HIV susceptibility related to HIV target cells and cervical ectopy. A study of young South African women living in a rural area endemic of urogenital schistosomiasis.

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Elisabeth Kleppa

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Norwegian Centre for Imported and Tropical Diseases,
Department of Infectious Diseases, Oslo University Hospital, Norway
and
Faculty of Medicine, University of Oslo, Norway

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ABBREVIATIONS AND DEFINITIONS

AAM	Alternatively activated macrophages
APC	Antigen presenting cells
CAA	Circulating anodic antigen
CCA	Circulating cathodic antigen
CCR5	Chemokine receptor 5 (HIV co-receptor)
CD14	Cluster of differentiation 14 (macrophage / monocyte marker)
CD3	Cluster of differentiation 3 (T-cell marker)
CD4	Cluster of differentiation 4 (T-helper cells)
CD8	Cluster of differentiation 8 (Cytotoxic T-cells)
DC	Dendritic cells
FCS	Foetal calf serum
FGS	Female genital schistosomiasis
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSV	Herpes simplex virus
IL 4	Interleukin 4
IL 10	Interleukin 10
IL 13	Interleukin 13
OR	Odds ratio
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
RCT	Randomized controlled trial
STI	Sexually transmitted infection
Th1 / Th2	Type 1 / Type 2 T-helper cells
Th17	Type 17 T-helper cells
Treg	Regulatory T-cells
WHO	World Health Organization

SUMMARY

More than 110 million Africans are estimated to be infected with *Schistosoma (S.) haematobium*, but it is still one of the so-called "neglected tropical diseases". Poor countries bear the majority of the disease burden, contributing to the maintenance of the cycle of poverty.

In Africa, women are at a higher risk of human immunodeficiency virus (HIV) infection than men, and this cannot be explained by behavioural factors alone. Biological risk factors seem to contribute to the differences in HIV prevalence between geographical regions and genders. This thesis will focus on female genital schistosomiasis and cervical ectopy, both factors hypothesised to facilitate the transmission of HIV through the genital mucosa. Genital schistosomiasis in women is characterized by lesions referred to as "sandy patches" that appear grainy or homogenous and are thought to be caused by the deposition of ova in the genital tissues. Sandy patches are often associated with abnormal mucosal blood vessels and contact bleeding due to the fragile mucosa. Cervical ectopy and female genital schistosomiasis (FGS) may be diagnosed by photocolposcopic examination, and both conditions are likely to be present before sexual debut. In addition to increased susceptibility to HIV infection, *S. haematobium* infection has been suggested to accelerate the progression of HIV infection, possibly through increased immune activation.

In this study, South African women attending high schools in KwaZulu-Natal were included. Blood, urine and cervical lavage samples were collected and a photocolposcopic examination performed.

We found that FGS was associated with a higher frequency of HIV target cells and HIV co-receptor expression in the genital compartment (the proportion of $CD14^+CCR5^+$ cells was higher in FGS-positive (FGS+) than FGS-negative (FGS-), $p = 0.036$) and in blood (both the proportions of $CD14^+$ cells ($p = 0.042$), and $CD4^+CCR5^+$ cells ($p = 0.018$) were higher in FGS+). Furthermore, praziquantel treatment decreased the proportion of HIV target cells in FGS-positive women. Both the $CD14^+$ cell population and CCR5 expression by $CD4^+$ cells decreased significantly in both compartments after anti-schistosomal treatment ($p = 0.043$ and 0.025, respectively).

We found no significant difference in CD4 cell counts in women with or without *S. haematobium* infection. Furthermore, we found that cervical ectopy was associated with the prevalence of chlamydia infection (adjusted OR 1.78, p = 0.033) and with HIV infection in the youngest study subjects (OR 2.19, p = 0.014).

Future studies are needed to show whether treatment of schistosomiasis and/or intervention against cervical ectopy may become tools in the battle against the HIV epidemic.

INTRODUCTION

This thesis presents results from a large on-going intervention study in KwaZulu-Natal, South Africa. The research focuses on various aspects of female genital schistosomiasis (FGS) and their possible relationship with HIV infection. Studies have indicated that between 33 and 75 % of women infected with *Schistosoma (S.) haematobium* may suffer from FGS, and it has been estimated that 19 million preschool and school girls may develop FGS during the next decade in sub-Saharan Africa [1].

In a previous study from Zimbabwe, Dr Eyrun Kjetland found that women with genital schistosomiasis were more likely to be infected with human immunodeficiency virus (HIV) than women without genital lesions [2]. In the wake of this worrying result, this project was started in South Africa in 2009 to address some of the numerous questions that remain unanswered regarding genital schistosomiasis; the mechanisms behind the association between HIV and female genital schistosomiasis, possible confounding factors and immunological effects of urogenital schistosomiasis.

This thesis, which is the first from this South African project, encompasses data from gynaecological investigations (including colposcopy), laboratory analyses of urine, blood and cervicovaginal lavage and a comprehensive questionnaire covering behavioural risk factors for sexually transmitted infections (STIs) and schistosomiasis. Results on FGS and cervical ectopy, factors that may render a woman susceptible to HIV and other STIs, are presented.

BACKGROUND

Schistosomiasis

Schistosomiasis was first discovered by Theodor Bilharz in 1851 and is also known as Bilharzia. It is a parasitic disease caused by trematodes of the *Schistosoma* species, and according to the World Health Organization (WHO), more than 240 million people are infected worldwide [3].
After malaria, it is the second most common parasitic disease of public health concern [4]. Still, schistosomiasis is categorized as one of the "neglected tropical diseases" [5]. The disease is poverty related and often found in areas lacking clean water and sanitation.

The most common forms of the blood fluke in humans cause intestinal (*S. mansoni* and *S. japonicum*) and urogenital (*S. haematobium*) disease. *S. haematobium* and *S. mansoni* are found in Africa and the Middle East, and *S. mansoni* is also found in the Caribbean and South America. *S. japonicum* is only found in Asia. The worms live in perivesical veins (*S. haematobium*) or mesenteric veins (*S. mansoni* and *S. japonicum*). Intestinal disease is characterised by abdominal pain, rectal bleeding, diarrhoea and sometimes hepatic fibrosis, which may lead to portal hypertension [6].
In 2009, WHO decided to name the disease caused by *S. haematobium* urogenital schistosomiasis [7]. *S. haematobium* ova deposited in the female genital tissue cause female genital schistosomiasis and will be the focus of this thesis.

Schistosome ova trapped in tissues elicit an immune response that may cause disease. Schistosomiasis is a chronic infection that may cause severe morbidity including bladder cancer and kidney failure (urogenital disease) and liver failure (intestinal disease). The parasite may also cause more inconspicuous changes such as poor school performance, fatigue and impaired development in children [4,8]. The loss of performance in infected individuals may have significant public health implications, and represents an additional burden to countries that are often poor and where other infections, such as HIV, are prevalent [8,9].

Distribution

S. haematobium is endemic in 53 countries, mainly in sub-Saharan Africa, the Nile valley and on the Arabian peninsula [10] (Figure 1). More than 240 million people require treatment for schistosomiasis, and at least 90 % of them live in Africa [3,4]. In endemic areas, studies have shown a prevalence of FGS between 33 % and 75 % [2,11–13]. More than 300 million women and girls in Africa are at risk of having schistosomiasis [14].
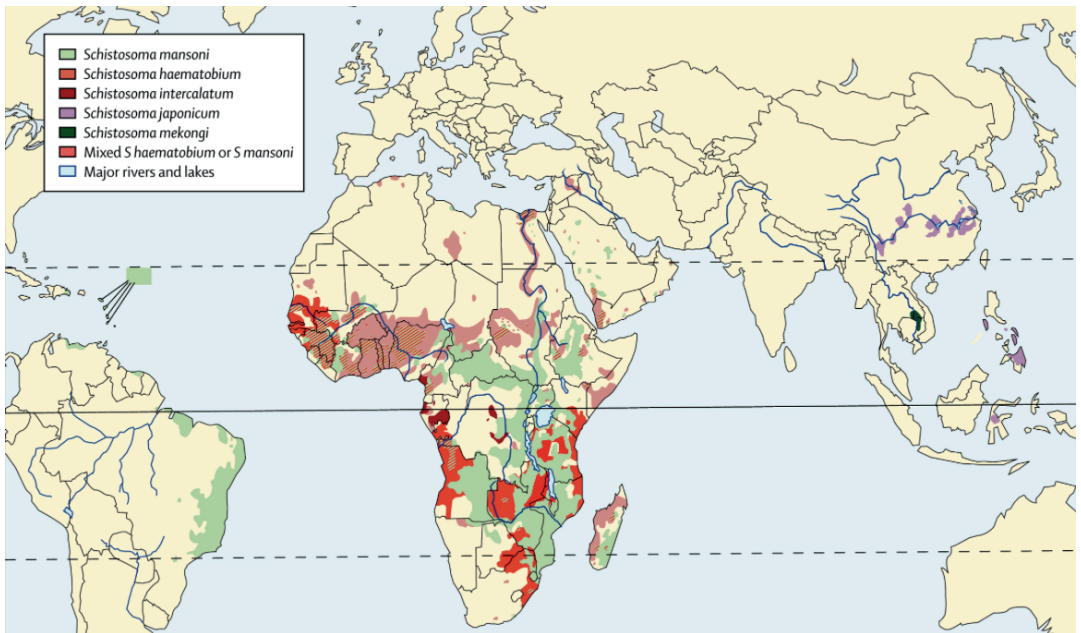
Figure 1. Distribution of schistosomiasis. Map showing the geographical distribution of *Schistosoma* species (From Gryssels et al. 2006 [15], based on updated data from Doumenge et al. 1984 [16])

Life cycle

The parasite is transmitted through contact with fresh water containing snails, the intermediate host, of the *Bulinus* species [6] (Figure 2). Snails shed cercariae that may penetrate human skin when in contact with fresh water bodies. After penetration, the cercariae develop into schistosomula and migrate to the portal vein where they mature for several weeks. The mature worms then migrate to venous plexuses in the urogenital tract, where worm pairs may live for as long as 30 years, on average 3-10 years [17]. The female worm produces thousands of eggs. The eggs migrate towards the urogenital tract, where they are shed in the urine and to a lesser extent in vaginal excretions, or, for a proportion, they are trapped in tissues. Ova have been shown to be excreted in urine with a peak around noon [18]. Each ovum contains a miracidium, which is released upon contact with water. The miracidium may then infect a freshwater snail and continue the life cycle of the parasite [15].
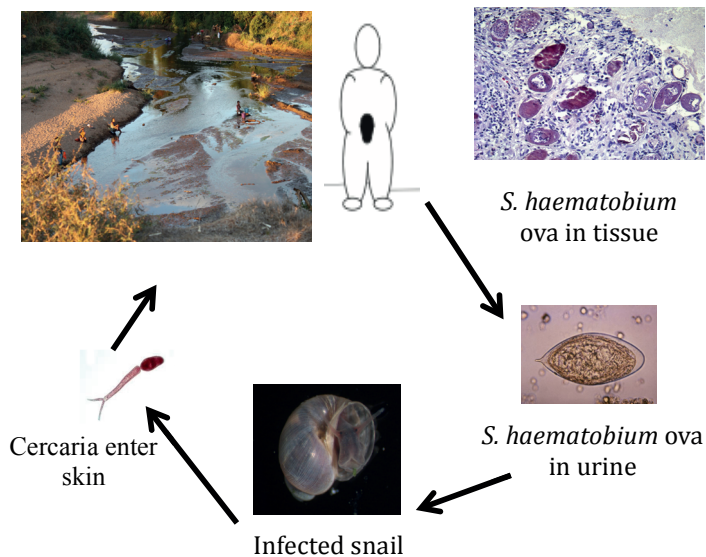
Figure 2. *Schistosoma haematobium* **life cycle.** (Photographs by Peter M Jourdan, Wikimedia Commons/Snek01 and Eyrun F Kjetland)

Schistosomiasis immunology

The cercariae and adult worms create a systemic immune response in the human host associated with high levels of IgE and eosinophilia [19]. Compared to the immune response to schistosome ova the response to adult worms is relatively moderate, and worms are therefore able to live for years in human veins [19]. This may be due to their ability to cover themselves with host antigens [7]. Partial schistosomiasis immunity develops for persons living in endemic areas [15]. The prevalence and intensity of the infection increase until the age of 8 - 15, and then decrease during adulthood [15]. Interestingly, children have been found to be more susceptible to infection than adults. This is not likely to only be caused by changing patterns in water contact, but also the adults' larger degree of acquired immunity [15]. Acquired immunity is thought to be mediated through IgE antibodies against worm antigens [15] and eosinophils might be of large importance for the susceptibility to reinfection [20].

Helminth protection requires both antibodies and T-cells [17]. Schistosomiasis has been suggested to shift the CD4 T cell response from a T helper cell type one (Th1) response towards a T helper cell type two (Th2) response and contribute to increased immune activation [21–24]. A study has indicated that African residents have higher immune activation compared with Italian individuals, possibly driven by environmental factors rather than being genetically determined [25]. Furthermore, Cohen et al. found increased levels of activated mucosal T-cells in the female genital tract in a cohort comparing Kenyan and American women, after adjusting for factors such as STIs [26].

The shift towards a Th2-type response is thought to occur once egg deposition starts [17]. However, the simplistic Th1/Th2 model has been challenged, and the importance of other populations of cells in the immune response has been recognized. How Th2 responses are induced is not completely understood, but T-regulatory cells (Treg, a subpopulation of CD4 cells) have been found to be involved in suppressing Th1-responses during schistosomiasis [27]. This control seems beneficial, as Th1 responses may lead to increased disease severity [27]. In addition, studies in mice have shown that a strong Th17-cell, also a subset of CD4 T-cells, response occurs in schistosomiasis [28]. A strong Th17 response may be associated with more severe pathology [28]. Chronic

schistosomiasis infection has also been associated with functional impairment of dendritic cells, another possible mechanism behind the Th2 immunological profile [29]. Macrophages represent another immune cell thought to be critical to the inflammatory response and fibrosis seen in chronic infections [30]. Macrophages are traditionally classified into two polarization states-the classically activated and the alternatively activated phenotype, mirroring the Th1 and Th2 lymphocytes [30]. Alternatively activated macrophages have been identified to be of major importance in chronic schistosomiasis infection [31–33]. These macrophages are associated with a Th2-dominated immune milieu and contribute to tissue protection [34].

Urogenital schistosomiasis

History of female genital schistosomiasis

The first report of FGS was written in 1899 [35], but many years passed before this disease attracted renewed attention in the scientific world. Genital lesions, called "sandy patches," were given their name due to their yellow sand-like colour [36]. The sandy patches resembled lesions seen in the schistosomiasis-infected bladder [37,38]. Numerous case reports have later confirmed the presence of schistosomiasis lesions in all female genital organs, causing various signs and symptoms such as infertility, genital tumours, tubal pregnancy and abdominal masses [39–51]. Genital lesions caused by *S. japonicum* and *S. mansoni* have also been described, though little is known regarding these species and genital tract disease [48,52–54].

Clinical manifestations

When cercariae penetrate the skin, a rash called "swimmer's itch" may develop. After a few weeks, the so-called "Katayama fever", a systemic hypersensitivity reaction, may occur and fever, fatigue, myalgia, cough and eosinophilia are typically found in this acute stage of schistosomiasis [55]. However, in endemic areas where people are chronically exposed to schistosomiasis, this acute

state is rarely described, possibly due to under–diagnosis (for example because of inter-changeable symptoms with malaria) or sensitisation in uteri [15].

S. haematobium ova may cause granulomatous inflammation in the genitourinary tract. In endemic areas blood in the urine is the most commonly known sign of schistosomiasis [56]. Chronic infection can cause bladder calcification and is associated with bladder cancer [15]. Fibrosis may occur in the bladder and ureters, causing hydronephrosis and subsequent kidney failure. Schistosomiasis is a chronic disease, and affected individuals may suffer from long-term anaemia, fatigue and malnutrition [24].

Schistosome ova trapped in genital tissues may cause gynaecological contact bleeding, friable blood vessels and inflammation [57]. Genital schistosomiasis may be found with or without egg excretion in the urine [2,58,59]. All segments of the female genital tract may be affected [60–65]. On gynaecological examination, lesions can be found in the cervical or vaginal mucosa (Figure 3). Some reports have described polypoid and ulcerous lesions, especially in the vulva and vaginal region [61], though later studies have not supported this observation [57,66]. It has also been hypothesized that vulval lesions are more common in younger women [67,68], but this has not been confirmed in epidemiological studies [57,68].

