), and sexual dysfunction was rare and comparable between the two groups.\textsuperscript{13}

The wives of circumcised men were asked about their sexual satisfaction before and after their husbands became circumcised. Only 2.9% of women said they were less satisfied, 57.3% said there was no change in their sexual satisfaction, and 39.8% said their sexual satisfaction had improved, primarily because of improved hygiene.\textsuperscript{14}

5. The Effectiveness of MC during Post-Trial Surveillance

With grants from NIH and the Gates Foundation, we are continuing to follow both trial participants and 15,000 people in the Rakai Community Cohort Study to assess the effects of MC on HIV incidence in the whole population as circumcision is increasingly made available through our service programs. As of 2010, over 40% of the male population in the Rakai Cohort have accepted circumcision, and there is evidence of a decline in HIV incidence in the male population (Fig 10). It will take more time for effects to be observed in women and in the whole population.

Figure 10. Male HIV incidence and the prevalence of circumcision in the Rakai Cohort before, during and after the circumcision trial
6. Research on mechanisms by which MC reduces the risk of HIV and viral STI acquisition

The three trials of MC in HIV-negative men (in Rakai, Kenya and South Africa) showed that 50-60% of male HIV infections occur via the foreskin, so it is important to understand the mechanisms whereby circumcision protects men. Heterosexual HIV transmission occurs via the genital mucosa, but it has been difficult to study mucosal mechanisms due to the need for biopsy. However, access to foreskin tissue left over from MC surgery provides an ideal model to study susceptibility or resistance to HIV infection, and to potentially provide insights that could lead to the development of mucosal vaccines or microbicides for HIV prevention. With support from the Gates Foundation and NIAID, we have, therefore, embarked on basic science studies of foreskin tissues.

HIV Target Cells in the Foreskin and Effects of Inflammation

The foreskin is rich in HIV target cells, including Dendritic cells (CD1A+ Langerhans’ cells) which first take up the virus, and CD4 and CD8 T-lymphocytes which rapidly disseminate the virus throughout the lymphatic system. Using immunohistochemistry (IHC) we have characterized and quantified these cellular targets (Figure 11 and 12).15

Figure 11. Immunohistochemistry of foreskin immunologic constituents. (Targeted cells are stained red)

Figure 12. Cellular anatomy of the foreskin. CD1A dendritic cells are in the epidermis and CD4/CD8 cells are predominantly in the dermis
The foreskin is vulnerable to inflammation which increases the density of the HIV target cells (Figure 13).

**Figure 13.a.** Focal Inflammation in the Foreskin (left) and densities of CD4 and CD8 cells in the presence of inflammation (lower panel, 13.b)

**Keratinization of the inner and outer foreskin and anatomic factors**

Keratin, a protein derived from dead mucosal epithelial cells provides a barrier to viral entry through intact skin. The external surface of the foreskin is heavily keratinized whereas the inner mucosal surface has a thin keratin layer, possibly allowing HIV entry (Figure 14).

Figure 14. Keratinization of the inner and outer foreskin surfaces: inner mucosa is lightly keratinized, whereas outer mucosa has a thick keratin layer.

The preputial space under the foreskin is warm and moist, possibly facilitating viral survival. The foreskin is retracted over the shaft during intercourse and the lightly keratinized inner mucosa is
exposed to potentially infected vaginal secretions. The foreskin is also vulnerable to trauma and ulceration which provide a portal for viral entry.

**Foreskin Surface Area and HIV Acquisition**

If, as hypothesized, the foreskin is a major route for HIV entry, we reasoned that the surface area of the foreskin should be associated with HIV risk. The foreskin was measured after surgery and Godfrey Kigozi showed that HIV acquisition was increased in men with larger foreskin surface areas.\(^{16}\) (Figure 15)

Figure 15. Larger foreskin surface area is associated with increased HIV incidence

In summary, MC reduces HIV risk by removing HIV target cells, reducing inflammation and ulceration and eliminating the moist subpreputial space. However, MC may have other effects on the genital microbiota and immunologic milieu.

**The effects of MC on the penile microbiota**

We hypothesized that by removing the foreskin we would affect the bacterial colonization of the penis, specifically reducing anaerobic bacteria in the low oxygen subpreputial environment. Therefore, in collaboration with Lance Price and Cidny Liu of TGen, we assessed bacterial colonization before and after circumcision, compared with colonization of uncircumcised controls. The microbiome was characterized and quantified using broad-range PCR primers to amplify the V3-V4 hypervariable region for 16s rRNA gene-based pyrosequencing analysis.\(^{17}\) Following circumcision there were declines in the abundance of anaerobic bacteria (*Clostridiales Family XI* and *Prevotellaceae*), which are pro-inflammatory organisms.\(^{17}\) (Table 3)

Table 3. Change in relative abundance of anaerobic bacteria following circumcision

<table>
<thead>
<tr>
<th>Anaerobes</th>
<th>Pre-MC %</th>
<th>Post-MC %</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Clostridiales Family XI</em></td>
<td>33.6</td>
<td>3.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><em>Prevotellaceae</em></td>
<td>21.5</td>
<td>0.2</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Since genital inflammation increases HIV risk, this decrease in proinflammatory anaerobes could contribute to the protection afforded by circumcision.
Bacterial communities also became more homogeneous following circumcision (Fig 16).

![Non-metric Multi-Dimensional Scaling (nMDS) plot of Control and Intervention Males at Baseline and Year-1 Follow-up](image)

**Figure 16.** Non-metric multi-dimensional scaling (nMDS) plot of genital microbiota in circumcised and uncircumcised men. The penile microbiota in control males did not change from baseline to the year 1 follow-up. However, in intervention arm men, the microbiota became significantly more homogeneous post-circumcision.

7. Operations and service delivery research on MC

With PEPFAR and WHO funding we have provided circumcision to 6700 men after the trial, and trained 280 circumcision providers (physicians, clinical officers, nurses and counselors) from many regions of Uganda. Cumulatively we have performed over 12000 procedures, inclusive of trial participants. Surgery is performed under local anesthesia in outpatient theaters (Figure 15) and extended to two satellite clinics.

![Circumcision under local anesthesia](image)

![Training nursing staff](image)
Two surgical methods have been assessed, the sleeve procedure and the dorsal slit method (Fig 17).

Assessment of competency following training in circumcision
Dr. V Kigundu assessed how many procedures were required before newly trained practitioners reach optimal competence. After training, operative time was 40 minutes and practitioners needed to perform ~100 surgeries before the time required for surgery declined to 25 minutes (Figure 18). Complication rates were high for the first 20 surgeries. These findings have important implications for programs because planners must allow for longer surgical times and thus fewer procedures following training, and they must provide supervision of surgeries to reduce early complications.

Table 4. Time required for surgery and complication rates by procedure and provider

<table>
<thead>
<tr>
<th></th>
<th>Sleeve N = 1050</th>
<th>Dorsal Slit N = 971</th>
<th>Physician N = 564</th>
<th>Clinical Officer N = 1457</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complications (%)</td>
<td>0.9</td>
<td>0.6</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Time (minutes)</td>
<td>28.5</td>
<td>24.5</td>
<td>24.3</td>
<td>29.9</td>
</tr>
</tbody>
</table>

To reduce surgical time we plan to assess the acceptability and safety of the Shang Ring in Rakai. The Shang Ring a newly developed minimally invasive surgically procedure developed in China. The procedure is reported to require only 5 minutes of surgical time. We have submitted a grant to conduct this study.
HIV CARE AND ANTIRETROVIRAL THERAPY (ART)

Antiretroviral services
Since 2004 the Rakai Program has provided HIV care to infected persons via our central clinic in the Rakai Health Sciences Center is Kalisizo and through 17 mobile Suubi (“Hope”) clinics which visit communities at biweekly intervals. Up to now, all HIV-infected persons in Rakai District have been eligible for this free service. The central clinic, includes X-ray and abdominal ultrasound, and a well equipped clinical diagnostic laboratory.

HIV+ persons who are not yet eligible for antiretroviral therapy (ART) (currently just over 3,000 individuals) are provided with a Basic Care Package of cotrimoxazole prophylaxis, treatment for opportunistic infections, insecticide impregnated bed nets and clean water containers with hypochlorite disinfectant. Patients are initiated on ART at a CD4 cell count of 250 or below, and/or WHO HIV clinical stage IV disease. Individuals whose CD4 cell counts are ≥350 at first visit are retested every 6 months; persons with CD4 counts between 250 and 350 are reassessed every three months.

Between Aug 2004 and May, 2010, the RHSP program initiated 2,275 adults and 115 children on ART, of whom 1,907 (90.3%) remain on treatment. Through an NIH RO1, the RHSP is conducting clinical and population-level studies of ART, including drug adherence, response to treatment, HIV drug resistance, and the effects of ART on HIV prevalence and incidence, and on HIV-related behaviors in the community. 95% of ART patients consented to enroll in the ART-Related Clinical Study (ARCS), with 94.1% follow up at 3 years; losses occurred primarily due to death (4.4%) and out-migration (2.1%). The median CD4 cell count at treatment initiation was ~172. 92.6% of ARCS patients have maintained good HIV viral load (VL) suppression on first line therapy. 56% of first line patients are on zidovudine (AZT), lamivudine (3TC) and nevirapine (NVP) and 32.5% on AZT, 3TC and efavirenz (EFV). Less than 5% of patients are on second line therapy because of first line treatment failure, defined as a viral load spike ≥10,000 copies/mL. The high rates of adherence and continuation have been achieved through intensive counseling, home-based outreach, a “treatment buddy” program, and a Peer Educator program which links patients to successful ART users and to doctors and counselor by cell phone.
Women represent 65.4% of ART patients. RHSP offers free family planning (FP) methods to HIV+ women (condoms, oral pills, injectables and implants): 31% of HIV+ women used FP before initiating ART, compared to 59% after ART initiation. 6.4% of female ART patients become pregnant annually. Pregnancy rates increase with higher CD4 counts after ART initiation.

**Research Related to Antiretroviral Care in Adults**

**Evaluation of WHO treatment and monitoring criteria**

RHSP showed that WHO clinical staging criteria had low sensitivity (51%) and positive predictive value (64%) for the detection of CD4 counts <200 cell/mm³, the criterion for ART initiation. Thus, WHO clinical criteria would miss nearly half the patients requiring therapy, highlighting the need for CD4 screening. ¹⁹

In resource poor settings, the detection of treatment failure is often based on WHO recommended CD4 count immunologic criteria. However, RHSP data show that WHO immunologic treatment failure criteria overestimates true virologic failures, when compared to the “gold standard” of viral load monitoring. ²⁰

Therefore, use of the WHO immunologic criteria would result in a substantial proportion of patients being inappropriately switched to expensive second line drugs despite adequate viral suppression. CD4 counts currently need to be performed on fresh blood samples and are thus limited to settings with laboratory capacity. To facilitate the conduct of CD4 counts in underserviced settings, we assessed assays of rearranged T-cell receptor beta genes on dried blood spots. The rearranged T-cell receptor beta count correlated well with total lymphocyte counts (R=0.497; p<0.001), which can be used to determine treatment eligibility and could increase monitoring capabilities. ²¹

**HIV transmission in HIV-1 discordant couples before and after ART**

We evaluated the effect of ART on HIV transmission among 250 HIV discordant couples (i.e., couples with one infected and one uninfected partner). All couples were offered couples counseling, facilitated disclosure of results and information on risk reduction strategies. Prior to initiation of ART, HIV transmission occurred at a rate of 8.4/100 person years but there were no HIV transmissions after positive partners started ART. (Reynolds et al, AIDS, 2010 in press)

**Task shifting and patient support**

A cluster randomized trial of Peer Educators (HIV+ ART patients trained to provide support to newer patients) showed that at >96 weeks, ART users with Peer Educator support had lower virologic treatment failures than patients without such support (Figure 19). This suggests that the Peer Educators helped to mitigate treatment fatigue among longer-term patients. Additionally, RHSP studies indicate that cell phones and a “warm line” can help Peer Educators by providing community-based personnel access to clinicians and advice on patient management. (Chang et al PLoS One, 2010 in press).
Randomized trial of Herpes suppression in HIV-infected individuals to slow disease progression
Interventions that slow HIV-1 disease progression could postpone the need for antiretroviral therapy (ART) and prolong life expectancy for HIV-infected persons. Herpes simplex virus type 2 (HSV-2) has been shown to increase HIV-1 replication and HIV viral load. We are conducting a randomized trial of HSV-2 suppressive therapy among 440 HIV/HSV-2 dually infected individuals who had not yet qualified for antiretroviral therapy by CD4 count (CD4<250). The study has achieved excellent retention rates (>95%) and is scheduled to be completed in October 2010.