FSG has been found to be associated with infertility [69,70]. It may also cause contact bleeding, itch and discharge and could therefore mimic a sexually transmitted infection [71,72]. Irregular menstruation, pelvic pain, dysuria and haematuria have also been found more frequently in villages with a high prevalence of FGS than in low prevalence sites [12]. Menorrhagia and irregular bleeding have been found to be associated with uterine schistosomiasis [73]. Some cervical schistosome lesions may be mistaken as cervical carcinoma and this can have serious consequences for the patient [43,60,61]. Whether cervical carcinoma and FGS are associated is still debated [74,75],
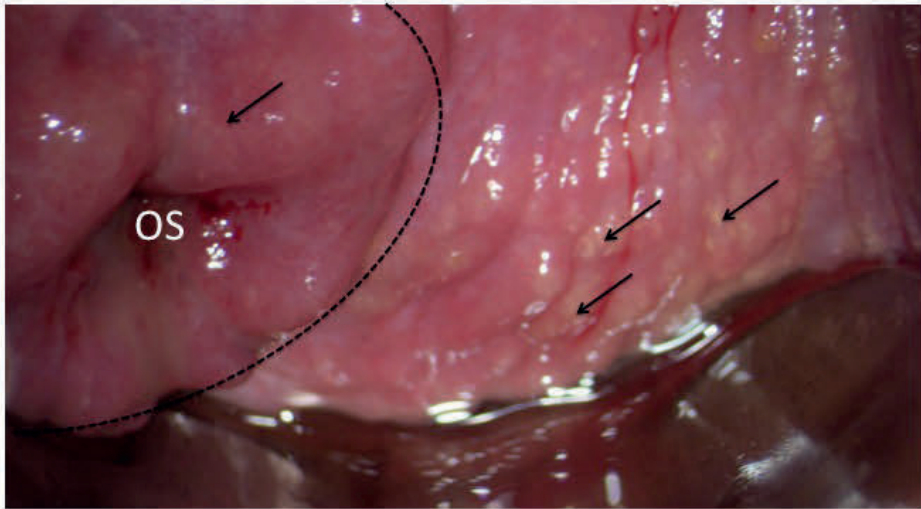
Figure 3. Female genital schistosomiasis lesions. Image from a colposcopic investigation showing the cervix (surrounded by a circle) and the vaginal wall. Numerous sandy patches appearing as grains, both single and clustered, are seen in the mucosa; the arrows point at some examples. (Photograph by Elisabeth Kleppa)

Due to the lesions in the genital mucosa, one could suspect an increased susceptibility to other infectious diseases [68]. Cross-sectional studies have shown an association between HIV and FGS (see chapter about FGS and HIV) [2,76], and schistosomiasis has also been hypothesized to be associated with other STIs [77]. The question of FGS being a possible risk factor for urinary tract infection remains unanswered.

Genital lesions may also be found in male genital organs such as the seminal vesicles, and in HIV-positive men this has been hypothesized to increase the risk of HIV transmission [78,79].

Histology

On histopathological examination, ova are most commonly found in the cervix followed by the vagina, ovaries, fallopian tubes, vulva and uterus [57,80]. Histological studies have shown that egg accumulation is often focal, possibly due to groups of worms settled in certain vascular regions [81]. In autopsy samples, the number of ova found in the tissues was related to the number of female worms, and the number of ova in tissues correlated to the number of eggs passed in the urine [82,83]. A large proportion of the *S. haematobium* worms was identified in the genitourinary organs (52 %), while 47 % were found in the mesenteric circulation [82].

Sandy patches have been described in the bladder and Von Lichtenberg et al. suggested that schistosomal bladder lesions progress from polypoid patches to sandy patches [84]. They described the sandy patches in the bladder as granular, grey to tan brown, with a surface that was rough and sandy when touched [84]. Similar findings were observed in chimpanzees infected with *S. haematobium*, where sandy patches in the bladder were found to contain numerous degenerated and calcified ova [83].

Histologically, the ova deposited in the genital tissues cause a granulomatous reaction [37], and both calcified and non-calcified ova can be found in the granulomas [85]. Studies have shown that the infiltrate around schistosome ova consists of monocytes, macrophages, lymphocytes, plasma cells, neutrophils, eosinophils, fibroblasts, mast cells, and blood platelets; monocytes and macrophages being the most predominant cells [85,86] (Figure 4). Studies of *S. mansoni* granulomas have shown that they develop by an initial macrophage and T-lymphocyte response followed by recruitment of other immune cells including numerous macrophages [87]. The macrophages fuse and form multinucleate giant macrophages, followed by collagen deposition and fibrosis [87,88].

In a study of *S. mansoni*, the lymphocyte populations in granulomas as well as in peripheral blood increased 8-16 weeks after infection [89]. T-cells seem necessary for the granulomatous reaction, as T-cell-depleted mice do not make true granulomas [90]. In murine *S. mansoni* infection, the granulomatous reaction around the schistosome ova in the liver is driven by a Th2 response [91].

Regulatory T-cells, IL-10 responses and alternatively activated macrophages have been found to be involved in the Th2 type response, which seems to modulate the granulomatous reaction and reduce tissue damage [19,91].

The urogenital lesions may persist over time and in a mice study, calcified *S. haematobium* eggs were injected in the mucosa [92]. The egg density decreased over time, but still created a granulomatous reaction similar to that caused by viable eggs [92].
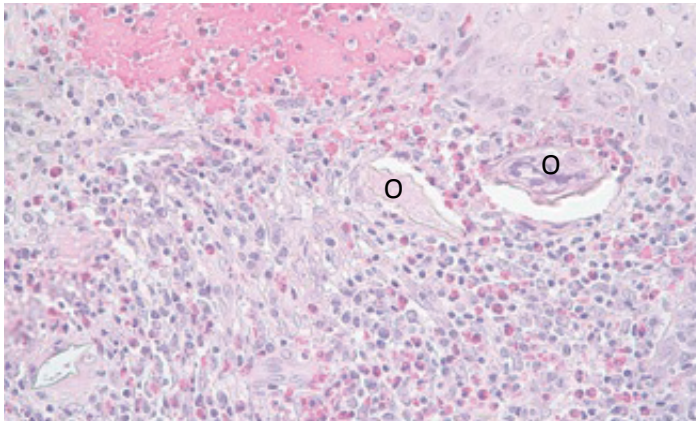
Figure 4. Histological image from genital tissue biopsy. *S. haematobium* ova (O) in tissue surrounded by a cellular infiltrate including numerous lymphocytes and eosinophils. (Photograph by Peter M. Jourdan)

Diagnosis

Traditionally, the diagnosis of urogenital schistosomiasis has been based on the finding of ova in urine microscopy. However, this method may not be able to detect low intensity infections or chronic infections with ova trapped in the tissues [93]. Genital lesions may be present without ova excretion in urine [57,58]. Antibody tests are of limited diagnostic value in endemic areas because the test may be positive for years even after successful treatment [94]. Antigen assays, such as circulating anodic antigen (CAA) and circulating cathodic antigen (CCA), have been shown to be specific and sensitive in detecting active schistosomiasis infection, but sensitivity in low intensity infections may be suboptimal [94]. Microhematuria, detected by urine strips, may serve as an indicator of urogenital schistosomiasis prevalence [95]. Indirect disease markers, such as pain when urinating or bloody urine, have been found to have poor predictive potential for urinary schistosomiasis [96].

FGS is diagnosed by identification of typical genital lesions by visual inspection of the lower genital tract, usually done by a colposcopic gynaecological examination (Figure 5). In consensus meetings in Copenhagen and Durban, it was agreed that one of three mucosal colposcopic findings may serve as a diagnosis of female genital schistosomiasis: 1) sandy patches appearing as single or clustered grains, 2) homogenous yellow areas or 3) rubbery papules [68]. Abnormal blood vessels are often seen in association with the lesions (Figure 5). The lesions are acetonegative [68].

The diagnosis of schistosomiasis in general, and FGS in particular, remains a challenge [97]. In many cases, magnification seems to be necessary, as the lesions are easily missed [98]. Neither the Pap-smear nor microscopy for ova in urine have proven to be sensitive tests for FGS [58,99]. Schistosoma PCR of vaginal lavage samples may be a supplement to gynaecological examination [100]. However, lesions containing dead, calcified ova that do not contain DNA are undetectable by PCR. Biopsy for diagnostic purposes remains controversial, especially in areas where HIV is highly prevalent [101,102]. There is a lack of diagnostic tools that are both sensitive and specific as well as inexpensive, safe and available in endemic areas [68]. Few colposcopes are available in resource-poor settings, and extensive training is necessary for the clinician to recognize the genital lesions [68].
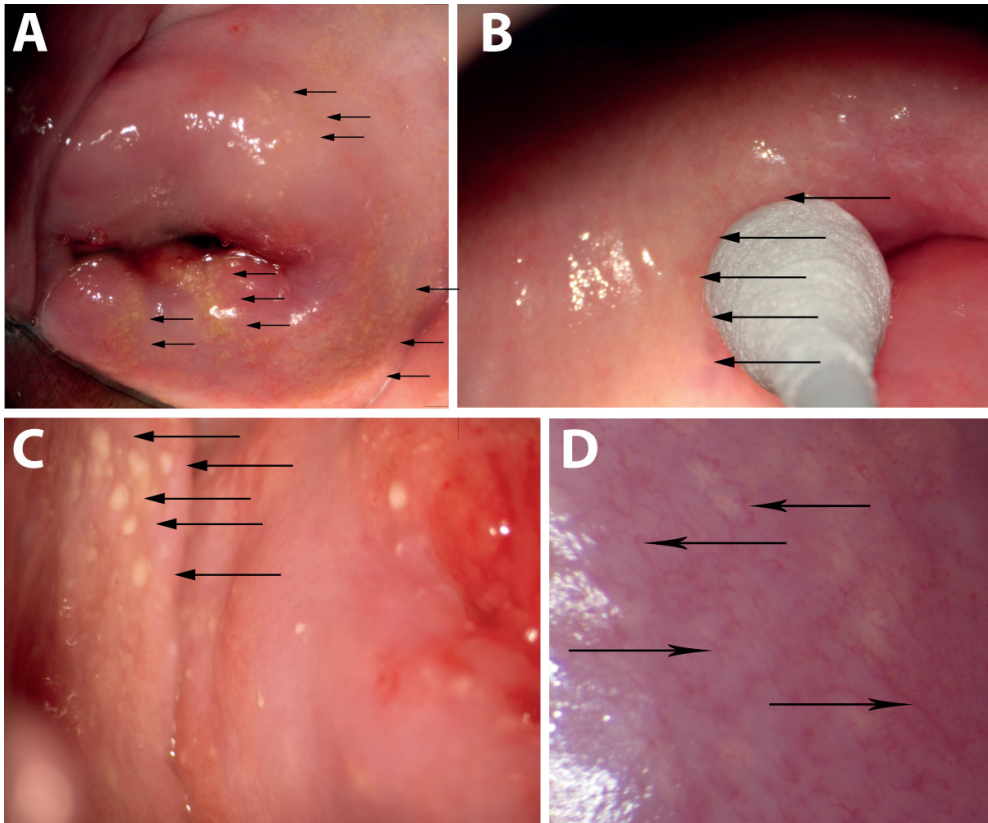
Figure 5. Mucosal colposcopic findings in female genital schistosomiasis. A) Grainy sandy patches, both single grains and clusters. Arrows point to examples. B) Arrows point to homogenous sandy patch on the cervix. C) Rubbery papules (arrows). D) Abnormal blood vessels (arrows point to examples). (Figure from [103], photographs by Bodo Sahondra Randrianasolo and Eyrun Kjetland).

Treatment

The antihelminthic drug praziquantel has been the schistosomiasis treatment of choice for decades. The tablets are taken as a single dose per os and kills adult worms [104]. Treatment should be administered 4-6 weeks after the transmission season, due to the limited effect on immature worms. Side effects are generally mild and include nausea, abdominal pain and vomiting, but the drug is considered safe and has not been associated with severe side effects. The WHO recommends school-based mass treatment in schistosomiasis endemic areas [105], but it has been argued that younger children might also need anti-schistosomal treatment due to early water exposure [106].

Although praziquantel has shown a high cure rate of urinary schistosomiasis, the effect on genital lesions is debated [104]. In a study of young girls, genital symptoms were associated with *S. haematobium* infection and genital schistosomiasis is therefore likely to be present from an early age [107]. Still, it is not known when FGS starts to develop and therefore the age at which treatment should be given is debated [108]. The treatment outcome may also vary between age groups, as a study showed that treatment of older women might have limited effect on genital lesions, while treatment early in life may prevent later damage [109].

It has been hypothesized that the duration of the infection may be of importance [110,111]. Another study found a drastic reduction of ova excretion one year after treatment for *S. haematobium* infection, and also a reduction in abnormalities in the lower urinary tract. However, vesical calcification, which is associated with chronic infection, persisted one year after treatment in four out of ten cases [110]. A recent study found that out of 33 women with *S. haematobium* ova in urine and positive schistosoma PCR, 24 % still had positive PCR 6 months after treatment and 33 % had cervical lesions that did not significantly decrease after treatment [112]. Repeated treatment may therefore be necessary to prevent irreversible lesions.

If sustainable control of the infection is to be achieved, public health measures such as improved sanitation and information to the community are important in addition to praziquantel treatment [113].

HIV

HIV was first described in 1983 and has since become a pandemic with more than 35 million infected in 2013 [114–116]. HIV is a retrovirus that untreated causes failure of the immune system and, ultimately, death [117]. Two types of HIV have been identified, HIV-1 and HIV-2 [117]. Most infections are caused by HIV-1, which will be referred to as HIV in this thesis. Especially sub-Saharan Africa has been severely affected by the infection, and even though the inhabitants of this region comprise only 10 % of the world's population they account for almost two thirds of all HIV cases [118].South Africa has the largest HIV positive population in the world-approximately 17 % of the general population is infected [119]. There is significant variation in HIV prevalence by province, ranging from 24.7 % in KwaZulu-Natal to 4.8 % in Western Cape [119]. In antenatal women aged 15 – 19 years, the HIV prevalence was 16.8 % in KwaZulu-Natal in 2011 [119]. In year 2000 STIs (including HIV) accounted for over 26 % of all deaths in South Africa [120].

In South Africa, approximately 59 % of adults living with HIV (3.5 million) are women [121]. The HIV prevalence may be up to eight times higher in young women than in young men, and behavioural factors cannot fully explain the difference [122–125]. Sexual transmission is the most common route of infection worldwide, and accounts for the majority of infections in developing countries [126]. It has been found that male to female transmission is more likely to occur than female to male transmission [122,127,128]. Understanding the mechanisms of HIV transmission and its risk factors is crucial in order to identify preventive interventions.

Genital immunology and transmission of HIV

Heterosexual transmission is the primary route of HIV infection in sub-Saharan Africa [129,130], and the genital mucosal tissue is the major entry point for the virus [131] (Figure 6). It has been estimated that worldwide, 30 – 40 % of all new HIV infections in women occur through vaginal intercourse [130]. The risk of male to female HIV transmission has been found to be 0.1-1 % per exposure [132–134]. However, the risk is higher when co-infections are present, and a meta-analysis found that the presence or history of genital ulcers in either member of a couple increased the infectivity per sexual act 5.3 times [134].

The mucosal transmission of HIV is still not completely understood [135,136]. Gp120, exposed on the surface of the HIV virus, binds the T-lymphocyte receptor CD4. This binding changes the viral envelope in a way that makes co-receptor binding, usually to the chemokine receptor CCR5, possible. This interaction leads to fusion of the virus with the cell and entry of the virus [137]. The variant of HIV using the co-receptor CCR5 is preferentially transmitted sexually [138,139]. The importance of CCR5 for HIV transmission is shown by the protection to HIV infection in those homozygous for the defective D32-CCR5 allele who therefore lack functional CCR5 receptors [140]. The most famous example illustrating the crucial role of CCR5 is the so-called "Berlin patient", an HIV-positive man who developed acute myeloid leukaemia. After stem cell transplantation from a donor homozygous for the defective CCR5 allele, HIV has not been detected in the patient [141,142]. Individuals who are heterozygous for the mutation have been found to be partially resistant to HIV infection [140].
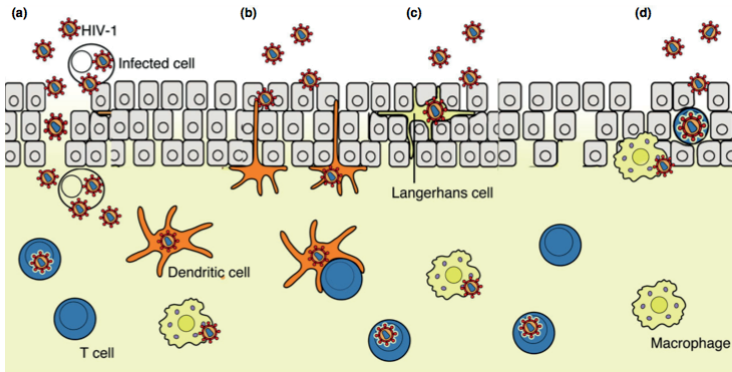
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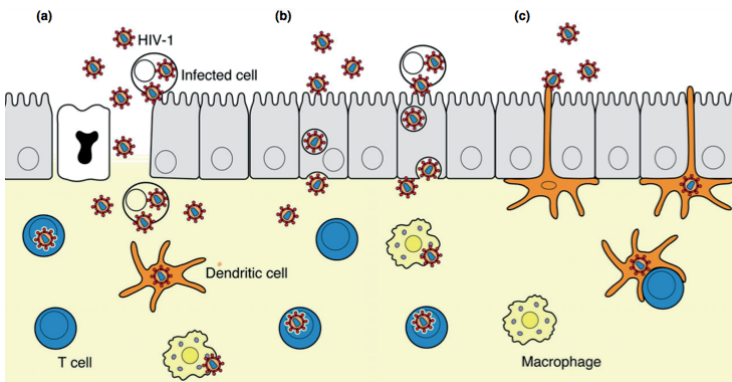
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Figure 6. HIV transmission in the female genital mucosa.

1. Pathways of HIV entry in the vaginal and ectocervical mucosa: a) Disruption of the mucosa by trauma or infection, ulcerations and erosion provide direct access to target cells. b) Dendritic cells at the epithelial/lamina propria interface capture virus and migrate into the lamina propria or further to draining lymph nodes. c) Langerhans cells may take up virions that enter the stratified epithelium. d) HIV directly infects CD4+ T-cells and macrophages within the squamous epithelium.