Research Related to HIV Prevention, Care and Treatment in Infants and Children

Prevention of mother-to-child HIV transmission (p-MTCT)
RHSP provides antiretroviral prophylaxis to HIV+ pregnant women using an innovative maternal self-medication strategy whereby mothers are provided with medications during pregnancy and treat themselves and their infants.22 This self-medication strategy is needed because of the high proportion of home deliveries (>70%). Infected mothers receive antenatal care, the Basic HIV Care Package (cotrimoxazole, bed nets, clean water and Fansidar for malaria prophylaxis), and both mothers and infants are evaluated in the home after delivery. Drug regimens used for p-MTCT are described

![Postpartum home visits to HIV+ and HIV-negative mothers](image1)

![Infant anthropometry](image2)

Figure 19.
Cumulative hazard of virologic failure in patients supported by Peer Educators (red) and controls
Evolution of prevention of mother- to-child transmission strategies
Prevention of mother-to-child HIV transmission (pMTCT) has evolved rapidly and the effectiveness of the prophylactic regimens was assessed by Joseph Kagaayi. From 2002-2007 mothers and infants only received single-dose nevirapine (sdNVP). From 2007--2008 mothers received Zidovudine (AZT) starting at 28 weeks of pregnancy and mothers and babies received sdNVP. Thereafter, mothers and infants received this regimen plus infant prophylaxis with NVP during breast feeding. Additionally, the criteria for starting pregnant women on ART was raised to 350 CD4+ cells/ul. Infant HIV infection declined progressively from 19% before prophylaxis was available to 1.8% with the current regimen (Fig. 20). The rates of transmission are now comparable to the U.S.

WHO/UNAIDS recommended formula feeding to prevent breast milk HIV transmission if it was “safe and affordable”. We evaluated mortality and HIV-free survival of breast and formula-fed infants of HIV-positive mothers. Free formula and education on safe preparation, especially avoidance of bottle feeding, was offered to mothers who opted not to breastfeed. The cumulative 12-month probability of infant death was six times higher in the formula-fed infants, and there were no differences in HIV-free survival.\(^{23}\) (Fig.21). In light of these findings we stopped formula and encourage breast feeding with NVP prophylaxis.

![Infant blood draw](image)

![Figure 20. Mother-to-Child HIV Transmission with Evolving Prophylaxis Strategies](image)
**HIV-1 resistance after single-dose nevirapine to prevent mother-to-child HIV transmission**

HIV is known to develop drug resistant mutations after single dose nevirapine (sdNVP). We assessed whether these resistant viruses can persist as stably integrated proviruses within the latent reservoir of resting CD4(+) T cells. Blood samples were assessed in women >6 months after receiving sdNVP. Resting CD4(+) T cells were isolated and activated to allow detection of stably integrated proviral DNA. Nevirapine resistance mutations (K103N and G190A) were detected in the latent reservoir of 8% of women and this could lead to a lifelong risk of re-emergent resistant virus potentially compromising future treatment.24,25

**Mother-to-child HIV transmission and placental malaria**

Mothers were asked to retain their placentas after delivery, in buckets of 10% formol saline provided by the RHSP. Placental malaria was diagnosed in paraffin embedded tissues using immunohistochemistry with a primary monoclonal antibody (3A4) for *P falciparum* histidine-rich-II protein (HRP-II) and a secondary peroxidase antibody. Maternal malaria was assessed by thick and thin smear, and Binax NOW ICT antigen test.

![Figure 21. Cumulative survival of formula and breastfed infants of HIV+ mothers](image1)

![Figure 22. Collection of placentas and HRP-2 staining of *P falciparum* in placental tissues](image2)
Among mothers who delivered between 1994-2000, prior to sdNVP prophylaxis, malaria was found in 31% of placentas. MTCT rates were increased in mothers with placental malaria (29.3%) compared to mothers without malaria (10.3%, RR=3.6, 95%CI 1.3-10.1). There was a dose response of increasing MTCT with higher HIV viral load and placental malaria, with 35.3% HIV transmission among mothers with both high viral load and concurrent placental malaria.26 (Figure 23).

Since provision of cotrimoxazole, insecticide impregnated bed nests and malaria prophylaxis with Fansidar, placental malaria has decreased to ~10% in HIV+ pregnant women.

Physical and neurodevelopmental outcomes in HIV Infected children

Under a NIH K award, Heena Brahmbhatt and colleagues assessed neurodevelopment (motor, cognition, vision and hearing) in HIV+ and HIV-uninfected children using age-appropriate and culturally adapted tests (Mullen Scales of Early Learning 0-7 year olds and Kaufman Assessment Battery (KABC-II) for children aged 7-18).

HIV-infected children had global deficits with significantly lower scores in virtually all domains evaluated. However, for younger children aged 0-6 years, receipt of ART and longer duration of time on treatment markedly improved neurodevelopmental indices (Fig. 24), whereas for older children aged 7-14, there was no significant improvement with ART (Fig. 24)
The improvement in developmental scores among younger but not older HIV+ children supports early initiation of ART, which also improves survival among HIV-infected infants and children.
HIV subtype effects on HIV transmission, disease progression and survival

The Rakai HIV epidemic is unique in that there is a predominance of HIV-1 subtype D infections (59.7%) with 15.1% subtype A, 21.1% recombinant viruses, 4.3% had multiple subtypes (i.e., superinfection) and 0.3% had subtype C. This diversity of circulating viral subtypes has facilitated studies of viral determinants of transmission and disease progression.