2. Pathways of HIV entry in the mucosa of the endocervix: a) Disruption of the mucosa and direct access of the virus to target cells. b) Columnar epithelial cells endocytose and transcytose virus across the epithelium into the lamina propria. c) Dendritic cells in the lamina propria extend processes between epithelial cells, capture virus, migrate into the lamina propria or to lymph nodes and trans-infect target mononuclear cells. (Adapted from Shen et al. 2014 [136])

Transmission is most likely possible both in the upper and lower genital tract [130,135,143]. Still, the cervix and especially the endocervix are the suggested sites of most HIV acquisition during heterosexual sex. The mucosal immune milieu is a critical determinant of HIV transmission [144,145]. It is likely that a sufficient number of HIV target cells are needed for transmission, and an intact epithelial barrier is probably one of the most important factors preventing mucosal HIV infection [146]. T-cells and antigen presenting cells (APC) are present in the cervical and vaginal mucosa, but the region where the ectocervix transforms into the endocervix may have enriched CD4 T-cell and APC populations, and may therefore be particularly susceptible to HIV infection [130,147–149]. The endocervix also contains immune cells, and these numbers increase when an STI is present [147,150]. Primary HIV-susceptible cells are situated under the genital epithelium, often clustered just beneath the basal membrane [130,151]. HIV target cells expressing HIV co-receptors have been found in the dermal papillae in the ectocervical mucosal epithelium [152,153].

The cells most likely to be infected in the female genital tract are resting and activated CD4 T-cells, dendritic cells or macrophages, all of which have been shown to be susceptible to HIV in vitro [139,154–157]. The vaginal macrophages are likely to be recruited from blood monocytes [157] and are more permissive to HIV infection than those from other tissues [158]. Genital dendritic cells express dendritic cell-specific intracellular adhesion molecule-3 grabbing non-integrin (DC-SIGN) and can present the virus to CD4 cells in the submucosa or regional lymph nodes [139]. Furthermore, the Th17 subset of CD4 T-cells in the cervical mucosa may be especially susceptible to HIV infection [159]. Dendritic cells in the endocervix and most T-cells and macrophages in the cervicovaginal mucosa express CCR5 [160,161].

Current data suggest that HIV has at least two routes to penetrate the genital epithelium and reach lymphoid tissues: by trans-epithelial migration of infected Langerhans cells [162,163], or by access to the lamina propria through loss of epithelial integrity resulting in direct infection of lymphocytes, dendritic cells and macrophages [163,164] (Figure 6).

The quantity and composition of HIV-susceptible cells in the mucosa may influence the probability of infection [165,166]. In cervical biopsies, the susceptibility to HIV was found to be associated with the number of HIV-susceptible cells and the expression of co-receptors [166]. Furthermore,

the density of CCR5 on T-cells has been shown to be associated with both the efficacy of HIV entry and viral replication [167,168].

The genital and blood compartments may be immunologically different, and local immune responses are of great importance for HIV susceptibility [169]. A higher proportion of genital mucosal T-cells expresses CCR5 and activation markers compared with blood [130,170,171]. It has been shown that mucosal samples from rhesus macaques, including samples from the cervix, have higher proportions of CD4, CD8 and CCR5 positive cells than blood samples [172]. Immune activation of resident immune cells is likely to increase HIV transmission [164,173].

Behavioural and biological risk factors for HIV

In a large South African study, Pettifor et al. found that young women were significantly more likely to be infected with HIV than young men [123]. Older partners, a high number of sexual partners and inconsistent condom use were also factors associated with HIV infection [123]. In a study from KwaZulu-Natal, both prevalence and incidence of STIs were geographically clustered and overlapped with HIV clusters [174]. These clusters were characterised by young age, not married or cohabitating couples and multiple partners [174]. Furthermore, it has also been shown that age at sexual debut, alcohol consumption and education level is associated with increased risk of HIV infection [133,175,176].

Urbanisation, wars, poverty, sexual abuse and other socioeconomic factors may render many young African women at risk of HIV [177,178]. Risk behaviour could be a consequence of women's position in the society where they often have little control of their sexual life [177]. It is likely that the economic and political history in South Africa has been of importance for the massive spread of HIV in this country. The migrant labour system has disrupted stable sexual relationships. In addition, access to STI treatment has been limited, especially in disadvantaged groups. Urbanisation, which has been shown to be associated with higher HIV prevalence, is also seen to a large extent compared with many other African countries [120].

A high HIV prevalence in young women have been reported, even with only one sexual partner and/or few episodes of sexual intercourse [122]. The male-female discrepancy in HIV prevalence is likely to be caused, at least partly, by greater susceptibility to HIV in women than in men [122]. HIV transmission is an inefficient process, and a number of biological factors have been suggested as contributing factors to the large variation in HIV prevalence between different areas [124]. Biological factors such as viral load in blood and genital secretions and the number and frequency of HIV target cells are likely to be of significance for HIV transmission [124]. Other biological factors of importance may be: viral factors (Clade C dominates in southern Africa and India), genetics (homozygosity for the defective CCR5 allele is found more often in people of European descent), co-infections (e.g. schistosomiasis) and systemic and mucosal immune factors associated with HIV transmission (increased HIV replication and activation of HIV target cells may be due to genetic differences or co-infections) [124]. Also cervical ectopy may increase the risk of HIV infection in young women [177,179]. One of the suggested mechanisms is the observation of a higher percentage of monocytes and CD4+CCR5+ cells in the genital tract in women with ectopy [180]. Hormonal contraceptives, especially injectable contraceptives, have also been suggested to increase the risk of HIV acquisition and/or progression. When comparing rates of HIV acquisition in women using or not using hormonal contraception, an adjusted hazard ratio of 1.97 was found in a large study of HIV-serodiscordant couples [181].

HIV and sexually transmitted infections

Numerous studies and meta-analyses have confirmed the association between recent STIs and both HIV acquisition and transmission, but this association varies between organisms and populations [134,182]. Genital ulcer disease [183], bacterial vaginosis [184], *Trichomonas (T.) vaginalis* [185,186] and HSV [187] have been suggested to increase HIV susceptibility. Still, it has been disappointingly difficult to prove the effect of STI treatment on HIV incidence [128,188]. One early study in Tanzania showed a large reduction in HIV incidence after STI treatment [189], however several large randomised controlled studies have later failed to show the same impact [190,191]. None of the large intervention studies included schistosomiasis treatment [192]. The population-attributable fractions, or new HIV infections attributable to STIs, decrease during an HIV epidemic,

and the effect of intervention may therefore vary in different phases of an epidemic [191]. Further, individuals with STIs may have co-infections including viral diseases that are not treated in regular syndromic treatment protocols [191]. However, mathematical modelling has shown that STI treatment may be an important and cost-saving HIV prevention strategy in most populations, also where the HIV epidemic is generalized [193]. Given the large variation in prevalence in different populations, it has been argued that preventive measures must be adjusted to the local context and start before the age when HIV prevalence starts to rise dramatically [194].

Sexually transmitted infections may increase the risk of HIV acquisition [182,183,195] through several overlapping mechanisms: 1) via direct, physical compromise of the normal epithelial barrier as a result of mucosal ulcerations and enhanced viral access to susceptible target cells in the submucosa, 2) by altering the mucosal environment and/or levels of innate immune proteins, 3) by increasing the susceptibility to additional genital co-infections, 4) by increasing the influx of activated HIV target cells to the mucosal site of HIV and 5) by impairing systemic HIV immune control and increased levels of HIV viral levels in blood and genital tract [129,195,196]. The latter factor could also result in increased risk of HIV transmission to an HIV-negative sexual partner.

Genital HSV-2 infection has been associated with a threefold increase in cervical CD4 T-cells expressing CCR5 [196]. Furthermore, cells isolated from genital tissue biopsy samples or peripheral blood from women with primary or secondary syphilis or HSV-1/2 infection all had significantly increased numbers of CD14 cells expressing CCR5 on the cell surface within tissue obtained from genital ulcers [197]. Patients with primary and secondary syphilis also had increased CCR5 surface expression on monocytes isolated from non-ulcerated tissue [197], and *T. pallidum* has been found to induce CCR5 expression on monocytes in blood in vitro [198]. An increased number of macrophages in the cervical epithelium has also been observed in women with cervicitis [199].

Female genital schistosomiasis and HIV

Infections with *S. haematobium* and HIV show a geographical overlap in Africa [200] (Figure 7). Furthermore, a recent analysis of *S. haematobium* and HIV prevalence in sub-Saharan Africa showed that the two infections correlated on a country level [201].

A possible relationship between HIV and FGS was suggested in the 90s [202], and since then two clinical cross-sectional studies have shown an association between the infections [2,203] (Table 1). In the Zimbabwean study, colposcopic gynaecological examinations were used to diagnose FGS. In the other study, Downs et al. found an odds ratio of 4 for having HIV if schistosome ova were found in urine or genital specimens [203]. The average age of the study participants in these two studies from Zimbabwe and Tanzania was 34 (mean) and 30 years (median), respectively.
In a later publication from Tanzania, CAA testing for *S. mansoni* infection was performed and an OR of 3.9 was found for being HIV infected given a positive test [76]. Although there seems to be epidemiological evidence of an association between schistosomiasis and HIV, there is a paucity of publications on the biological mechanisms behind the association.

Author	Study group	Country	Association between HIV and FGS
Kjetland et al. 2006[2]	527 women 20 – 49 years old	Zimbabwe	Adjusted OR = 2.9; 95% CI: 1.11–7.5, p = 0.030
Downs et al. 2011[203]	457 women 18 – 50 years old	Tanzania	OR = 4.0, 95% CI: 1.2–13.5

Table 1. Clinical studies investigating the association between urogenital schistosomiasis and HIV.

Several studies have discussed the cost of anti-schistosomal treatment based on the association between HIV and FGS and concluded that mass treatment for schistosomiasis is likely to be cost-effective for the prevention of HIV [204–207]. Mathematical models of disease dynamics show that treatment of HIV co-factors, such as schistosomiasis, may slow or even reverse the spread of HIV infection [208]. Mass treatment of schistosomiasis could therefore potentially have major benefits for African women [1].
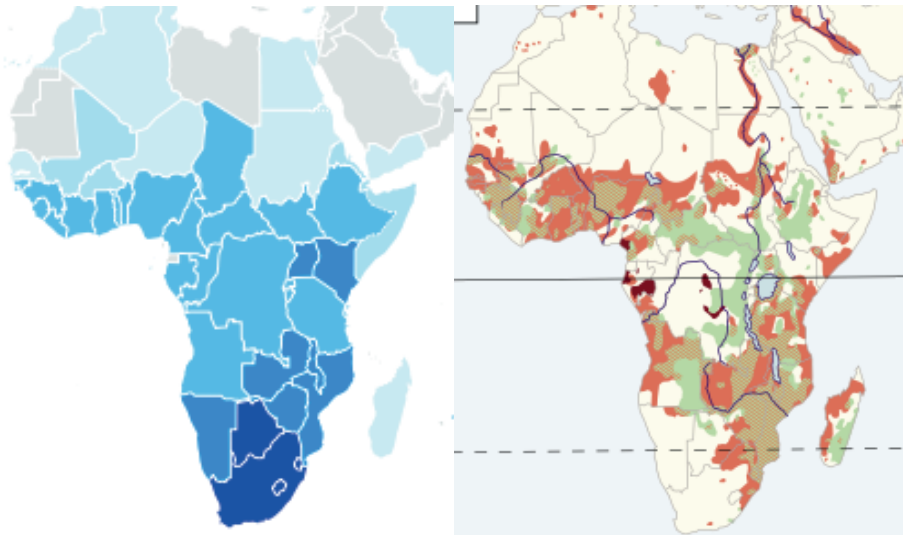
Figure 7. Maps showing distribution of HIV and schistosomiasis infection. Distribution of HIV infection prevalence by country (left, darker blue indicates higher prevalence) and *S. haematobium* (orange, right) in Africa. (Adapted from UNAIDS 2013 [114] and Gryseels et al. [15]).

Mucosal schistosomiasis immunology and HIV

Monkeys infected with *S. mansoni* have been found to be more susceptible to mucosal HIV infection than monkeys without schistosomiasis [209]. Schistosomiasis-positive macaques who were exposed to the HIV virus intra-rectally were more likely to become infected, while this difference was not found when the animals were exposed intravenously [210]. The authors therefore concluded that the increased susceptibility to HIV induced by schistosomiasis is likely to

be at the mucosal level [210]. Immune cells situated around schistosome eggs in the genital mucosa may provide an entry route for the HIV virus [211] (Figure 8). HIV in semen has been hypothesised to attain easy access to deeper genital cell layers in women with genital schistosomiasis, parallel to mechanisms observed in women with STIs. The virus may enter through the friable epithelium or broken blood vessels, and lesions might be present before the woman becomes sexually active [66,107,212]. Increased vascularity and contact bleeding might also contribute to increased susceptibility to HIV in women with FGS [213].
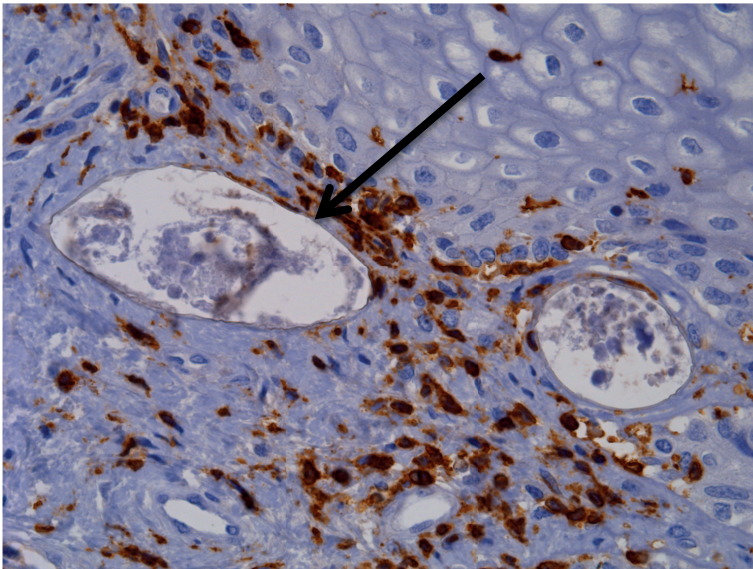
Figure 8. Biopsy from the female genital mucosa. Section of biopsy showing *S. haematobium* egg (arrow) surrounded by CD3+ T-lymphocytes (brown). (Photograph by Peter M. Jourdan)

A histopathological study showed higher numbers of HIV target cells, both CD4 T-cells and macrophages, surrounding schistosome ova in cervical biopsies [211]. Calcified ova were surrounded by the highest densities of genital CD4 T-cells, while macrophages surrounded viable schistosome ova [211]. A recently developed mouse model for *S. haematobium* has shown that granulomas rich in HIV target cells form around *S. haematobium* eggs in the bladder as well as in the vaginal wall [214,215].

Systemic schistosomiasis immunology and HIV

Although the influence of co-infections, such as parasitic and helminthic infections, on the HIV epidemic has been discussed for years, many questions remain unanswered [124,216,217]. It has been suggested that schistosomiasis not only causes increased HIV susceptibility, but also lead to rapid progression of the infection and higher risk of HIV transmission [200,218]. Schistosomiasis infection could therefore exacerbate the progression of HIV infection once established [219].

In patients infected with schistosomes, immunological alterations that may facilitate rapid replication of HIV in the systemic circulation has been observed [66,220]. HIV replicates preferentially in Th2 cells, which may be induced by helminth infections [221,222]. Peripheral blood mononuclear cells (PBMCs) from HIV-positive patients with schistosomiasis showed decreased proliferation and decreased Th2 –like cytokine production compared with HIV-negative patients with schistosomiasis. This observation supports the hypothesis that HIV has a predilection for Th2 cells [223]. Cells from individuals infected with schistosomiasis, intestinal helminths or filariasis have been shown to be more susceptible to HIV infection in vitro [224–226]. The immune response to helminths and the following activation of Th2 type cells may therefore provide an optimal milieu for HIV infection and progression to AIDS [227]. Furthermore, in a Kenyan study, individuals infected by *S. mansoni* had a higher level of HIV co-receptor expression on their mononuclear cells in blood than cured individuals [228].

Schistosome infection may lead to increased immune activation, which may increase viral replication [23,224]. In monkeys, it has been shown that SHIV replication was up-regulated in animals co-infected with *S. mansoni* [118]. The consequence of increased HIV replication in co-infected persons may be more viral genotypic heterogeneity, causing increased CD4 T-cell loss [23,227,229–231]. Plasma HIV RNA has been found to be closely related to genital tract HIV RNA levels [232], and genital inflammation has been shown to increase the HIV viral load in cervicovaginal secretions [233], both factors that may lead to increased transmission from the HIV-infected person.

In a study from Zimbabwe, individuals with schistosomiasis with or without HIV were randomized and received praziquantel either at inclusion or after three months [219]. Those who received treatment at baseline had a significantly lower increase in plasma HIV RNA load than those who received treatment after three months (mean plasma HIV RNA difference between baseline and follow-up for the groups: -0.21 $\log_{10}$ copies/mL [95% CI, -0.39 to -0.02 copies/mL]; p=0.03) [219]. Other studies investigating the effect of helminth treatment on HIV viral load have not shown a similar effect, or even a transient increase in HIV viral load [21,234].

In summary, these studies indicate that schistosomiasis may increase HIV replication and progression, and possibly facilitate transmission due to a higher viral load.

Urogenital schistosomiasis and CD4 count

The HIV virus invades and kills CD4 T-cells, which are important markers for monitoring the progress of the infection. Helminth infections may trigger immune responses that have been hypothesized to decrease CD4 T-cell numbers [235].

The mean CD4 cell count differs between populations and is higher in women than in men [236–240]. Studies on the effects of helminths on CD4 levels have shown conflicting results [217,219,241–243]. Treatment of helminth co-infection may slow the decline in CD4 count in HIV-positive individuals, possibly due to suppression of the Th1 response by helminths [244]. In Zimbabwe, Kallestrup et al. included both HIV-positive and -negative persons with schistosomiasis in a randomized controlled trial, and found that early praziquantel treatment was associated with an increase in CD4 cell count [219]. However, a Ugandan study on HIV-infected persons' CD4 cell counts did not find a difference between the *S. mansoni* positive and negative groups [241]. A review article concluded that further studies are needed in order to determine the effect of anti-helminthic treatment on CD4 counts [245]. Since the CD4 count at HIV seroconversion may be of importance for the progression of HIV infection, the interaction between helminths and CD4 levels warrants further investigation [246].