HIV subtype effects on transmission were assessed by Noah Kiwanuka for his PhD dissertation in 268 discordant couples; subtype A had greater infectivity than subtype D. Transmission with subtype A was 15.5/100 person years (py) compared to 10.5/100py with subtype D (p = 0.01). Molecular studies showed that only a subset of viruses in an infected individual are transmitted, and these transmitted viruses have characteristic signature amino acid sequences, a shorter envelope and lower V3 charge. Phylogenetic analyses showed that transmitted viral variants were closer to ancestral sequences, suggesting selective infectivity. Moreover, viral load set points are similar in donor and recipient infections, suggesting shared characteristics of transmitted viruses.

We evaluated the effect of HIV subtype on disease progression in 350 HIV-1 seroconverters observed prior to the availability of antiretroviral therapy. Progression to AIDS was defined as a CD4(+) cell count decline below 250 cells/mm³ or progression to clinical AIDS and death. The median time from infection to AIDS was shorter for subtype D (6.5 years), recombinant infections (5.6 years) and multiple subtype infections (5.8 years) than subtype A (8.0 years, p = 0.02). Relative to subtype A, the risk of progression to AIDS was increased in subtype D (HR = 2.13, CI 1.10-4.11), recombinant viruses (HR= 2.16 CI 1.05-4.45), and multiple infections (HR = 4.40, CI 1.71-11.3). (Figure 24) Similarly, the relative risk of death was 5.65 (CI 1.37-23.4) for D, 6.7 (CI 1.56-28.8) for recombinant infections and 7.67 (CI 1.27-46.3) for multiple infections. These findings may impact decisions on when to initiate antiretroviral therapy and have implications for future trials of HIV-1 vaccines aimed at slowing disease progression. In a related study, Tom Lutalo showed that the median survival time in Rakai was shorter than reported in other African populations with predominantly subtype C infections.

![Figure 24. HIV-1 subtype and progression to AIDS (left panel) and death (right panel)](image-url)
If subtype D is less transmissible and more rapidly fatal than subtype A, one would expect that subtype D will decrease over time, and analyses of subtype distributions show such viral evolution is occurring. In 1994 subtype D constituted 70.0% and subtype A 16.3% of infections, whereas in 2002 the proportion of D declined to 61.5% and A increased to 23.3% \((p = 0.015)\).\(^3\)

Phylodynamic analyses viruses from Rakai suggest that subtypes A and D were introduced in the 1940s and 1950s, with markedly increased spread during the 1970s during the time of civil war and the Tanzanian invasion of Uganda. It is noteworthy that subtype D is found in northern Tanzania.\(^3\)

Studies of co-receptor tropism show that D but not A viruses utilize CXCR4 co-receptors which are associated with late stage disease, possibly explaining the pathogenicity of D viruses. Also, subtype D viruses have a higher replication capacity (Figure 25).

**HIV Superinfection**

The immune response to HIV is insufficient to contain the virus and to prevent re-infection with subsequent viruses of the same or different subtype, leading to superinfection. Inter-subtype superinfection results in recombinant or multiple subtype infections observed in 25.4% of HIV-infected individuals, and the likelihood of such inter-subtype infections is increased in persons exposed to multiple infected sex partners (Figure 25). (O Laeyendecker  CROI 2008)
Using follow up data from the cohort we have cloned viruses and sequenced the gp41 envelope gene in individuals and couples with repeat observation. Figure 26 shows the viruses observed in a married couple (red female, blue male) at two time points (2000 and 2005-06). The couple were originally infected with a C subtype but the female partner was dually infected with a subtype D virus in 2000 (Fig 26.a). By 2005, the woman had transmitted the subtype D virus to her male partner (Fig 26.b). We will ultimately be able to estimate the rate of superinfection in the population. This is of relevance to vaccine development since if superinfection is frequent, it would suggest that systemic immunity might not protect individuals from subsequent infection with HIV.

Fig 26.a. HIV infected couple (female red, male blue) in 2000. The man and the woman share a subtype C virus, and the woman is superinfected with subtype D.

Fig 26.b. The same couple observed in 2005. The female has transmitted the subtype D virus to her male partner.
**Viral Diversity by Tissue Compartments**

For his MSc., Dr. Ronald Galiwango assessed viral diversity in foreskin and blood of circumcised HIV-infected men. He showed that the evolutionary distance of viral swarms in the foreskin tissues relative to the population viral pool was shorter (0.057) than the evolutionary distances for viruses in the blood (0.070) in 83.3% of subjects with amplifiable viruses (p= 0.012). This suggests sequestration of the initially transmitted virus in the preputial tissues (Figure 26).

![Discrete main branch serum and preputial clustering](image)

**Clinical Epidemiology Research**

**Variation in rates of disease progression**

Via long-term RCCS follow up Rakai can determine dates of HIV infection and disease outcome in order to classify HIV-infected persons into long term non-progressors (LTNP) defined as CD4 counts >600 at 7 or more years post-infection, standard progressors (SP) defined as deaths between 5-9 years post-infection, and rapid progressors (RP) defined as deaths within 4 years of infection. Among persons with long-term follow up, the probability of being a LTNP was 19.2%.34

**The role of microbial translocation in HIV disease progression.**

HIV rapidly destroys the gut associated lymphoid tissue (GALT) and increases intestinal permeability leading to microbial translocation to the blood stream. In cross-sectional U.S. studies, microbial translocation has been associated with more advanced disease progression. In Rakai, Dr. Andrew Redd assessed microbial translocation and cytokine responses longitudinally in HIV+ individuals categorized as long-term non-progressors (LTNP), standard progressors (SP) and rapid progressors (RP) using multiple translocation markers (lipopolysaccharid LPS, endotoxin core antibody EndoCAb, and soluble CD14).35 None of these markers changed significantly during the course of disease, suggesting that microbial translocation and subsequent inflammatory responses may not be related to disease progression in HIV-infected Ugandans (Figure 27), possibly because of prior exposure to intestinal bacterial and helminth infections.
Hormonal contraception and HIV disease progression