Cervical ectopy

Ectopy occurs when the columnar epithelium, normally found in the endocervical channel, extends out onto the ectocervix (Figure 9 and 10). It is a normal finding in young women, and after adolescence the process of squamous metaplasia covers the columnar epithelium and the cervical ectopy decreases [247,248]. It has been shown that approximately 50 % of 13 – 15-year-olds have cervical ectopy, and the percentage with ectopy increases during adolescence before decreasing during childbearing age [247,249].

The ectopic zone is constituted of only one layer of columnar epithelial cells in contrast to the stratified squamous epithelium covering the rest of the ectocervical surface. The columnar epithelium is thin, fragile and in close contact with the underlying vascular stroma [250]. The area covered by columnar epithelium may therefore be less protected against viruses on the surface [247].

It seems biologically plausible that ectopy may be a risk factor for HIV infection. More HIV target cells have been found in columnar than in squamous epithelium [250,251], and higher levels of HIV viral shedding in the genital tract have been correlated to ectopy [252,253]. The possible relationship between contraceptives and cervical infections has also been hypothesized to be mediated by ectopy, as hormonal contraceptives might increase cervical ectopy [254,255].
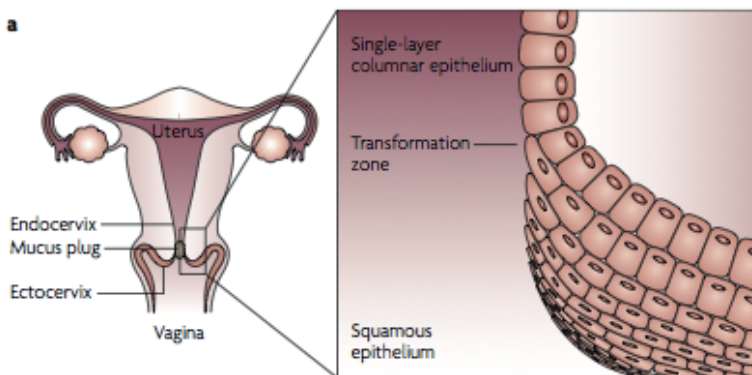
Figure 9. Female genital tract. Figure showing an overview of the female genital tract (left) and the transition from columnar to squamous epithelium (right). (From Hladik et al. 2008 [130])
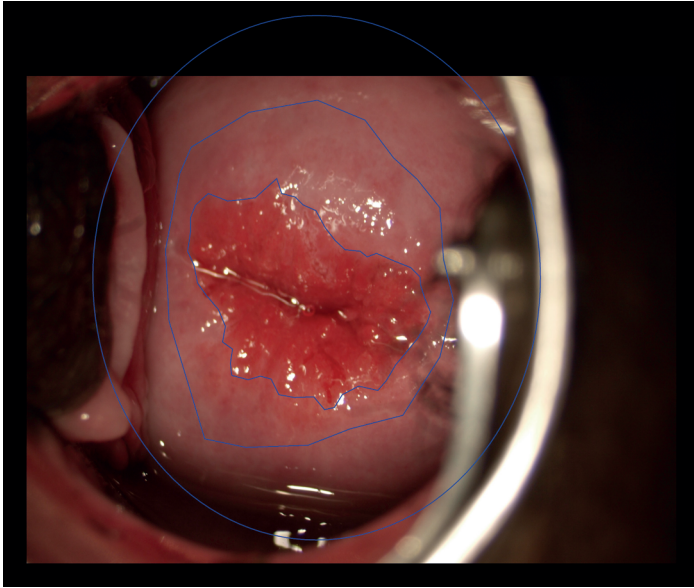
Figure 10. Colposcopic image of the cervix. The inner line surrounds the columnar epithelium (ectopy, see Discussion; Diagnosis of ectopy). (Photograph by Elisabeth Kleppa)

Ectopy has been hypothesized to be associated with an increased risk of STIs including HIV [250,256,257]. A larger area of cervical ectopy and/or transformation zone during adolescence has been suggested as one factor behind the increased susceptibility to HIV in young women [144,177]. In a study from Kenya, the presence of cervical ectopy was an independent predictor of HIV infection in women [256]. But although some studies have found a strong association between HIV acquisition and cervical ectopy, others have not been able to show the same tendency [248,250,258,259].

Some epidemiological studies have found an association between ectopy and *Chlamydia trachomatis*, human papilloma virus and cytomegalovirus [250,260–263]. It is difficult to determine cause and effect in these associations, but women with ectopy were found to be more likely to contract *C. trachomatis* infection [264]. *C. trachomatis* is an intracellular bacterium that resides in columnar epithelial cells, making cervical ectopy a favorable condition for infection. Gonococcal infection has not been found to be associated with cervical ectopy [264,265]. To our knowledge, the relationship between FGS and ectopy has not been studied previously.

HYPOTHESES AND RESEARCH QUESTIONS

The main objective was to study HIV target cells and cervical ectopy in young South African women living in an area where urogenital schistosomiasis is endemic.

1.   It has been hypothesized that FGS may increase the risk of HIV acquisition. We therefore wanted to explore the association between female genital schistosomiasis and the density of potential HIV target cells and expression of the HIV co-receptor CCR5 in blood and cervical cytobrush samples, and to evaluate the effect of anti-schistosomal treatment on these cell populations.

Research questions:
- Is FGS associated with the proportion of HIV target cells and CCR5 expression in blood and cervical cytobrush samples?
- Does anti-schistosomal treatment influence the proportion of HIV target cells and CCR5 expression?

2.   It has been hypothesised that cervical ectopy may increase the risk of STIs including HIV. In paper 2, we therefore investigated the association between cervical ectopy, genital schistosomiasis and sexually transmitted infections, including HIV.

Research questions:
- Is cervical ectopy associated with STIs including HIV?
- Is cervical ectopy associated with FGS?

3.   It has been hypothesized that urogenital schistosomiasis may exacerbate the progression of HIV and that HIV may alter the manifestations of schistosomiasis. We therefore studied the association between urinary and genital *S. haematobium* infection and CD4 cell counts in HIV-negative and -positive women.

Research questions:

- Is urogenital schistosomiasis associated with the number of circulating CD4 cells in HIV-negative and -positive women?
- Does HIV status and/or CD4 cell count influence egg excretion in women with urinary schistosomiasis?

MATERIALS AND METHODS

Overview of the project

After working for several years with applications for funding and ethical approval, Eyrun Kjetland, Myra Taylor and their co-workers were ready to start the research project in 2009. A research site in KwaZulu-Natal was chosen, an area endemic of HIV and schistosomiasis. The site was close to laboratory facilities in Durban, but still rural and similar to typical rural sites in other developing countries in sub-Saharan Africa. The main aim of the project was to determine whether there is an association between HIV and FGS. In addition, several sub-studies looking at various aspects of FGS and the relation to co-infections were planned, including the ones presented in this thesis. These sub-studies are all part of a high school cohort, which will be referred to as the total cohort in this thesis (Figure 11).
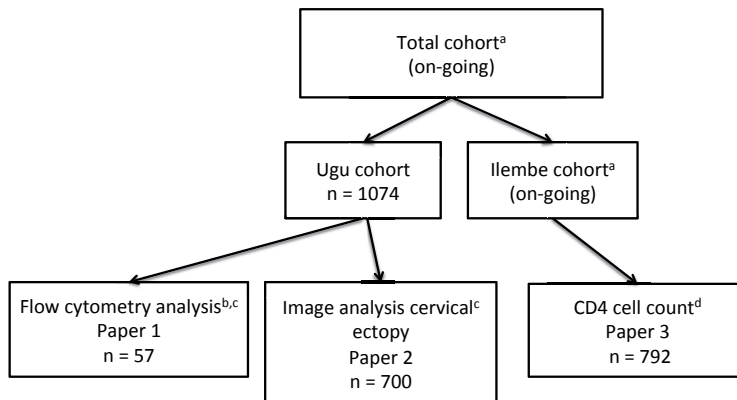
Figure 11. Inclusion of the participants in the different sub-studies presented in this thesis.

[a]Number of participants not presented as the study is on-going.

[b]The number of women included in the Ugu cohort at the time this study was done was 870.

[c]There were 53 women who were included in the cohorts of both paper 1 and paper 2.

[d]Number of women recruited between May and October 2013.

The preparations for this project have been extensive, building a research facility in a rural area was challenging and time-consuming work. In 2010, the research clinic was set up with examination rooms, a waiting area and freezers with generator backup to prepare for the frequent power cuts in the area. Equipment, gynaecological chairs, reagents and medication (anti-schistosomal treatment and STI treatment) were bought, and protocols and questionnaires were written, translated and amended several times. In parallel, the community liaison was a continuous process, with numerous meetings with local chiefs, headmasters, departments, hospitals and supporting professionals such as psychologists, school nurses and laboratory professionals.

Research staff were employed and trained locally. In total, more than 50 persons, mostly women, were employed at different stages of the project. This included data enterers (2), nurses (7), HIV counsellors (3), field and office managers (3), drivers (4), a logistician and research assistants (27). The research assistants, mostly young women, had no formal education. They were trained by, amongst others, the project doctors Elisabeth Kleppa, Kristine Lillebø and Eyrun Kjetland (EK, KL, EFK), Professor Myra Taylor, experienced research assistant Pumla Mkhiva and visiting collaborating senior scientists. The research assistants' tasks varied, they would interview the study participants using the study questionnaire, go through the individual informed consent procedure, assist and translate from isiZulu to English for the doctors in the investigation room, do urine microscopy, data entry, hold school meetings (see Enrolment chapter) and entertain the study participants in the waiting area. The project nurses were responsible for STI treatment at the clinic, but also helped with other tasks when necessary. HIV counsellors were hired to offer HIV counselling and testing to the study participants.

In 2010, the inclusion of women from high schools in Ugu district started. The women were brought from their school, sometimes several hours away from the research clinic, and returned to school (or to their house if the school was closed) at the end of the day. We could not recruit study participants during holidays when the schools were closed or during exam periods when the women were unable to be away from school. We also had to stop working during a school strike that lasted for more than five weeks. Still, we were able to recruit the intended number of women to the study during 2011 and 2012. In 2012, a second research clinic was opened in Ilembe district, north of Durban, in order to reach the targeted number of participants. By doing so, we were able to reach a larger area without transporting the included women too far. The data presented in this thesis were

collected between 2011 and October 2013. The project is still on-going and objects, methods and further results will be presented in future publications and theses.

Areas of contribution

This PhD-project started in 2010 and consisted of several phases with different tasks and responsibilities. The candidate (EK) spent the first 2.5 years in South Africa doing fieldwork, and also went back for shorter periods of time after returning to Norway. During the first year in South Africa, Eyrun Kjetland and the candidate planned the clinical work and prepared the study site. This involved various tasks such as finding a site for the research clinic, preparing questionnaires, employing more research assistants and nurses and ordering equipment, medication and laboratory reagents. The research clinic with investigation and interview rooms, a waiting area and a small laboratory were prepared (see photographs in Appendices). Questionnaires were written in English before being translated into and back-translated from isiZulu. For the clinical examination, an electronic form was made where the entered information was stored directly on a local server in accordance with EU directives (see data collection tools). Images from the colposcopic investigation were also stored on this safe server. In order to prepare the community for the planned study, we had several meetings with the local Departments of Health and Education, the local chiefs in the districts and principals and school governing bodies of the high schools in the area.

When the first study participants were recruited in 2010, the candidate performed the clinical investigations together with another female doctor who joined the project in 2011 (KL). This involved preparing the women for and conducting the gynaecological colposcopic investigations. The doctors (EK, KL, EFK) also headed the first part of the clinical follow-up after the STI results were ready and made sure that the women received the treatment they needed from the study nurses. The candidate also took part in the recruitment of young women and participated and gave talks in numerous school meetings with students, parents and teachers. In the early phases, our presence as clinicians was particularly important, as the recruitment process was continuously evaluated and adjusted and the research assistants were still undergoing training. We had to make sure that the correct information was conveyed to the study participants and teachers, that the informed consent

procedure was done correctly, and that practical procedures (giving the participants a study ID, separating pages with ID and name, storage of forms and questionnaires, arranging transport, etc.) were coordinated. As the project evolved, the field workers were able to work more independently, a higher number of study participants came to the clinic every day and the doctors spent the majority of their time in the clinic. On a normal day, 20 women came to the research clinic.

In a large project like this, an overwhelming number of practical tasks that must be taken care of in order to ensure efficient and high-quality work. The management was also responsible for the human resources aspect, from salaries to the employees' safety and wellbeing. Eyrun Kjetland was the manager of the project when present. However, in her absence, EK was the deputy coordinator, leading the project on site for approximately nine months, in close collaboration with the local field manager.

In 2011 and 2012, the candidate planned and headed a nested study (paper 1). Women with or without genital schistosomiasis were included and genital and blood samples collected. The clinicians (KL and EK) collected the samples at the clinic in the morning. Then, EK and Sipho Zulu (Masters student at University of KwaZulu-Natal (UKZN)) brought them to the laboratory at the Durban medical school and analysed them in the afternoon together with collaborating scientists from UKZN. EK and Veron Ramsuran (UKZN) performed the gating and subsequent analyses.

Towards the end of 2012, the candidate returned to Norway and spent most of her time doing data cleaning, analysis, writing scientific papers, attending mandatory PhD-courses and international meetings and conferences. Together with PhD candidate Sigve Holmen, the image analysis for paper 2 (cervical ectopy) was planned and performed using images that had been collected in the Ugu cohort.

Study sites

The study was conducted in Ugu and Ilembe districts in KwaZulu-Natal, South Africa. South Africa has the largest HIV epidemic in the world and KwaZulu-Natal is one of the provinces hardest hit by the infection [178]. Among antenatal women in South Africa, 29.5 % were HIV-positive in 2011 [119]. Of the general population (15-49 years), 24.7 % were HIV-positive in KwaZulu-Natal in 2011; this is the highest prevalence in the country [119].

The Ugu district is one of the districts hardest hit by the HIV epidemic in KwaZulu-Natal; 41.7 % of antenatal women were HIV-positive according to a survey from 2011 [119]. In Ilembe, the same study showed an HIV prevalence of 35.1 % in antenatal women [119].

Figure 12. Topographic map of South Africa. Main rivers (blue) are scattered in large parts of the country, including the East Coast. Magnification (right) of KwaZulu-Natal with the blue arrow pointing at Ilembe district and the red arrow pointing at Ugu district. (From United Nations [266]) and Wikimedia Commons / Htonl) .

The Ugu district is located at South Africa's eastern coastline and covers an area of 5866 km^2 [267] (Figure 12). In 2006, approximately 704 000 people lived in the district. The majority speak isiZulu as their first language and most (98 %) live in rural areas. In 2011,the official unemployment rate in Ugu was 30 % [267]. Ugu is one of the poorest districts in the country, and according to a survey from 2001 62 % of the households did not have access to running water [268]. There are four district hospitals, 37 clinics and 40 mobile clinics serving this district [268].

The Ilembe district is also coastal and is located north of Durban. The district covers an area of 3260 km^2. The district is mostly rural and similar to Ugu district with a total population of approximately 606 000 (2011), mainly isiZulu speaking [269].

KwaZulu-Natal has a tropical to subtropical climate with hot and wet summers (November – February) and cooler and dry winters (June – August) [270] (Figure 13). In large parts of this region, the temperature is sufficient for schistosomiasis transmission. There are numerous big rivers in this area, used for recreational purposes, laundry and for fetching water for households (Figure 12).
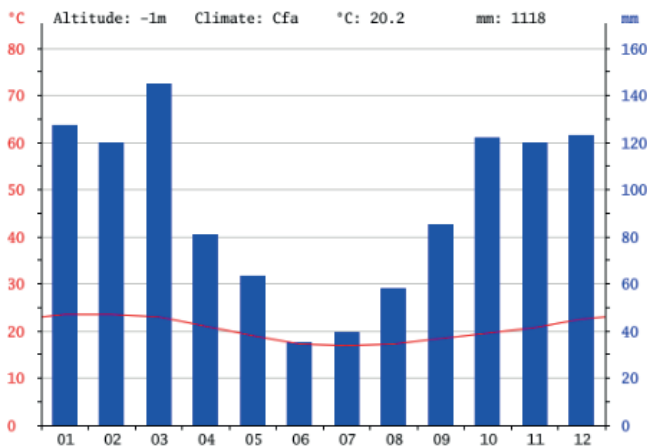
Figure 13. Average yearly temperature and rainfall in Port Shepstone, Ugu District, KwaZulu-Natal. Average yearly rainfall per month in mm (right) and average temperature in C° (left). (From http://en.climate-data.org)

Enrollment

Almost three quarters of South Africans between the age of 7 and 24 years attend school [125]. All the participants in the sub-studies of this thesis were part of a cohort of high-school students in rural KwaZulu-Natal, South Africa (see photographs in Appendices). Women were included in the study from 2010 to 2013. There are a total of 267 high schools in the chosen districts; we included women from 31 schools (Ugu, papers 1 and 2) and 26 schools (Ilembe, paper 3). The larger high schools situated below 300 meters above sea level were eligible and were randomized into the larger main project. The details on the randomization process will be presented with the results of the main on-going project in a forthcoming publication.

The inclusion process of the study participants consisted of several stages. First, the schools were contacted and a meeting with the principal or a representative was arranged. The project field manager, sometimes accompanied by one of the project doctors, was normally in charge of this meeting. During the meeting the study was explained and the school received copies of permissions from the Departments of Health and Education. If the schools wanted, an information meeting for parents and guardians was held.

The next step was an information meeting for the female students above the age of 16 years. The information meeting was coordinated as an assembly meeting or class-by-class. At the end, a short screening questionnaire was given to all, filled in by the woman, and collected at the end of the meeting (see Appendices). Both the field manager and several research assistants participated in order to make the process as efficient as possible. The questionnaire included questions regarding interest in participation, water contact and sexual experience.

At a later stage, eligible women were contacted. A trained research assistant or nurse explained and read the consent form (in isiZulu) to each woman who signed if she was willing to participate in the study (see Appendices). The informed consent procedure took place at the school. Then, in agreement with the school, a date for the visit to the research clinic was decided.

A female driver transported the young women in small groups to the research clinic where they received information followed by the collection of samples (blood and urine), an interview and clinical gynaecological examination. Lunch was provided in the waiting area. Women with genital symptoms were treated at the research clinic according to the South African syndromic treatment guidelines [271]. Family planning was also offered by the project nurses.

Months after, when the STI results were ready, the women were offered medical treatment and information about the diagnosed STIs at a follow-up visit.

Inclusion and exclusion criteria

In the main project, sexually active women above the age of 16 were included after the screening. This age limit was chosen due to the fact that women had to be sexually active in order to participate in a gynaecological examination. Under the age of 16, the proportion of sexually active women was likely to be lower and including them would therefore have led to a lot of unnecessary screening and disturbance of the schools. We therefore included women from the highest grades in high school (grades 10 to 12). Due to the fact that some women come back to school after dropping out (for example after giving birth), we had an age range from 16 to 34 years.

At the clinic, blood and urine samples were collected from all and an interview (see Data Collection Tools and Appendices) was done. Women who were found to be pregnant or virgins at the clinic did not go through a gynaecological examination.
Of the women who came to the clinic, 20 % did not undergo the gynaecological examination (see flowchart paper 2). The most common reasons why the gynaecological exam was not performed were that the woman was nervous or did not feel ready (47 %), was a virgin (25 %) or that she was pregnant (14 %).

For the different sub-studies there were additional inclusion and exclusion criteria (for details and numbers, see also fig. 2, paper 1 and fig. 1, paper 2).

Paper 1:
Inclusion: FGS-positive cases were included based on findings of pathognomonic sandy patches by photocolposcopic investigation. FGS-negative cases were included if they had no sandy patches and no schistosome eggs by microscopy of urine.
Exclusion: Women were excluded if they reported having HIV, were menstruating or had visible signs or symptoms of an STI. FGS-negative cases were excluded if they had a positive PCR result for detection of schistosome DNA in urine or cervicovaginal lavage. Furthermore, women who were found to be HIV-seropositive, or had cervical sampling incorrectly done, were excluded.

The sample size of this study was limited due to the high costs and large amount of work for each sample analyzed. To our knowledge, this study was the first of its kind and was therefore planned as a pilot study that will hopefully be repeated in a larger sample in the future (see Discussion page 66).

Paper 2:

Inclusion: One image was selected per patient if at least 25 % of the ectocervical surface was visible, the cervical os was visible, a part of the cervical curvature was visible, and the focus was adequate for identifying anatomical landmarks.

Exclusion: Images were excluded if there was heavy inflammation rendering the evaluation impossible or if there was a non-cervical element rendering the extent of ectopy nonevaluable (such as blood, discharge or a medical instrument).

All the images available at the time of analysis that filled the inclusion criteria were analyzed.

Paper 3:

Inclusion: Consecutive females recruited from May - October 2013.

A sample size calculation had shown that in a population with 20% urogenital schistosomiasis (assumed prevalence), we needed to recruit 600 participants in order to detect a difference in mean CD4 cell count of 80×10^6 cells / L with a power of 80% [107].

Gynaecological examination

The gynaecological examinations were performed by trained female medical doctors (EK, EFK, KL). The investigating clinicians were trained in colposcopy for approximately six weeks by a senior medical doctor (EFK) with ample experience in diagnosing FGS. During this period, the co-investigators followed the colposcopic examination on a screen connected to the colposcope. The senior clinician could therefore show the lesions to the observers while investigating the woman. EFK was also present in the investigation room during the clinicians' first three weeks of independent clinical work to ensure that the diagnosis was correct.

In the examination room, a female nurse or research assistant speaking the local language was always present in addition to the medical doctor. If the study participant preferred to speak isiZulu, the research assistant translated the conversation (English and isiZulu). Pregnant women and virgins were not examined. An autoclaved metal speculum was used. Cervicovaginal lavage samples were collected by spraying 10 mL of sterile saline on the cervical surface four times before drawing it back into a syringe and depositing it into tubes for frozen storage. Subsequently, a Pap (Papanicolaou) smear was done. Colposcopic examination of the entire cervicovaginal surface was performed (Olympus OCS 500 Colposcope with a mounted Olympus E420 10 megapixels (Mpx) or a Leisegang colposcope with a Canon EOS 40D 10 Mpx) and images stored. When cell atypia and/or cervical condyloma were suspected, iodine solution was applied in order to classify the lesion according to Reid's colposcopic index.

Data collection tools

An extensive questionnaire was made and used in the study (see Appendices). Questions on STI risk factors (sexual behaviour), schistosomiasis risk factors (water contact), obstetric history and gynaecological symptoms were asked. During the process of making the questionnaire, several sessions with young local women were held, in order to ensure that the questions were culturally acceptable and understandable. The research assistants and project nurses translated the questionnaire from English into isiZulu. The questionnaire was also back-translated to make sure that the translation was appropriate. The gynaecological questions were made by the clinicians in collaboration with an experienced professor in gynaecology with ample experience in tropical medicine (Mathias Onsrud).

The research assistants were trained in interview techniques by the study clinicians and experienced collaborating scientists. The interview was conducted one to one by a nurse or research assistant at the clinic in isiZulu, and the interviewer wrote the answers in English on the printed questionnaire. For quality control purposes, the clinician also checked the questionnaire in the investigation room when the study participant was present and asked additional questions if necessary. The paper forms were labelled with the participants' study ID, and the pages with name and tracking information (telephone number, address, etc.) were separated and stored in locked cabinets at a different location (UKZN, Durban) to ensure anonymity.

After the gynaecological exam, the clinician documented lesion size, type and localization in an electronic examination form. Additional factors such as contact bleeding, observed discharge and testing with acetowhite were also documented. The lesions were recorded as sandy patches (homogenous and/or grainy) with or without the presence of abnormal blood vessels. This information was stored directly on a local server (encrypted and password protected) together with the colposcopic images from the investigation. This information did not contain names or other patient identification data- only study ID number.

Samples (urine, blood, cervicovaginal lavage) were stored at the clinic in freezers (- 80 °C) with a generator backup (see sampling chapter). All samples were labelled with the participants' study ID number.

Sampling

A urine sample was collected from all participants between 10 a.m. and 2 p.m. to ensure optimal schistosome egg yield [272]. Merthiolate-formalin solution (2 %) was added to 10 mL of urine. Urine samples were centrifuged and the whole pellet was deposited on one or more glass slides. Schistosome ova in urine were counted by microscopy [273]. The research assistants collected the urine sample, processed it and did the microscopy. The research assistants who performed this task had no formal education, but had gone through a course of microscopy with an experienced parasitologist at UKZN before commencement of the study. This parasitologist also checked 10 % of the urine samples each week for quality control purposes.

Blood was collected by the project nurses in EDTA tubes for CD4 analyses in addition to serum and plasma for frozen storage (Vacutainer tubes, Becton, Dickinson and Company (BD), Franklin Lakes, NJ, US).

Analysis of sexually transmitted infections

Cervicovaginal lavage samples were analysed by strand displacement assay (SDA) for *Neisseria gonorrhoea* (ProbeTec CT/GT, Becton, Dickinson and Company (BD), Franklin Lakes, NJ, US) and *Chlamydia trachomatis* (ProbeTec CT/GC, BD). PCR was used to detect *T. vaginalis* (in-house PCR, Laboratory of Infection, Prevention and Control (IPC), University of KwaZulu-Natal (UKZN), Durban, South Africa) and *Herpes simplex virus* (in-house PCR, IPC). Syphilis was detected in frozen serum samples using Macro Vue test 110/112 for Rapid plasma regain (RPR), (BD), and Immutrep for *Treponema pallidum* hemagglutination assay (TPHA), (Omega diagnostics Group PLC, Alva, Scotland, UK). HIV testing was done using Bioline Rapid Test HIV (NJ, US) and confirmatory Sensa Tri-Line HIV Test Kit (Pantech, Durban, South Africa). The sensitivity and specificity of the tests are summarized in Table 2.

Sample	Method	Brand name; producer	Infection	Sensitivity and specificity (references)
Urine	Microscopy (ova)		*S. haematobium*	For detection of *S. haematobium* infection: Specificity close to 100 %, sensitivity: variable, estimated: 70 % [274,275]
CVL[a]	Strand displacement assay	ProbeTec CT/GT; BD	*N. gonorrhoea*	Sensitivity and specificity close to 100 % shown in urine and endocervical swab specimens, limited data for CVL [276,277]
CVL[a]	Strand displacement assay	ProbeTec CT/GT; BD	*C. trachomatis*	Sensitivity and specificity close to 100 % shown in urine and endocervical swab specimens, limited data for CVL [276]
CVL[a]	PCR	In-house PCR; IPC, UKZN	*T. vaginalis*	One study has shown sensitivity and specificity close to 100 % in CVL [278]
CVL[a]	PCR	In-house PCR; IPC, UKZN	*Herpes simplex virus*	PCR has been shown to be sensitive in CVL [279]
Serum	Rapid plasma reagin	Macro Vue test 110/112 for Rapid plasma reagin; BD	Syphilis (RPR)	Sensitivity: 73 – 100 %, specificity: 98 % [280,281]
Serum	Treponema pallidum hemagglutination assay	Immutrep; Omega diagnostics Group PLC	Syphilis (TPHA)	Sensitivity > 95 %, specificity > 99 % [282,283]
Serum	Rapid test, antibody detection	Bioline Rapid Test HIV1 / 2 3.0; MSD	HIV	Sensitivity: 100 %, specificity: 99 – 100 % [284]

Table 2. Sensitivity and specificity of STI and schistosomiasis tests.
CVL: Cervicovaginal lavage, BD: Becton, Dickinson and Company, PCR: Polymerase Chain Reaction, UKZN: University of KwaZulu-Natal, IPC: Laboratory of Infection, Prevention and Control.
a See discussion (page 67)

Analysis of CD4, CD14 and CCR5 expression

Blood and cytobrush samples were collected for flow cytometry analysis in a subsample. Blood (3x 10mL) was collected into sterile acid-citrate-dextrose anti-coagulated Vacutainer tubes. The investigation commenced by collecting cervical cells using an endocervical cytobrush (EndoCervex-brush, Rovers, Oss, The Netherlands). The brush was rotated 360° in the cervical os and placed in a transport medium (R10 made of RPMI supplemented with 10 % foetal calf serum (FCS), HEPES buffer, L-glutamine, streptomycin, and penicillin) at room temperature. Blood and cervical samples were transported to the laboratory for processing within four hours. PBMCs (2 x 10^6) were isolated followed by staining and analysis.

Cells were stained for 20 minutes in the dark, first with ViVid LIVE/DEAD (Invitrogen, NY, USA), then with CD3 APC H7 (T-cells), CD4 PerCP Cy 5.5 (T-helper cells), CD8 Qdot (cytotoxic T-cells), HLA-DR- phycoerythrin (PE), CD38 Alexa 700, CD56 PE Cy 7 (natural killer cells), CD14-fluorescein isothiocyanate (FITC) (monocytes/ macrophages) and CCR5 APC (all from BD, San Jose, CA, US). Flow cytometry analyses were done using fresh peripheral blood mononuclear cells. Data was acquired using an LSR II flow cytometer (BD) and FACSDiva software (BD). The data were then analysed using FlowJo software (Tree Star, Inc. version 9). Samples yielding a minimum of 3000 events after gating for CD3$^+$ cells were included for further statistical analyses.

Measuring ectopy on colposcopic images

Obtaining reliable measures of the ectopic area on the cervix is difficult. Most studies done on this topic have relied on visual inspection during the examination; this has been shown to be a less reliable method than computerized planimetry [285,286]. The images from a colposcopic investigation may also contain only a part of the cervix and in the traditional image analysis these photos cannot be used.

One image was selected per patient based on the following inclusion criteria: at least 25 % of the ectocervical surface visible, the cervical os visible, a part of the cervical curvature visible, and focus adequate for identifying anatomical landmarks. The number of images available per patient varied

from 1 to 46, with a median of 4 images per patient. During the measurements, images were excluded if there was heavy inflammation rendering the clinical evaluation impossible or if there was a non-cervical element rendering the ectopy non-evaluable (such as blood, discharge or a medical instrument). The image files from the photocolposcopic investigations were stored using high-quality JPEG compression along with data from the clinical investigation. All measurements were performed on the two-dimensional images using the open source image analysis software ImageJ (U. S. National Institutes of Health) (Figure 14). It allows for a visually guided delimitation of structures and a subsequent measurement of the selected area. We further calculated the ectopy area as a fraction of the total ectocervical area. Furthermore, the images were saved with the measurement overlays intact for later verification and refinement by a senior gynaecologist.

The ectocervical area was delimited by using the cervical os as the centre of an elliptical shape that was manually fitted to conform to the visible cervical boundaries. The original squamocolumnar junction was defined as the outer delimitation of any of the following structures: glandular openings, Nabothian cysts and primary rugae extending from the cervical os. In cases where such anatomical landmarks were absent or too sparse, the original squamocolumnar junction was not measured. Finally, the current squamocolumnar junction (confining the area of ectopy) was defined as a distinct transition of colour from deep red to brighter red: The columnar epithelium is single layered and appears dense red, whereas the transformation zone is covered in squamous epithelium, appearing brighter in colour.

The area constituting the transformation zone was defined as the difference between the area within the original squamocolumnar junction and the area within the current squamocolumnar junction. If the current squamocolumnar junction was not visible (inside the cervical canal), the transformation zone would be equal to the area central to the original squamocolumnar junction. In cases where the original or current squamocolumnar junctions were partially masked by medical instruments, blood, or as a result of the angle of inspection or photographic section, the ectocervix was divided into sectors.
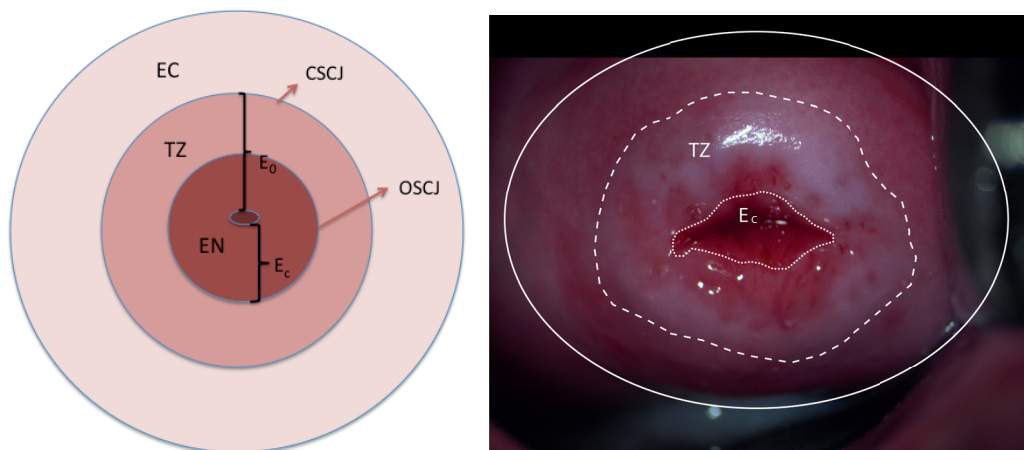
Figure 14. Cervical ectopy. Figures showing the zones on the cervix: EC= Ectocervix, TZ= Transformation zone, EN= Endocervix, CSCJ= Current squamocolumnar junction, OSCJ= Original squamocolumnar junction, E_O= Original ectopic area, E_C= Current ectopic area

(Figure by Sigve D. Holmen and Elisabeth Kleppa)

In order to evaluate the method, two persons measured a random selection of fifty images for analysis of inter-observer agreement (SH, EK; Table 3). One observer also analysed the same fifty images again half a year later, blinded to the results of the first measurement, for analysis of intra-observer agreement.

Images	Observer 1	Observer 2	Observer 2 2nd time
Ectopy ≥ 10 %	13	10	10
Ectopy < 10 %	37	40	40
Kappa, inter-observer	0.831 ($p < 0.001$)		
Kappa, intra-observer		0.750 ($p < 0.001$)	
Ectopy could not be evaluated	0	0	0
Ectopy evaluated in sector	5	3	5
TZ could not be evaluated	12	10	6
TZ evaluated in sector	16	9	17

Table 3. Observation characteristics and inter- and intra-observer agreement.

Statistical analyses

All statistical analyses were performed using IBM SPSS Statistics version 20 (Armonk, NY, USA). A significance level of 5 % was used in all the statistical analyses. In article one, nonparametric statistics were used, as the data did not follow a normal distribution. The Mann-Whitney U test was used to compare the results from the flow cytometry in FGS-positives and -negatives. To compare results from samples collected before and after treatment, the Wilcoxon signed rank test was used. Pearson's chi-square test or Fisher's exact test was used to compare the groups for cervical ectopy and STIs. Univariate and multivariate logistic regression models were constructed to analyse the associations between ectopy and gynaecological pathology.