There is concern that hormonal contraception in HIV+ women may increase HIV disease progression. For her PhD, Chelsea Polis assessed progression to AIDS or death in 625 women who acquired HIV during Rakai Community Cohort followup, 21% of whom used hormonal contraception. The median survival times were comparable in users and non-users of contraception, but the former women had improved longer-term survival (Figure 28). (Polis et AIDS in press)

Fig 28. Survival from infection to AIDS or death among hormonal contraception users (HC) and non-users (No HC). Adjusted HR = 0.70, CI 0.5-0.97, p = 0.03)
HIV and Liver Disease
Among Rakai Community Cohort participants, the prevalence of prior hepatitis B virus (HBV) infection was 41% in HIV+ compared with 40% in HIV-negatives (multivariate adjusted risk 1.3, CI 1.00-1.71, p =0.05). Chronic HBV infection was similar in both groups (5%). Only 1% of participants had serological evidence of hepatitis C infection.

Using Transient Elastography FibroScan technology we assessed cirrhosis and fibrosis in 500 HIV infected and 500 uninfected persons. Significant fibrosis was found in 17% of HIV+ and 11% of HIV-negative persons (p = 0.008). This high prevalence of fibrosis in HIV+ persons was comparable to that observed in treatment experienced HIV cohorts in Europe and the US. The risks were increased among persons with CD4 counts <100, especially if they were not on ART, chronic HBV infection and alcohol use. Risks were also increased among fisherman suggesting a possible link to shistosomiasis. (Dr. L Stabinski et al, AIDS, submitted)

HIV and Chronic Kidney Disease
HIV has been associated with higher rates of chronic kidney disease in HIV+ African Americans. With Dr. Greg Lucas, we assessed rates of impaired glomerular filtration rate (GFR) in HIV-infected and uninfected adults. Reduced GFR was more common in HIV+ (8.4%) than HIV-negative individuals (4.7%, p =0.002), GFR in HIV-infected persons also showed greater decline over time (p=0.019). However, the rates of reduced GFR in the Rakai population are lower than those observed in African-Americans. (G Lucas et al JAIDS in press)

RESEARCH on SEXUALLY TRANSMITTED INFECTIONS (STIs)

Herpes simplex virus type 2 (HSV-2)
The prevalence of HSV-2 infection was 33.8% among male circumcision trial participants, and incidence was 4.9/100 py. Incidence was higher among persons with high risk sexual behaviors (multiple sex partners, non-use of condoms and consumption of alcohol before sex.)36

Acute HSV-2 acquisition is associated with increased risk of HIV infection (RR = 5.28, CI 2.79-9.98), as is chronic HSV-2 (RR = 2.78, CI 1.64-5.68).37 However, it is difficult to assess whether this association is causal or whether it is due to exposure to HSV-2 and HIV co-infected partners leading to transmission of both viruses. If the relationship is causal, then HSV-2 infection should precede HIV infection; however, among individuals who acquired both viruses, only 18.8% acquired HSV-2 before HIV, 25% acquired HIV before HSV-2, and 56.3% acquired both viruses during the same follow up interval. This suggests that exposure to co-infected partners and concurrent transmission of both viruses may be common.

Among women with symptomatic genital ulcer disease (GUD) 75.0% of ulcers were due to HSV-2 and 6.3% were due to T pallidum (syphilis).38 Among men, 64% of ulcers were due to HSV-2, 4% were syphilis, and 4% were H. ducreyi (chancroid).39

Bacterial Vaginosis
Bacterial vaginosis (BV) is a disruption of the normal lactobacilli predominant flora that has been associated with adverse pregnancy outcomes and HIV acquisition. BV is common in Rakai, affecting over 45% of women. We conducted a study of the natural history of BV in 311 women who provided self-collected vaginal swabs at weekly intervals over two years. Dr. Marie Thoma analyzed these data for her PhD. Women who initially had either normal flora or BV, tended to persist in these states for most of the
period of observation (76.1% and 73.6%, respectively), whereas women with initially intermediate flora fluctuated, 36.0% regressing to normal and 36.6% progressing to BV (Fig 29).

![Vaginal flora status at current visit](image1)

![Vaginal flora status at prior visit (t-1) and over all observations](image2)

We assessed factors associated with persistent BV (defined as women with >75% of observation time with BV). Persistent BV was decreased with older age, pregnancy, consistent use of condoms and male partner's circumcision. The most unique finding was that women who used unprotected and potentially contaminated water for bathing had an increased risk of persistent BV.

![Typical unprotected water sources in Rakai](image3)
Disclosure of HIV Status Among Couples

Couples disclosure of HIV status is important for prevention of HIV transmission. Prior to the availability of ART in 2004, approximately 50% of HIV+ individuals stated that they had disclosed to their partners, but only a low proportion, 25%, of partners confirmed that disclosure had taken place. After ART became available, self-reported disclosure increased to 80% and 57% of partners confirmed being told their partner’s HIV status. (Figure 32, S Haberlin, in preparation).

Disclosure of HIV results has been facilitated by discussion groups including HIV+ and HIV-negative couples, followed by counselor facilitated face-to-face meetings with HIV+ couples and by the formation of couples clubs. Currently, ~80% of HIV-discordant couples have shared their HIV results.

Stigma against HIV-Infected Individuals

HIV-infected patients were asked about their experience with overt (enacted) stigma. 11% of women and 6% of men reported stigmatization in the prior 6 months (p=0.001). This is substantially lower than in other African studies suggesting that community education and sensitization can reduce bias (S Haberlin, submitted, AIDS Care).