The CD4 counts in paper 3 followed a close to normal distribution, and the Student's t-test was used to compare mean CD4 counts for women with and without sandy patches, abnormal blood vessels and schistosome eggs in urine. Linear regression was used when investigating the association between mean egg intensity in urine and CD4 count.

Ethical considerations

Research ethical committees in South Africa and Norway approved the study (Biomedical Research Ethics Administration, University of KwaZulu-Natal (Ref BF029/07), KwaZulu-Natal Department of Health, (Reference HRKM010-08) and the Regional Committee for Medical and Health Research Ethics (REC), South Eastern Norway (Ref 469-07066a1.2007.535)) (see Appendices). Furthermore, the Departments of Health and Education in Ugu district, KwaZulu-Natal gave permissions. All participants signed individual, written informed consents (see Appendices). STI treatment was offered in accordance with the South African syndromic treatment protocol and also if the woman tested positive on laboratory analyses. Anti-schistosomal treatment was offered to all by the Department of Health as part of a mass drug administration campaign. Voluntary HIV testing and follow-up was done in accordance with South African guidelines with pre- and post-test counselling. HIV-positive patients were referred to local clinics for follow-up and treatment. South Africa provides universal access to antiretroviral treatment.

At each step (from school meeting to the clinical exam), the woman was reminded that she could withdraw without consequences any time, and treatment would still be available.

RESULTS

Paper 1

Schistosoma haematobium may cause female genital schistosomiasis (FGS), characterized by genital mucosal lesions. There is clinical and epidemiological evidence for a relationship between FGS and HIV. We investigated the impact of FGS on HIV target cell density and expression of the HIV co-receptor CCR5 in blood and cervical cytobrush samples in HIV-negative women. Furthermore, we evaluated the effect of anti-schistosomal treatment on these cell populations.

The study followed a case-control design with post-treatment follow-up, nested in an on-going field study on FGS. Blood and cervical cytobrush samples were collected from 25 FGS-negative and 19 FGS-positive women for flow cytometry analyses. The FGS+ women were given anti-schistosomal treatment, and 14 were seen at the follow-up visit. Urine samples were investigated for schistosome ova by microscopy and polymerase chain reaction (PCR).

In the FGS+ group, 12 (63 %) of the women had *S. haematobium* ova in the urine, while none of the FGS+ women seen post treatment had ova in the urine. FGS was associated with a higher frequency of $CD14^+$ cells (monocytes) in blood (11.5 % in FGS+ vs. 2.2 % in FGS-, p=0.042). Frequencies of $CD4^+$ cells expressing CCR5 were higher in blood samples from FGS+ than from FGS- women (4.7 % vs. 1.5 %, p=0.018). The $CD14^+$ cell population decreased significantly in both compartments after anti-schistosomal treatment (p=0.043). Although the frequency of $CD4^+$ cells did not change after treatment, frequencies of CCR5 expression by $CD4^+$ cells decreased significantly in both compartments (from 3.4 % to 0.5 % in blood, p=0.036; and from 42.4 % to 5.6 % in genital samples, p=0.025).

The results support the hypothesis that FGS may increase the risk of HIV acquisition, not only through damage of the mucosal epithelial barrier, but also by affecting HIV target cell populations, and that anti-schistosomal treatment can modify this.

Comments and additional results: Fourteen women with genital schistosomiasis were seen post-treatment. None of these women had schistosome eggs in the urine after praziquantel treatment. As the group was small, we did not evaluate the effect of treatment on the genital lesions. This will, however, be analysed in a larger group at a later stage and published in a future paper.

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Thirty samples were available for *Chlamydia trachomatis* analysis and chlamydia was associated with CCR5 expression in $CD4^+$ cells in cervical samples only (p = 0.001). The proportion of CD4 T-cells was, however, similar in genital samples with and without chlamydia (p = 0.495). Both for chlamydia and the other STIs investigated, there were no significant differences for the variables investigated in blood.

There were no statistically significant differences in cell frequencies in the FGS-positive women after treatment when compared with the FGS-negative women (data not published).

Cervical ectopy was measured in 53 of the women included in the study. This was however done at a later stage, and not included in paper 1. Subsequent analyses of the genital samples showed no significant differences between women with and without ectopy (data not published).

Paper 2

It has been hypothesized that ectopy may be associated with increased susceptibility to STIs. In this cross-sectional study, we explored the association between sexually transmitted infections (STIs including HIV) and cervical ectopy.

We included 700 sexually active young women (see Figure 1, paper 2). We did computer-assisted measurements of the ectocervical area covered by cylindrical epithelium (ectopy) in 694 colposcopic images and STI analyses on cervicovaginal lavage and serum samples. All participating women answered a questionnaire about sexual behaviour and use of contraceptives. The mean age was 19.1 years. More than half of the women had given birth (383/682). Ectopy was found in 27.2 %, HIV in 27.8 %, chlamydia in 25.3 % and gonorrhoea in 15.6 %. The median degree of ectopy was 4.0 % (range 0.0 % - 69.5 %). We found that age, parity, chlamydia and gonorrhoea, years since menarche, years since sexual debut and number of sexual partners were associated with ectopy. In multivariate analysis with chlamydia infection as the dependent variable, women with ectopy had increased odds of having chlamydia infection (Adjusted OR (AOR) 1.88, p = 0.011). Gonorrhoea was not associated with ectopy after adjusting for other STIs in a multivariate

model (AOR 1.15, p = 0.578). In women under 19 years of age, we found two-fold higher odds of being HIV-positive for those with ectopy (OR 2.19, p = 0.014).

In conclusion, cervical ectopy is associated with *Chlamydia trachomatis* infection and HIV in the youngest women.

Comments: The analysis of the association between HIV and STIs on the one hand and FGS on the other hand is outside the scope of this paper, and will be published in forthcoming papers from the project.

Paper 3

Schistosoma (S.) haematobium causes urogenital schistosomiasis and has been hypothesized to adversely impact HIV transmission and progression. On the other hand, it has been hypothesized that HIV could influence the manifestations of schistosomiasis.

In this cross-sectional study, we explored the association between urogenital *S. haematobium* infection and CD4 cell counts in 792 female high-school students from randomly selected schools. The absolute CD4 cell count was used (dual platform technology using markers for CD45 and CD4). We also investigated the association between low CD4 cell counts in HIV-positive women and the number of excreted schistosome eggs in urine.

Sixteen percent were HIV-positive and 31 % had signs of urogenital schistosomiasis (as determined by genital sandy patches and / or abnormal blood vessels on ectocervix / vagina by colposcopy or presence of eggs in urine).

After stratifying for HIV status, participants with and without urogenital schistosomiasis had similar CD4 cell counts. Furthermore, there was no significant difference in the prevalence of urogenital schistosomiasis (sandy patches, abnormal blood vessels or schistosome eggs in urine) in HIV-positive women with low and high CD4 cell counts. There was no significant difference in the number of eggs excreted in urine when comparing HIV-positive and HIV-negative women. Furthermore, there was no significant difference in mean egg excretion in HIV positive women grouped by CD4 cell count (CD4 cell count < 350, 350-500 and > 500, p = 0.695).

Our findings indicate that urogenital schistosomiasis does not influence the number of circulating CD4 cells.

Supplementary table to Paper 3 (Figure 2):

		HIV negatives (n)	HIV positives (n)
Sandy patches	Yes	62	16
	No	452	93
Abnormal blood vessels	Yes	248	39
	No	269	70
Schistosome eggs in urine	Yes	127	21
	No	504	100
Sandy patches or schistosome eggs in urine	Yes	172	30
	No	368	80

Table 4. Number of CD4 cell counts done in each subgroup.

Synopsis of the results

Table 5 shows the results in view of the study hypotheses and research questions. In general, the results show that women with local mucosal findings had a higher frequency of HIV target cells (FGS) and a higher HIV prevalence (cervical ectopy, youngest group). Urogenital schistosomiasis was not associated with the number of systemically circulating CD4 cells.

Table 5. Results in view of hypotheses and research questions

Hypotheses	Research questions	Papers	Results	Hypothesis supported
FGS may increase risk of HIV acquisition	Is FGS associated with the proportion of potential HIV target cells and CCR5 expression?	1	<u>Blood</u>: Yes, higher proportion of $CD14^+$ cells and $CD4^+CCR5^+$ cells <u>Cervix</u>: Yes, higher proportion of $CD14^+CCR5^+$ cells	Yes
FGS may exacerbate progression of HIV	Is FGS associated with the number of circulating CD4 cells?	3	No	No
Treatment of FGS may reduce risk of HIV acquisition	Does anti-schistosomal treatment influence the proportion of HIV target cells and CCR5 expression?	1	<u>Blood</u>: Yes, decreases proportion of CD14+ cells and $CD4^+CCRR5^+$ cells <u>Cervix</u>: Yes, decreases proportion of $CD14^+$ cells, $CD14^+CCR5^+$ and $CD4^+CCR5^+$ cells	Yes
Cervical ectopy may increase risk of STIs	Is cervical ectopy associated with STIs, including HIV?	2	Yes (w/chlamydia, and for girls < 19 years, w/HIV)	Yes
FGS may increase risk of STIs through increased risk of cervical ectopy	Is cervical ectopy associated with FGS?	2	No	No
HIV may influence the manifestations of FGS or urinary schistosomiasis	Is FGS associated with CD4 cell count in HIV+ women?	3	No	No
	Does HIV status influence egg excretion in women with urinary schistosomiasis?	3	No	No
	Does CD4 cell count in HIV+ women with urinary schistosomiasis influence egg excretion?	3	No	No

Limitations: See Discussion (material and methods)

DISCUSSION

Discussion of methods

Enrollment

We recruited a large cohort from the highest grades in randomly selected high schools. Only sexually active women were included, and the women we saw are therefore not likely to be representative of the whole high school population. They do, however, represent students at risk for sexually transmitted infections. Since high school students were included, the results may not represent findings of cervical ectopy, FGS and STIs in older women or children. It is possible that young girls also have genital schistosomiasis lesions, but since gynaecological examinations cannot be performed in virgins, this is not possible to diagnose with colposcopy.

Recruitment was done over a period of almost three years. Even though we have no reason to believe that there were large changes in the district that might have affected the study results during this timeframe, we do not know for certain whether any risk factors for HIV or FGS changed.

Two questionnaires were used in this study. Firstly, a short one-page questionnaire (isiZulu) was distributed to all women at the school after a short information session. They filled in this form themselves, sometimes in busy, crowded environments like a classroom. Information about the study including confidentiality of the collected information and test results was given on beforehand. However, the sensitive nature of the questions, for example the ones regarding sexual experience, may have been difficult to answer truthfully in such settings. One would expect some underreporting of sexual activity, which would lead to a lower participation rate in the study.

Also in the longer interview at the clinic, underreporting of sensitive issues is to be expected. Questions on especially sensitive issues such as number of sexual partners, use of alcohol and other

possible HIV risk factors, are likely to be especially prone to underreporting. Also questions that may be related to poverty, such as lack of access to clean water sources, may be regarded as stigmatising.

Sampling and analysis

Cytobrush sampling has been shown to be an adequate way to study cervical cell responses [287,288]. Genital cells captured by a cytobrush include CD4 T-cells, monocytes/macrophages and Langerhans cells, all HIV-susceptible and expressing CCR5 [289]. Cells from the cytobrush have been found to be relatively robust during transport and storage [290]. However, we cannot know for certain that the cells captured by the endocervical cytobrush are representative of the cells most likely to be infected by the HIV virus in vivo. In addition, a number of cells die during transport. Compared with the cervical cytobrush technique, cervical biopsies would offer some advantages, but was contraindicated in our research setting due to the possible increased risk of HIV acquisition [101].

There was a break of 7-8 months between two rounds of laboratory work (paper 1) in order to see patients post-treatment. Most of the FGS-positive patients were therefore seen during the first round of recruitment. Flow cytometry is a sensitive analysis and settings may change over time. Even though a detailed protocol was followed, we cannot exclude the possibility of differences in the results between the two rounds. Some variables such as $CD14^+$ cells and $CD4^+CCR5^+$ cells in the cervical samples were not significantly different in FGS-positive and -negative women, but showed a significant decrease after treatment. This finding may be caused by the limited sample size. It would have been interesting to look at the co-expression of CD4 and CCR5 in the $CD14^+$ population, but the data were not gated for this cell population.

It is a possible limitation for the flow cytometry analyses that multivariate analysis was not possible due to the small sample size. Adjusting for the effect of STIs on the various parameters could therefore not be done. Furthermore, women who had no ova in urine and no genital schistosomiasis lesions were defined as schistosomiasis-negative cases. However, some negative cases may have

had urogenital schistosomiasis including healed genital lesions. If the negative controls had been exposed to *S. haematobium*, the impact of schistosomiasis infection may have been underestimated.

We did meticulous STI testing in the collected samples. PCR and strand displacement assay techniques, both generally sensitive and specific detection methods, were used to test the cervical samples for STIs. However, there is limited data published on STI testing in cervicovaginal lavage, but studies of HSV and HPV in lavage have shown high sensitivity and specificity [279,291] (Table 2).

Gynecological examinations and image analysis

Diagnosis of FGS

Diagnosing FGS poses several challenges. The crush biopsy from cervical lesions with direct inspection of ova in the microscope has been considered to be the gold standard [68,102]. However, the iatrogenic lesion may create an entry point for HIV in endemic areas. Biopsy may be a safe approach if sexual abstinence can be ensured one day prior to, and fourteen days following the procedure [101]. In many areas, it is not possible to ensure this for all patients, making it an inappropriate diagnostic approach. There is a need for objective, safe, inexpensive and reliable methods to diagnose FGS. The widespread use of cell phones and IT technology on the African continent, make computerized image analysis based on the colour properties of the lesions a potential diagnostic tool in the future.

Schistosomiasis is a focal disease, and women from the same area may portray different stages of the infection — from acute disease with newer lesions to chronic infection with lesions containing dead calcified ova. Detailed information on the colposcopic appearance of genital lesions during these different phases of infection is still warranted [68].

The FGS diagnosis was made by the clinician performing the photocolposcopic examination. As in other operator-dependent investigations, colposcopy requires extensive training. FGS lesions may

be subtle and appear together with or mimic an STI [72] . Discharge and inflammation may make the diagnosis more difficult [57]. Grainy sandy patches are thought to be pathognomonic to FGS, while homogenous sandy patches and abnormal blood vessels may be less specific [57]. As the diagnosis depended on the clinician who performed the colposcopic examination, inter-observer variability may have occurred. Extensive training of the clinicians (see Gynaecological Examination chapter) was done to prevent this, but as we have not measured inter-observer variability, it cannot be ruled out. However, colposcopy must still be regarded as the best diagnostic tool under these circumstances, as genital lesions can be found with or without ova in urine, and antibody tests cannot distinguish between current or past infection or identify genital lesions [68,103,292].

Diagnosis of ectopy

Cervical ectopy is a normal finding in young women [247]. Measuring the area of cylindrical epithelium can be difficult, but computerized planimetry has been shown to be more accurate than a clinician's estimate using direct visual assessment [285].

We chose to use the same cut-off for cervical ectopy as several other studies; at least 10 % of the cervix covered with cylindrical epithelium [248,256]. This is an artificial cut-off that is not based on any biological rationale. It is possible that the absolute area, rather than a fraction, is more important for the susceptibility to HIV and other STIs. However, this was not possible to calculate in our images.

Still, using images of high quality from the gynaecological investigations, we were able to perform precise measurements of the fraction of ectopic area on the cervix. Images that showed only a part of the cervix could be included, and the computerized measurements therefore allowed us to analyse a large number of images. Calculations of inter- and intra-observer variability indicated that computer measurements are reliable.

Discussion of results

Main findings

This study is based on previous work that has shown an association between HIV infection and female genital schistosomiasis [2,203]. Schistosomiasis has also been hypothesized to alter the progression of HIV in co-infected individuals [218]. We have studied mucosal and systemic immunological and colposcopic aspects of HIV and FGS in young South African women.

We found that FGS was associated with a higher frequency of HIV target cells and HIV co-receptor expression in blood and in the genital compartment. This may indicate that FGS-positive women could be more susceptible to HIV infection than FGS-negative women. Praziquantel treatment decreased the proportion of HIV target cells in FGS-positive women in both blood and the genital compartment. This supports the hypothesis that anti-schistosomal treatment may reduce HIV susceptibility.

We also investigated the relationship between blood CD4 cell count and urogenital schistosomiasis in HIV-negative and HIV-positive cases, but did not find a significant association. This finding indicates that urogenital schistosomiasis does not influence the number of circulating CD4 cells and therefore does not seem to exacerbate HIV infection.

Furthermore, cervical ectopy was associated with chlamydia infection as well as HIV in the youngest study subjects. This supports the hypothesis of cervical ectopy being a risk factor for sexually transmitted diseases including HIV.

To summarize, these results may seem to support the hypotheses on HIV susceptibility but not the hypotheses on the effect on progression of HIV.

A general limitation when discussing the results is the cross-sectional study design (exception: follow-up post-treatment of FGS-positive women in paper 1). Inferring causality should therefore, if ever, be done with great caution.

HIV target cells

CD14$^+$ cells

Monocytes circulate in the blood and may differentiate into macrophages in tissues. Macrophages have been found to play an important role in the response to schistosomiasis [31]. These cells often express the CD14 molecule [293]. Both CD14$^+$ and CD4$^+$ cells are potential HIV target cells, and the availability of these cell populations may influence HIV susceptibility [131,139,165].