Prevention of interpersonal violence and sexual coercion

Interpersonal violence and sexual coercion are major problems in Rakai; 30% of women report experiencing personal violence and 24% report sexual coercion in the past year and 14% report that their sexual debut was coercive. Sexual coercion is associated with reduced contraceptive use, increased unwanted pregnancy and illegal abortion. Coercion at first sex is also associated with the subsequent risk of HIV infection. Women who were coerced at sexual debut reported higher rates of subsequent abortion than those who reported consensual sexual debut.[Polis, 2009 #432]
To address this problem Jennifer Wagman (PhD candidate) initiated a community-randomized study (SHARE) to assess the effects of increased awareness of the problem and the need for behavioral change, building networks for mutual support, and establishing violence prevention policies. The evaluation is ongoing, but qualitative studies indicate that sexual coercion was considered to be normal in intimate partner relationships due to gender inequalities and women’s lack of authority.

**Family Planning Outreach**
Tom Lutalo conducted a community-randomized study of improved family planning outreach via community reproductive health workers. The study showed increased rates of use of modern methods of family planning, and a reduction in pregnancy rates.

**Community Engagement and Education**
Community engagement is central to all Rakai Program activities and is provided by the Health Education and Community Mobilization (HECM) Section via meetings with community and religious leaders, community meetings, drama sessions, video shows and school outreach. The Community Advisory Board (CAB) advises the Program on research and services, and assists with communications. There are additional outreach efforts with specific high risk groups such as persons living with HIV, bar maids, commercial sex workers, motorcycle (bod-boda) drivers and prisoners.
ONGOING and FUTURE STUDIES

The Impact of male circumcision (MC) and ART on HIV incidence and risk behaviors in the Rakai Community Cohort Study (RCCS) population

With support from the Bill & Melinda Gates Foundation we are assessing the impact of ART and male circumcision on HIV incidence in the Rakai population. It is anticipated that as MC coverage increases in Rakai it will initially reduce HIV acquisition in men, which will result in lower HIV exposure of women and a subsequent decline in female incidence. This study will also monitor risk behaviors to determine whether perceptions of the protective effects of MC or ART result in an increase in sexual risk behaviors (i.e., risk compensation). The RCCS provides one of very few sites worldwide where such population based research can be carried out, given its well characterized populations, extensive data on HIV transmission rates and on behaviors of individuals and within couples, detailed information on all ART patients and MC acceptors as well as non-acceptors.

Clinical studies of male circumcision

By intensive weekly follow up of 100 HIV-infected and 100 uninfected men who request MC as a service and who consent to follow up, we are rigorously assessing wound healing following surgery. We are also assessing keratinization of scar tissue using poly-L lysine coated contact slides and staining for keratin. HIV and HSV-2 shedding during wound healing are being determined.

Immunologic and virologic studies of foreskin tissues and genital swabs

The male circumcision trials clearly demonstrate that at least half of HIV infections in men occur via the foreskin, and the foreskin tissues left over from MC provide access to mucosal tissues which could not otherwise be obtained. Thus, the foreskin is providing a model for investigating correlates of HIV infection and protection.

Under a grant from the Gates Foundation are working with Dr. Rupert Kaul at the University of Toronto and Dr. Taha Hibrod at the Karolinska University in Stockholm, in order to assess genital immunology in men and women to determine correlates of mucosal protection from HIV infection in highly exposed but persistently uninfected men and women in HIV-discordant couples. These studies will examine innate immune factors, cytokines and chemokines, cellular immunity and IgA in exposed but uninfected individuals compared with infected and with HIV unexposed participants. Pilot studies have shown the feasibility of quantifying soluble immune factors in genital swabs and cellular immunity in flash frozen foreskin tissues recovered at time of circumcision.

Cytokine and chemokine levels in preputial swabs of Rakai men.

Levels of IL-1, IL-6, IL-8, IP-10, MCP-1 and RANTES were assayed in cryopreserved prepuce swabs collected during the Rakai MC trial.
These studies may provide insights leading to the development of mucosal vaccines or novel microbicide formulations.

With a NIH grant we are working with Dr. A. Haase at the University of Wisconsin to detect early HIV infection in foreskin tissues. This will provide insights into the process of early viral entry and the determinants of productive or aborted infection.

**Genital microbiota, inflammation and HIV risk**
Under a NIH RO1 are assessing whether genital anaerobes cause inflammation and up-regulation of soluble and cellular immune factors. We will then conduct studies of genital bacterial communities and inflammatory markers among persons who become HIV infected compared with uninfected controls. If, as hypothesized, microbial induced inflammation is associated with the risk of HIV acquisition, it could provide new methods for HIV prevention such as the use genital aseptic microbicides (e.g., chlorhexadine).

**Long term clinical effects of antiretroviral therapy (ART).**
Over 2 million persons in Sub-Saharan Africa have initiated combined ART, and in high quality programs, treatment response is good. However, data from the US and Europe indicate that patients on long term ART may experience substantial non-communicable disease co-morbidities, including cardiovascular, kidney and liver disease, neurological and psychoneurological disorders, and what has been described as more rapid aging. We have submitted a grant to NIH to conduct a longitudinal study of Rakai ART patients, HIV+ persons not yet ART-eligible, and a comparison group of age, gender and community-matched HIV-negative persons. Participants would undergo detailed annual clinical and laboratory assessments, and evaluation of their functional status. Such comprehensive data are critically needed to plan the prevention and management of co-morbidities, and the provision of long term health care and social support for HIV+ African patients before and after the initiate ART.
FACILITIES

The RHSP has over 400 full time Ugandan professional and support staff.

The Rakai Health Sciences Center, the RHSP’s field station, houses clinical facilities including 12 exam rooms, outpatient operating theaters, X-ray, abdominal ultrasound, Fibroscan\textsuperscript{R} for liver transient elastography, pharmacy, laboratory and data management facilities.