We found a higher percentage of CD14$^+$ cells in blood in FGS-positives compared with -negatives, and a decrease in both the genital compartment and blood after praziquantel treatment. CD4 T-cells did not show corresponding changes. In a study of cervicovaginal biopsies, Jourdan et al. quantified the HIV target cells in schistosome-infected female genital mucosa. They found that viable *S. haematobium* ova were significantly associated with a higher density of macrophages in genital tissue in adult women, whereas calcified ova, typical of chronic *S. haematobium* infection, were not associated with macrophage density [211]. The young women in our cohort may have had more recent infections, dominated by live ova. On the other hand, the study participants were recruited from an area endemic of *S. haematobium* and were therefore likely to have been exposed to the infection from a very young age. Immunity to schistosomiasis infection is only partial and reinfection is frequent [15]. Both acute and chronic infections may therefore coexist in the same population. Variation in the duration of infection in populations from different geographical locations is a possible explanation to the discrepancies between the studies.

CD4$^+$ cells

When comparing FGS-negative and -positive women, we did not find any difference in the proportion of CD4 T-cells in genital samples. Likewise, praziquantel treatment did not influence CD4 T-cell levels. However, significantly higher levels of CCR5 expression on CD4 T-cells were found among schistosomiasis positive cases. This finding may suggest that even if the proportion of CD4 T-cells is similar in FGS negative and positive women, the cells may be more susceptible to HIV in the FGS positive group.

In contrast to our findings, Jourdan et al. found a higher number of CD4 T-cells surrounding calcified schistosome ova in histological genital samples [211]. The young women in our cohort may have had more live schistosome ova, suggesting recent infection, and less calcified ova in the genital mucosa. It is however also possible that the effect of schistosomiasis is greater on the monocyte/macrophage cell population than on the CD4 T-cell count. Local mucosal effects may also be of greater importance than the systemic effects.

Genital schistosomiasis lesions have been reported to be refractory to praziquantel treatment [111]. However, our study indicated that in women with genital lesions, the frequency of HIV target cells expressing CCR5 decreased after treatment. It is therefore possible that praziquantel may have an effect on HIV susceptibility, even if macroscopically visible lesions are still present.

It has been hypothesized that schistosomiasis infection has a negative influence on HIV progression in co-infected persons [23,219]. However, in our samples, mean CD4 counts were similar in *S. haematobium* positive and negative cases, both amongst those with HIV and those without. This finding may indicate that schistosomiasis infection does not lead to lower CD4 cell counts and more rapid progression of the HIV infection through loss of CD4 cells. A possible limitation to the study is the number of samples in each group (Table 4). However, from the power calculations done prior to the study, we would expect to detect a relatively small difference (80×10^6 cells / L) with our sample size.

FGS and cervical ectopy; biological risk factors for HIV?
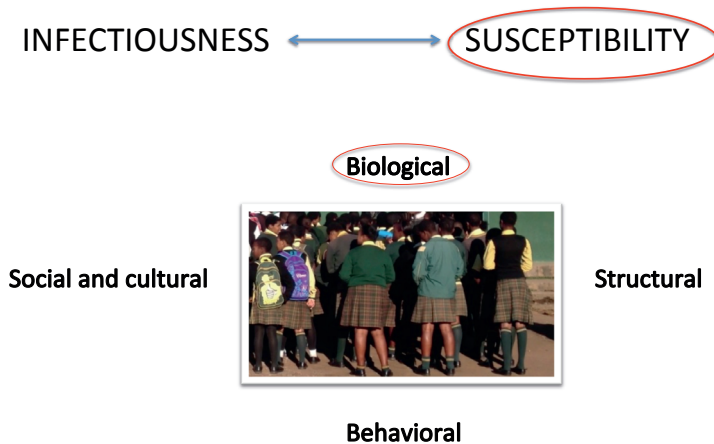
Figure 15. Factors that may influence HIV susceptibility. Social and cultural, behavioural, structural and biological factors may influence HIV susceptibility [174,294].

There are a number of possible reasons why young South African women are at such a high risk for HIV infection: social, structural, behavioural and biological (Figure 15). Both genital schistosomiasis and cervical ectopy are biological factors that have been suggested to be associated with increased susceptibility to HIV [2,250]. However, the biological mechanisms behind these associations have been sparingly investigated.

A relevant question is whether schistosomiasis is a risk factor for HIV, or whether HIV makes the schistosomiasis infection more severe with increased egg excretion. In Zimbabwe, a study looked at the excretion of schistosome eggs in HIV-positive and -negative individuals and did not find any difference in ova excretion [295]. In contrast, Mwanakasale et al. found that HIV-positive individuals excreted fewer schistosome ova than HIV-negative individuals, and it was hypothesized that egg excretion depended on a functioning immune system [17,296]. A lower prevalence of haematuria, a commonly used indirect way of diagnosing schistosomiasis, has also been reported in individuals co-infected with HIV and *S. haematobium* infection [296]. In our work, we did not find a significant difference in egg excretion in HIV-positives and –negatives. This is an important

finding when investigating the association between HIV and schistosomiasis, since egg excretion in urine is often used for diagnosing schistosomiasis. According to our results, the association between HIV and urogenital schistosomiasis found in previous studies, is not caused by altered egg excretion in the HIV-positive group.

Genital schistosomiasis lesions are likely to be present at the time of sexual debut [107] and there is no evidence of an age-dependent increase in FGS prevalence in the overall population [201]. These findings support the hypothesis that FGS precedes HIV infection. Furthermore, it is biologically plausible that genital schistosomiasis lesions make the genital mucosa more susceptible to sexually transmitted HIV infection. This may be caused by mechanisms similar to sexually transmitted infections, such as a broken epithelial barrier and an increased number of HIV target cells surrounding schistosome ova [211,297].

Cervical ectopy is likely to be present at the time of sexual debut, as the size of the ectopic area normally decreases with age in adult women [247]. Studies have shown that CD4 T-cells and CCR5 expression is found more frequently in the endocervical, columnar epithelium than in the ectocervical squamous epithelium [150,250,251]. HIV target cell availability in the genital mucosa may be higher in women with cervical ectopy. It has therefore been suggested that ectopy is likely to be a risk factor for HIV rather than HIV causing increased ectopy [250].

In the youngest women (under 19 years old) participating in our study, we found that cervical ectopy was associated with HIV. We also found higher proportions of HIV target cells in women with FGS. The virus may therefore have a higher chance of encountering a target cell after gaining access to the submucosa in women with FGS or cervical ectopy.

Even though a correlation between HIV and *S. haematobium* prevalence has been found on a country level [201], the two infections do not always show a geographical overlap. FGS is hypothesized to be one of the numerous HIV risk factors. Further studies are needed in order to determine the impact of FGS on the HIV epidemic in different populations. Similarly, not all studies have found an association between ectopy and HIV [250]. Larger, longitudinal studies are therefore warranted.

CONCLUSIONS AND PERSPECTIVES FOR FUTURE RESEARCH

In this work, we explored two genital tract conditions that might pose a risk for HIV in young women. Both FGS and cervical ectopy are associated with changes in the genital mucosa that could potentially increase HIV susceptibility. Congruent with this hypothesis, we found that FGS was associated with a higher frequency of HIV target cells and HIV co-receptor expression in the genital compartment ($CD14^+CCR5^+$) and blood ($CD14^+$ and $CD4^+CCR5^+$). Praziquantel treatment decreased the proportion of HIV target cells in FGS-positive women ($CD14^+$ and $CD4^+CCR5^+$) in both compartments. This finding might imply that anti-schistosomal treatment could reduce the risk of HIV acquisition, but larger studies on the effect of anti-schistosomal treatment on HIV incidence are warranted.

Urogenital schistosomiasis has also been hypothesized to exacerbate the progression of HIV. However, no significant difference in CD4 cell counts between women with and without *S. haematobium* infection was found. This finding indicates that *S. haematobium* infection does not exacerbate HIV infection through loss of CD4 cells. However, urogenital schistosomiasis may influence the levels of HIV viral load in blood and genital secretions and therefore exacerbate HIV progression and/or HIV transmission to an HIV-negative partner. This should be further explored in future studies. Our findings indicated differences between systemic and mucosal samples, and both compartments should therefore be investigated.

We found that cervical ectopy was associated with the prevalence of chlamydia infection and with HIV infection in the youngest study subjects. Cervical ectopy was not significantly associated with FGS. Little is known about treatment options for cervical ectopy, and counseling on barrier contraceptives is currently the clinician's only option when cervical ectopy is found in a young woman [298]. Studies on the treatment of potential HIV risk factors such as cervical ectopy are needed.

Young women are at a disproportionally high risk of contracting STIs including HIV [174,299]. Deciphering biological risk factors for HIV is crucial in order to understand transmission and development of the epidemic as well as to design efficient prevention strategies. A high number of young women infected with schistosomiasis are at risk of HIV infection, and the interaction between the infections may have significant implications. Inexpensive mass treatment with anti-schistosomal drugs could potentially reduce the development of FGS and reduce the spread of HIV [1].

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Appendices

[___]___]___]___]

VIBE

Oslo University Hospital

UNIVERSITY OF KWAZULU-NATAL

Usuku/ Date |___|___|___|___|___|___|
                                     (yy) (mm) (dd)

1. **Isibongo** / Surname(s) _____
   Amagama / First name(s) _____
   Igama lesidlaliso/ izithakazelo/ amanye amagama
   Nickname/praise names/other names _____

2. **Wazalwa nini?** /When were you born? |___|___|___|___|___|___| yy mm dd

3. **Ukuliphi ibanga lemfundo?/** Which grade are you in? |___|___| **Section**: |___|

4. **Ikheli leposi** / Postal address _____
_____
_____
_____

5. **Inombolo kamakhalekhukhwini /**Cell |___|___|___| - ___|___|___|___|___|___|___|

6. **Inombolo yocingo lwasekhaya** /Landline |___|___|___| -|___|___|___|___|___|___|___|

Sibuza lemibuzo elandelayo ngoba isichenene singadala inkinga kwisibeletho. Lokho akwazeki kahle hle kodwa inhlangano yomhlaba jikelele isikuveze njengenkinga ebalulekile kwabesifazane abasakhula.
We are asking the questions on the next page because Bilharzia may create reproductive tract problems. This is not well known but the World Health Organisation has recently recognised this as an important health problem for young women.

Izimpendulo ozosinika zona ziyimfihlo asikho isidingo sokutshela abanye abantu nawe ungabuzi abanye abantu ukuthi baphendule bathini ngoba akukhombisi inhlonipho..
The answers you give below are completely confidential. You do not need to tell anyone about this and you should not ask others what they answered as this is impolite.

7. **Sayina lapha uma usukulungele ukuphendula imibuzo ephepheni elilandelayo:** Sign here if you are prepared to answer the questionnaire on the next page:

_____
(**sayina/** signature)

Sicela ukekelezele impendulo yakho:/ Please put a circle around your answer:

1. Uneminyaka emingaki? / How old are you? Age (years) |___|___|

2. Sengike ngawathinta amanzi asemfuleni, damini noma asexhaphozini. I have touched water from a river, dam or pond.	Yebo	Cha	Mhlawumbe

3. Kangaki? How often?	Nsukuzonke	Maviki-onke	Nyangazonke	Hhayi njalo

4. Sengike ngaba nesichenene. I have had Bilharzia.	Yebo	Cha	Angazi
5. Singike ngalashelwa isichenene. I was treated for Bilharzia before.	Yebo	Cha	Angazi
6. Khona oseke waba/onaso isichinene ekhaya. I have a family member who has /has had Bilharzia.	Yebo	Cha	Angazi
7. Nginazo/sengike ngaba nazo izinhlungu uma ngichama. I have/ have had pain during urination (now or before).	Yebo	Cha	
8. Ngino/ngake ngaba nomchamo obomvu. I have/ have had red urine (now or before).	Yebo	Cha	
9. Bengithanda ukubonana nodokotela ngasese. I would like to talk to a doctor in private.	Yebo	Cha	
10. Ngike ngiphiso umchamo esithubeni (noma ngihleli phansi) I sometimes have a sudden urge to urinate (even when sitting still)	Yebo	Cha	
11. Nginodadewethu omncane osasesikoleni. I have younger sisters who are in school.	Yebo	Cha	
12. Ngino dadewethu omdala osasesikoleni. I have older sisters who are in school.	Yebo	Cha	
13. Sengiqalile ukuya esikhathini. My periods have started.	Yebo	Cha	
14. Abanye bomndeni wami banenkinga yokukhulelwa. Someone in my family has problems falling pregnant.	Yebo	Cha	
15. Nginaye/sengike ngaba naye umuntu engiya naye ocansini. I have/ have had a sexual partner.	Yebo	Cha	
16. Kuyenzeka ngophe esithubeni. I sometimes bleed between my periods.	Yebo	Cha	
17. Nginalo/sengike ngaba nalo ukutshezi olungajwayelekile enkomeni. I have/ have had abnormal vaginal discharge.	Yebo	Cha	
18. Bengithanda ukuza nomunye ukuzobona udokotela. I would like to bring someone with me to see the doctor.	Yebo	Cha	
19. Ngingathanda ukukhulelwa kuleminyaka emibili ezayo. I would like to fall pregnant within 2 years.	Yebo	Cha	
20. Kumkhandlu Ugu senza ucwaningo olukhulu mayelana nesichenene ezimpilweni zabesifazane. Kulolucwaningo wamukelekile ubheke ekuthi awunaso.Mangase kwenzeke ukhethwe kucwaningo ungathanda ukubamba iqhaza? In Ugu district we are doing a large study on the effects of Bilharzia on female health. In this study you will be invited to check if you have female Bilharzia. If you are selected to the join the study- would you like to participate?	Yebo	Cha	Mhlawumbe

Version 12.08.2011
Baseline Teen id |___|___|___|___| - 1
Interviewer _____
Interview date dd/ mm/ yy ____/____/_____
QC BY_____(sign, red pen)

Please use a circle or tick where there are pre-entered responses

Isiqalo: Igama lami......ngisebenzela iVIBE project njengoba kwakushiwo ngaphambilini. Ngiphinde ngikubonge ngokunginikeza imvume yokuthi ngisebenze nawe. Ngithanda ukugcizelela amanye amaphuzu ngaphambi kokuthi siqale.

Yonke lembuzongxoxo imayelana nesichenene sesibeletho sabesifazane kanti futhi yonke into esizoyixoxa izohlala phakathi kwethu..Ayikho imibuzo elungile nengalungile. Unelungelo lokuyimisa impendulo ngxoxo noma isiphi isikhathi. Ukusebenzisana kuyoba intokozo / Introduction*: I am ....and I am working for the VIBE project as mentioned during the informed consent procedure. Thank you again for giving me permission to work with you. I would like to emphasize some points before we start.*

All the questions below are related to Female Genital Bilharzia and everything discussed here will be confidential. There are no wrong or right answers. You are free to stop the interview at any time. Your cooperation will be appreciated.

1) **Isikole** / school: _____

2) **Igama lesikole sebanga eliphansi ophuma kuso** / Name of the primary school you came from_____

3) **Uyasebenza na? njenge?**/Are you working? As? _____

4) **Iminyaka** / Age |___|___|

5) **Wazalelwaphi?** / Where were you born ?_____

6) **Inkolo** / Religious affiliation [Christian] [Muslim] [Shembe] [Leganyana] [Methodist] [other_____

7) **Ulimi lwasekhaya** / Home Language [Zulu] [Xhosa] [English] [Other_____

MENSTRUATION

8) **Ugcine nini ukuya esikhathini?** / **LMP**: ____/____/_____dd / mm / yy or NA

Ukhulelwe? Uma kungu yebo angalinda izinyanga ezimbili emva kokuteta.
AYIKHO IMIBUZO EZOBUZWA, MNIKE IPHEPHA LOLWAZI
Pregnant? If yes, she can wait until 2 months after delivery NO MORE QUESTIONS
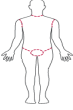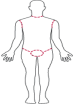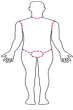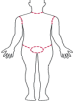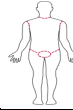
9) **Waqala uneminyaka emingaki ukuya esikhathini?** / Age of your first menstruation? |___|___|

10) **Ubanaso isilumo kangangokuba kumele uhlale ekhaya?** / Do you normally have painful periods so that you have to stay home from school? [yebo] [cha]

11) **Ubanaso isilumo esikwenza ufise ukuthatha amaphilisi ezinhlungu?** / Do you normally have painful periods so that you wish to take painkillers? [yebo] [cha]

12) **Indlela yokuhlela** / Contraceptive method:
   a) **Uyajova?** / Are you on contraceptive injection? [yebo] [cha]
   b) **Amaphilisi okuhlela** / contraceptive pills [yebo] [cha]

13) **Uya ngendlela efanayo nyanga zonke** / Are your periods regular [yebo] [cha] [NA= Post Partum / if injectable contraceptive / pills]

14) **Uya izinsuku ezingaki esikhathini? /** How many days does your period normally last? |___|___| [NA= Post Partum / if injectable contraceptive / pills]

15) **Kuye kwenzeke wophe ungalindele?** / Do you have times of unexpected bleeding or spotting in between your normal periods? [yebo] [cha] [NA= Post Partum / if injectable contraceptive / pills]

16) **Ujwayele ukopha kakhulu kuze kube namahluli?** / Do you normally have periods with heavy bleeding and clots? [yebo] [cha] [NA= Post Partum / if injectable contraceptive / pills]

PREGNANCY

17) **Usukhulelwe kangaki sekukonke? /** How many times have you been pregnant? |__|__|

Month Year	Abortion = 1. Miscarriage =2. Birth =3	Trimester: 1= 1st (before 3 mnths) . 2= 2nd (between 3 to 6 mnths). 3=3rd (6 to 9 months)	Complications, please describe

18) **Bangaki abantwana abaphilayo?** / How many children are alive? |__|__| [NA=99]

URINE AND BILHARZIA

19) **Wake waba naso isichenene?** / Have you ever had Bilharzia? [yebo] [cha] [angazi]
20) **Wake walashelwa isichenene nini**?/ Have you ever been treated for Bilharzia, when?
Never |__| OR [age] |__|__|1st time |__|__|2nd ime |__|__|3rd time
21)