Rakai Health Sciences Center

Main building

Inner courtyard

Outdoor bandas rve as additional conference rooms and patient waiting areas

New RHSP HIV clinic under construction
Laboratory Capacity

In 2003 a 4000 square foot laboratory facility was built by RHSP and equipped by the NIAID ICER collaboration. The laboratory opened in late 2004 and currently supports both basic bench and clinical research and provides clinical laboratory support for the RHSP antiretroviral treatment program. This is a state-of-the art biohazard II level, full service laboratory equipped with hematology (Coulter ACT-5 Diff), chemistry (Roche Cobas), Flow cytometry (Facscalibur), molecular virology and microbiology for HIV viral load, Hepatitis B viral load, genital ulcer multiplex PCR (4 thermocyclers -GeneAmp PCR system 9700 and BI 7900 real time PCR), microbiology and histopathology equipment for tissue processing and sectioning.

Additional equipment includes standing centrifuges, plate washers, 6 laminar flow biosafety air hoods (Labotech Biosafety level 2) for cell and sample processing, 6 CO2 incubators, 2 hot plates, balance/weighing scales, 8 steri-loop bacteriology incinerators, water baths, plate rockers, 6 dry incubators, microfuges, microtiter plate mixers, hot plates, vortex mixers, heat blocks, multi channel and repeat pipettors, microscopes (Zeiss Axiostars with co-observation equipment, ZeissAxiostar inverted microscopes, Zeiss fluorescent microscopes), PCR/UV workstations, electrophoresis gel rigs (large and small), refrigerated centrifuges, 3 dead air box benchtop for molecular biology and a 3 spectrophotometer plate reader (VERSAmaxPlate), Auto still Distiller, Anaerobic Jars, traceable thermometers, traceable timers, tachometer and barometer, 20 -80°C freezers and refrigerators.

The laboratory participates in and has been fully successful in the external quality assurance programs of the College of American Pathologists (CAP) and the Virology Quality Assurance (VQA) programs. Samples collected from the field or obtained from the clinic are processed and sorted in the accession lab. Biologic samples are then sent to the microbiology lab for malaria or tuberculosis testing. Samples needing a viral load test are sent to the molecular lab and those requiring a CD4 test are sent to flow cytometry lab. HIV antibody testing is performed in the serology lab.
Data Management

Data facilities include an air conditioned data room with 30 work stations, separate server room, local area network (LAN), internet connectivity via satellite connection including direct access to the National Library of Medicine satellite linkage, and data manager offices. 3 GPS Magellan units (one stationary and two mobile) are available for satellite mapping of all households. In addition, there is secure storage room for forms awaiting entry. Field data collection is being converted from paper questionnaires to ultramobile PCs which will allow transfer of data entry staff to other responsibilities. Hard copy questionnaires, consent forms are stored in locked safe facilities for at least seven years following data collection.
Backend facilities
A clinical and research endeavor such as RHSP would not be possible in rural Uganda without an extensive physical support infrastructure. Support equipment includes two 200 KVA generators, two 50 KVA generator, electrical inverters and stabilizers, safety equipment, autoclaves for decontamination and sterilization, glassware washers, and a millipore reverse osmosis water purification system. The RHSP also has water storage tanks, and a Maximaster biohazard incinerator meeting international standards for biohazard disposal.

Transport equipment
At the Rakai Health Sciences Center in Kalisizo, the RHSP has 20 Mitsubishi Double-Cabin pickup trucks, (each can carry a team of 8 persons plus equipment and clinic materials), two vans, two SUVs and 2 Suzuki Samurai. All vehicles are 4 wheel drive. Vehicles are essential since there is no public or private transport to carry teams to the field and to transport personnel, supplies and samples to and from the field, Kampala, Entebbe, and the airport. The Program also owns 26 motorcycles which are used for field personnel for pMTCT services, supervision of field-based HIV counselors, and outreach to ART and HIV care patients. The RHSP maintains its own fuel tanks and filling station and a vehicle maintenance/repair facility.
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TRAINING

Training is supported by three grants from the NIH Fogarty International Center (AIDS Training Research Program, HIV Associated Malignancies and Training in Population and Health). The following are the degree and non-degree trainees between 2007-10.

JHU Masters Trainees
- Ssekubugu, Robert (MHS in International Health candidate 2011, Johns Hopkins University)
- Bwanika, John Baptist (MHS in Demography, Johns Hopkins University 2008-2010)
- Galiwango, Ronald (ScM in Molecular Biology and Immunology, Johns Hopkins University 2008-2010)
- Musiige, Adrian (MPH, Johns Hopkins University 2008-2009)
- Ssempijja, Victor (ScM Biostatistics, Johns Hopkins University 2007-2009)
- Okui, Lillian Aaca, MD (MPH, Johns Hopkins University, 2007-2008)
- Kiggundu, Valerian, MD (MPH, Johns Hopkins University, 2007-2008)
- Nakigozi, Gertrude, MD (MPH, Johns Hopkins University 2006-2007)

Summer Epidemiology at JHU
- Nabukenya, Dorothy - summer 2009
- Musoke Mukasa, Richard - summer 2009
- Kigozi Ssebagalla, Darix - summer 2009
- Ssetube, Absalom - summer 2007
- Nkale, Fausta - summer 2007
- Ruwangula, Andrew - summer 2007
- Musiige, Adrian - summer 2007
- Mawemuko, Susan - summer 2007

Makerere University/JHU Sandwich PhD
- Kigozi, Godfrey, MD (PhD Candidate)
- Nalugoda, Fred (PhD Candidate)
- Gertrude Nakigozi (PhD Candidate)
- Tom Lutalo (PhD Candidate)

In country-masters/bachelors
- Kairania, Robert (MA, ongoing)
- Sekasanvu, Joseph, (MSC, ongoing)
- Ssekasi, Barbara (MBA, ongoing)
- Nanteza, Betty (BSC, ongoing)
- Nalubega, Immaculate (BA, ongoing)
- Mpoza, Bryan (BBLT, ongoing)
- Kighoma, Nehemiah (MSc, completed)
- Mbaziira, Mathias (BP&L, ongoing)
- Kimera, Edward (MA, ongoing)
- Muwanika, Richard (BBLT, Makerere University 2007-2011)
- Kiwanuka, Deus (BA, Gender and Development, Makerere University 2007-2011)
- Baale, Eugene (BSC, Kyambogo University 2007-2011)
- Mukakalisa, Maria (MSc, Health Services Management, Uganda Martyrs University, Nkozi 2008-2009)
- Kakembo, Rebecca (MBA, Uganda Martyrs University, Nkozi 2007-2008)
- Balikuddembe, Ambrose (MA, Developmental Studies, Uganda Martyrs University, Nkozi 2007-2008)
- Nakalanzi, Margaret (BBLT, Makerere University 2004-2007)
- Manyi, Yeku (BSc, Makerere University 2004 -2008)

Training in other countries
- Mawemuko, Susan (MBA candidate, Bath University 2009-2010)
- Nkalubo, Violet (DCH, AMREF NBI 2008)
- Nkale, James (DCH, AMREF NBI 2007)
- Senkunja, James (DCH, AMREF NBI 2007)
- Nampijja, Resty (DCH, AMREF NBI 2007)
- Nabasumba, Alice (MBA, UK 2006-2007)
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to human immunodeficiency virus types 1 and 2 in serum and urine samples in a rural community-based 

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Chapters in books 2007-10


Letters, reviews 2007-10


Wawer MJ, Serwadda D, Sewankambo NK, Gray RH. A Population-Based Approach to Understanding a Very Clever Virus: a Brief History of HIV Research and Services in the Rakai Health Sciences Program (RHSP), Uganda. 2008 February CROI.


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Current Grant Support

Title: “Genital Anaerobes, Inflammation and HIV Risk; Rakai”
Sponsor: NIH/NIAID   Grant No: R01HD050180
Period: 06/01/2010-05/31/2013
PI: Ronald H. Gay
Total Cost: $2,519,405

Title: “ARV Effects on HIV Epidemiology and Behaviors, Rakai, Uganda”
Sponsor: NIH/NICHD   Grant No: R01HD050180
Period: 07/15/2005-05/31/2011
PI: Maria J. Wawer
Total Cost: $6,555,999

Title: “Community Level Effects of MC on HIV Epidemiology with nested Clinical Research”
Sponsor: Bill and Melinda Gates Foundation   Grant No: 22006.03
Period: 11/01/2008-11/01/2013
PI: Maria J. Wawer
Total Cost: $17,163,850

Title: “Circumcision: HIV, STIs and Behavioral Effects in a RCT and post-RCT surveillance”
Sponsor: NIH/NIAID   Grant No: U01AI075115
Period: 09/01/2007-08/31/2012
PI: Ronald H. Gray
Total Cost: $7,338,214

Title: “Circumcision: HIV, STIs,Behavioral Effects: In Situ Analyses of HIV Transmission in Foreskin” (Supplement)
Sponsor: NIH/NIAID   Grant No: 5U01AI075115-03
Period: 09/01/2009-08/31/2010
PI: Ronald H. Gray
Total Cost: $163,378

Title: “Circumcision: HIV, STIs and Behavioral Effects in a RCT and post-RCT surveillance” ARRA HPV Supplement
Sponsor: NIH/NIAID   Grant No: 3U01AI075115-03S1
Period: 09/22/2009-08/31/2011
PI: Ronald H. Gray
Total Cost: $737,205

Title: Rakai Adolescent Project (RAP)
Sponsor: NICHD RO1   Grant No: R01HD061092
Period: 06/01/2009-05/31/2014
PI: Dr. John Santelli  Columbia University
Total Cost: $1,071,038

Title: Morbidity/Mortality and Disability in Children Study
Sponsor: NIH   Grant No: K01TW007403
Period: 09/16/2009 - 08/31/2010
PI: Heena Brahmbhatt
Total Cost: $426,941
Title: HIV/HAART and Pregnancy/Contraception in Rakai, Uganda  
Sponsor: NIH/NICHD  
Grant No: R01HD060460  
Period: 04/01/2009-03/31/2012  
PI: Heena Brahmbhatt  
Total Cost: $881,523

Title: Study of Early Initiation of HAART in Children with HIV Infection in Rakai, Uganda  
Sponsor: WW Smith Charitable Trust  
Grant No: A0801  
Period: 01/01/2009-12/31/2010  
PI: Heena Brahmbhatt  
Total Cost: $199,000

Title: Male circumcision, risk compensation and intensive health education and VCT on HIV prevention in Rakai, Uganda  
Sponsor: JHU Center for Global Health  
Period: 01/01/2010-12/31/2011  
PI: Xiangrong Kong  
Total Cost: $15,864

Title: Training in HIV Associated Malignancies, Rakai, Uganda  
Sponsor: NIH/Fogarty International Center  
Grant No: 3D43TW000010-21S2  
Period: 09/21/2008-05/31/2011  
PI: Chris Beyrer  
Total Cost: $436,068

Title: International Training/Research in Population and Health  
Sponsor: NIH/Fogarty International Center  
Grant No: D43TW001508  
Period: 09/21/2008-05/31/2011  
PI: Ron Gray  
Total Cost: $892,176

Title: A Randomized double-blind, Placebo controlled trial of Acyclovir Prophylaxis versus Placebo among HIV/HSV-2 co-infected individuals  
Sponsor: NIAID/NIH ICER  
Period: 01/2007 – 12/2010  
PI: Steven J Reynolds

Title: Hepatitis B and HIV co-infection, Uganda  
Sponsor: NIH ICER  
Period: 05/2008-09/2010  
PI: Lara Stabinski

Title: Adolescent Reproductive Behavior and Fertility in Rakai  
Sponsor: In Depth  
Period: 09/2010-04/2011  
PI: Tom Lutalo, Fred Nalugoda  
Total Cost: $49,900

Title: PEPFAR  
Sponsor: CDC Uganda  
Period: 04/01/2007 – 03/31/2012  
PI: David Serwadda  
Cost: Variable by year, 2009-2010 period total cost - $2,310,083