	Wake waba nayo inkinga noma yiphi ngokuchama njenge: / Have you ever had any problems with urination like:	Esontweni eledlule / This last week	Kudala phambilini / Sometime before	Akukaze / Never
a.	**Zinhlungu uchama** / Pain when you urinate			
b	**Ukuzwa sengathi ufuna ukuchama esikhaleni noma uhlezi unganyakazi uze ucishe uzichamele** / Sudden urge to urinate causing a leak, even when sitting still [urge incontinence]			
c.	**Ukuzwa sengathi ufuna ukuchama esikhaleni noma unganyakazi ngaphandle kokuthi ucishe uzichamela** / Sudden urge to urinate, even when sitting still [urge] (no leakage)			
d.	**Iconsi lomchamo uma ugxuma, ukhwehlela noma uhleka** / Drop of urine if you jump, cough or laugh [stress incontinence]			
d.	**Umchamo obomvu** / Red urine			

WATER CONTACT

22) **Manje ngizokubuza ngezinhlobo zezinto ozenzayo noma owake wazenza ngamanzi, uzenza noma wazenza kangakanani, uhlala isikhathi esingakanani emanzini nokuthi umzimba uthintana kangakanani namanzi.** /Now I will ask you what kind of water activity you have or have had, how often you do them, for how long you stay in the water and how much of your body that is in contact with the water:
Umfula/river **Amadamu**/dam **Amanzi amile**/standing water. **Amanzi avela kulezizisuka**/ water from these sources. **None**

23) **Kukhona isikhathi empilweni yakho owake wahlangana nalamanzi?** [yebo] **[cha]**
Is there a period in your life when you had water contact? (make crosses for each period)

|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|___|
 1   2   3   4   5   6   7   8   9 10 11 12 13 14 15 16 17 18 19 20 21 22 yrs

24) **Sicele uchaze ngalezi zikhathi** / Please explain about **this (these)** period(s

	Kangaki/ *How often?* **Nsukuzonke** /*Daily (4).* **Kujwayele** /*Often (3).* **Kwesinye isikhathi** /*Sometimes (2)* **Kuthukela/ qabukela** / *Rarely (1).* **Ngeke** / *Never (0)*	**Uke uhlale kangakanani / Uhlala kangakanani emanzini?** / *For how long did/ do you stay in the water?* **Ngaphezulu kuka 5 h** / *More than 5 h (4).* **3-5 Amahora** / *3-5 hours (3).* **Ngaphansi kwamahora amathathu** / *Less than 3 hours (2).* **Kuze kube yimizuzu ewu-60** / *Up to 60 minutes (1)*	**Umzimba wake wawathinta / uwathinta kangakanani amanzi ngesikhathi wenza lezizinto** /*How much of your body was/is in contact with water during this activity?*
Uke noma wake wadlala/ ukubhukuda?/ Did or do you play / swim?			
Uke noma wake wawasha / wageza?/*Did or do you wash / bathe?*			
Uke noma wake wazihlanza izingubo? / *Did or do you do laundry?*			
Uke noma wazihlanza izingubo zokulala? /Did or do you wash blankets?			
Uke noma wake wawakha amanzi?/*Did or do you collect water?*			
Uke noma wake wadoba? /Did or do you *fish?*			
Uke uwele umfula zize izinyawo zakho zithintwe amanzi?/*Did or do you ever cross the water , so your feets become wet?*			

SYMPTOMS AND EVENTS

25) **Kubukeka kunjani ukuphuma ngaphansi kuwena?** / How is your <u>current</u> discharge?
    a) Kumbala muni/ Colour   1 2 3 4 5 6 7 8 [NA=99] (please circle)
    b) Okusagazana / Trace of blood 0 1 2 3 4 5 6 [NA=99]

26) **Wake wezwa ukungaphatheki kahle esithweni sakho sangasese njengo:** / Have you previously felt any discomfort in your private parts like**:**	**Esontweni eledlule /** This last week	**Kudala phambilini** Sometime	**NA /** Never
a. **Ukuluma** / Itch			
b. **Ukushisa / Ukushoshozela** / Burn / Sting			
d. **Isilonda /** Sore / ulcer			
e. **Isimila / isigaxa** / Lump / tumour			
f. **Okuphumayo okusagazana /** Bloody discharge			
g. **Okushubile/ Okusasigaxana okuphumayo** Thick/ lumpy discharge			
h. **Okusamanzi okuphumayo /** Watery discharge			
i. **Iphunga elingajwayelekile /** Abnormal smell			
j. **Okuphumayo okunombala ongajwayelekile /** Abnormal coloured discharge			
k. **Kukhona osuke wakushutheka esithweni sakho sangasese njenge nsipho?.** / Did you put substances inside your vagina? Such as soap ?			
l. **Wake wagcatshwa esithweni sangasese noma waxilongwa ngezinsimbi ezicijile** / Ever had genital cutting or used a sharp instrument in the genital area (not episiotomy)			
m. **Ubuhlungu besinye** / Lower abdominal pain			
n. **Ubuhlungu ngaphansi kwezimbambo** / Upper abdominal pain			

CONTRACEPTION & STDs

27) **Uke wakusebenzisa okulandelayo kuleli sonto eledlule** / Have you used the following the last week:
    i)   **Ikhondomu yabesifazane /** female condom [yebo] [cha]
    ii) **Ikhondomu yabesilisa** / male condom [yebo] [cha]

28) Uyazi ukuthi ziyini izifo zocansi STI? / Do you know what an STI is? [yebo] [cha] [angazi]
STD izifo ezithelelana ngokocansi, zibonakala kube khona okuphumayo okungajwayelekile, ukuluma, kube bomvu, amaqhubana noma isilonda esithweni sangasese. /STI's are diseases that you get through sexual contact, and symptoms include an abnormal vaginal discharge, itchiness, redness, lumps or sores in your private parts.

29) **Wake walashelwa izifo zocansi ngenyanga edlule?** / Have you been treated for an STI the last 4 weeks? [yebo] [cha] [angazi]

30) **Ucabanga ukuthi unaso isifo socansi njengamanje?** / Do you think you have an STI now? **Kungani** Why? _____

31) **Usulashelwe kangaki izifo zocansi?** / How many times treated for an STI? |___|___|

INTERCOURSE

32) Kukhona okukhumbulayo lapho wake wopha waba nezinhlungu esiswini esithubeni / Can you remember any specific event(s) when you had unexpected bleeding and sudden severe stomach pain in thepast. No [___] OR ____/____/____ mm / yy *(Research dr concl:_____)*

33) **Waqala uneminyaka emingaki ukwenza ucansi?** / What was your age when you had sex for the first time? |___|___|

34) **Hloboluni locansi owake walwenza? /** What kind of sex have you had?
   a) **Ukuphathaphatha isitho sangasese kuphela** / Petting [yebo] [cha]
   b) **Ukusoma** / Thigh sex [yebo] [cha]
   c) **Olokukhotha isitho sangasese** / Oral [yebo] [cha]
   d) **Olwasesithweni sowesifazane** / Vaginal [yebo] [cha]
   e) **Olwasembotsheni yokuzikhulula** / Anal [yebo] [cha]
   f) **Ugcine nini ukwenza ucansi**? / When was the last time you had sex?
      ____/____/_____dd / mm / yy
   g) **Uyisebenzisile Icondom ugcina ukwenza ucansi? /Did you use a condom the last time you had sex** ? [yebo] [cha]

35) **Ubanazo izinhlungu uma wenza ucansi?** / Do you have pain during sex? [yebo] [cha] [NA if no sex the last 6 months]

36) Uma kuwu yebo, ngabe kuba:/ If yes, is it:
   a) **Ubuhlungu obujulile** / Deep / thrusting pain [yebo] [cha] [NA if no sex the last 6 months]
   b) **Ubuhlungu uma kungena** / Superficial / upon entering [yebo] [cha] [NA if no sex the last 6 months]
   c) **Ubuhlungu ngaphansi kwezimbambo** / Pain under the rib cage [yebo] [cha] [NA if no sex the last 6 months]

37) **Ucabanga ukuthi kujwayelekile ukopha kancane emva kokwenza ucansi /** Do you think it is normal to have a little bleeding after sex? [yebo] [cha] [angazi]

38) **Uke wabona wopha emva kocansi?** / Have you ever seen bleeding after sex? [yebo] [sometimes / ngesinye isikhathi] [cha]

39) **Uyalwenza ucansi ngesinye iskhathi uma usesikhathini** / Do you sometimes have intercourse during your menstrual period [yebo] [cha]

40) **() Uke uzizwe ucindezelekile ukwenza ucansi nomuntu ngenxa yezipho/imali akunike yona?** / Have you ever felt pressured to have sex , because of the gifts or money you have been given ? [yebo] [cha]

41) **Wake wahlukunyezwa ngokocansi noma waphoqwa ukwenza ucansi** / Have you ever been sexually abused or forced to have intercourse? [yebo] [cha] IF YES:

42) **Sekuke kwenzeka kulezinsuku ezintathu ezidlule?** / Has it happened the last 3 days? [yebo] [cha] [NA]
☆ **Ngiyadabuka ukuzwa lokho, ngiyazi kunzima kuwena ukukhuluma ngalokhu. Kuwumthwalo onzima kakhulu ukuwuthwala ngokwakho.** I am so sorry to hear that, that must have been very hard. I know it is very difficult for you to talk about this. It's a very heavy load to have to carry on your own.**Ukhona osuke wakhuluma naye ngalokhu.** Have you talked to anyone about it? [yebo] [cha]
Ungathanda ukukhuluma nomaluleki ngaloludaba ?/Would you like to talk to a mentor about it ? [yebo] [cha]

RELATIONSHIP AND HIV STATUS

Ngaphambi kokuba uphendule imibuzo elandelayo sifuna ukukuqinisekisa ukuthi lolulwazi luyimfihlo futhi angeke lwatshelwa noma ubani. Imibuzo elandelayo imayelana nobudlelwane kanye nesimo sakho sesandulela ngculazi. Ngiyazi eminye yalemibuzo inzima ukuyiphendula kodwa ngicela usize wenze okusemandleni akho. Lemibuzo siyibuza wonke umuntu./ Before you answer the next questions, we want to assure you that the information you give will not be told to anyone. The next questions are about relationships and HIV status I know some of these questions are hard to answer but please do your best. We are asking the same questions to everybody.

43) **Wayeneminyaka emingaki umaqondana wakho omdala kunabo bonke?** / How old was your oldest partner? |__|__|

44) **Uma ucabanga wake waba nomaqondana onesandulela ngculazi** / Do you think you have had an HIV positive partner ? [yebo] [cha] [angazi]

45) **Uke waba nnmaqondana odla imishanguzo /** Have you ever had a partner who is taking ARV's? [yebo] [cha] [angazi]

46) **Unaye umaqondana njengamanje?** / Do you have a steady partner at the moment? [yebo] [cha]

47) **Usuwenze ucansi nabantu abangaki empilweni yakho?** / Lifetime sexual partners? |__|__|

48) **Ulale nabantu abangaki ngenyanga edlule?** / Number of sexual partners you have had the last month? |__|__|

49) **Ezinyangeni ezintathu ezedlule ugcina ukwenza ucansi waphuza utshwala noma izidakamizwa ? /** In the past 3 months; have you had sexual intercourse under the influence of alcohol or drugs? [yebo] [cha]

50) **Wake wahlolelwa isandulela ngculazi** / Have you been tested for HIV? [yebo] [cha]

51) **Ungakhululeka ukungitshela ngesimo sengculazi, ingabe unaso isandulela ngculazi?** / Would you feel comfortable telling me your status, do you have HIV? [yebo] [cha] [angazi] [Patient declined information]

If no or declined information go to ALCOHOL

UMA KUNGU YEBO: Ngiyaxolisa ukuzwa lokho, kufanele ukuthi kunzima. / IF YES: I am sorry to hear that, it must be hard for you.

52) **Waze nini ngesimo sakho? /** When did you get to know ____/____/____ mm / yy

53) **Ukhona osuke wamutshela ngalokhu?** / Have you told anyone about it? [yebo] [cha

54) **Ngabe CD4 count uyayihlola**? / Is your CD4 count monitored? [yebo] [cha]
   a) **Uma kuwuyeboibingakanani CD4 count?** / If yes, last CD4 count? |__|__|__|
   b) **Nini?** / When? ____/____/____ mm / yy

55) **Uyayithatha imishanguzo?** / Are you taking ARV's? [yebo] [cha]

56) **Ungathanda ukuthi sinihlelele iqembu lokululekana nabanye abafundi abanesimo esifana nesakho?**/Would you like us to organise a support group for HIV positive learners and would you join? [yebo] [cha] [angazi]

☆ **Wahlolwa watholwa unegciwane lengculazi futhi neCD4-count ayibhekwa. Ingabe lokhu kuyiko?** / You have been tested positive for HIV and your CD4-count is not monitored. Is this correct?
Uma kungu YEBO: Sibona ukuthi uhlolisiswe ngokushesha. Ngakho ke, uzothola incwadi evela kuthina ezokusiza ukuthi uxhumane nomtholampilo./ If yes: We recommend that you have a thorough investigation as soon as possible. Therefore, you will receive a letter from us that will help you to contact your clinic.

ALCOHOL / IZIDAKAMIZWA / DRUGS

Imibuzo elandelayo ingophuzo oludakayo. Izimpendulo onginika zona angeke zatshelwa muntu. / The following questions are about alcohol. The answers you give will not be told to anybody.

57) **Waqala nini ukuphuza utshwala?** / When was the first time you drank alcohol? [iminyaka / age] |__|__| [Ngeke / Never]

58) **Ingabe abangani bakho bayazisebenzisa izidakamizwa?** / Do your friends use drugs? [yebo] [cha] [angazi]

59) **Uyazisebenzisa izidakamizwa?** / Do you use drugs? [yebo] [cha]

60) Wake wasebenzisa okunye noma okungaphezulu kwalokhu okulandelayo? / Have you ever used one or more of the following

- a) **Insangu** / dagga [yebo] [cha]
- b) **Yibensin** / bensin [yebo] [cha]
- c) **Okunye 1** / Other 1[specify] _____
- d) **Okunye 2** / Other 2 [specify] _____
- e) **Izidakamiswa ezijovwayo** / Injectable drugs? [yebo] [cha]
- f) **Yiglue** / glue [yebo] [cha]
- g) **Umgwinyo** / ecstacy [yebo] [cha]

FAMILY AND LIVING

61) **Qala ngomdala kunabobonke endlini: /** Start with the oldest in the household:

Relation: M = mother. F= father. G= Legal guardian. B = brother. S = sister. Gm = grandmother. Gf = grandfather. U = uncle. A = aunt. C = cousin. Sf = Stepfather. Sm = Stepmother. Ss = Stepsister. Sb =Stepbrother. FR- friend, H=housekeeper. Ch= Child (son/daughter of study subject). Y= Husband / boyfriend of study subject. O = other. Ni = Niece. Ne=Nephew. X=study subject					
Ubani ohlala kulendlu yakini? Who lives in your house?	**Isilinganiso seminyaka** App. age	**Umsebenzi** Work		**Umfundi** Student		**Izinga lemfundo eliphezulu [Ayikho / Ephansi / Ephezulu / Ephakeme]** Top education (0=None) (1=Primary) (2=High school) (3=Tertiary) (10=don't know)
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	
		Yes	No	Yes	No	

OKUNYE UKUBAMBA IQHAZA / FURTHER PARTICIPATION

Sithanda ukukubonga ngokubamba kwakho iqhaza kulokhu, Sizama ukusiza abanye ngalolucwaningo.Sizobuye sibuye sizovakasha sizophinda ingxoxo mbuzo nokuxilonga / We want to thank you for participating in this research. We are trying to help others by this research. We will have a follow-up visit, when we will repeat today's questionnaire and examination.

INFORMATION ABOUT CLINICAL FINDINGS

Uma ucwaningo luthola lezizifo ezilandelayo ungafuna ukwazi?/ If the study discovers the following diseases, do you want to know:

1. **Izifo ezithathelana ngokocansi zingadala ukulimala kwenqondo, ukungabatholi abantwana noma umdlavuza olaphekayo. Ungafuna ukwazi uma unalezozifo /** Some sexually transmitted diseases may cause brain damage, infertility and treatable cancer. You want to know if you have such a disease [yebo] [cha] [angazi]

2. **Igciwane lengculazi liyathibeka futhi ungaphila isikhathi esideuze uguge uma wazi ngesimo sakho. Uyafuna ukwazi? /** HIV is treatable and you can live until you get old if you have your result. You want to know [yebo] [cha] [angazi]

3. **Ezinye izifo ezingatholakala ocwaningweni.Uyafuna ukwazi? /** Other diseases that maybe discovered. You want to know [yebo] [cha] [angazi]

Do you have any questions for me ?

Is there anything you want to talk about ?

TRACKING DATA PAGE

Person 1

1. **Isibongo /** Surname[s] _____
2. **Amagama** / First name[s] _____
3. **Isidlaliso / Isithakazelo / Noma elinye igama** / Other names _____
4. **Isikole** / school: _____ Grade: |___|___| Section: |___
5. **Ubani uthishawakho manje?** / Who is your current teacher ?_____
6. **Usuku lokuzalwa** / D.o.b. ____/____/____ dd/ mm/ yy **Wazalelwaphi?** / Where_____
7. **Uhlala kuphi isikhathi esiningi? [Ikheli lala uhlala khona]** / Where do you live most of the time? Physical address _____
8. **Ujwayele ukulala kangakanani lapha? [sonke isikhathi] [ingxenye yesikhathi] /** How often do you sleep here? [All the time] [Most of the time]
9. **Ikheli leposi** / Postal address _____
10. **Inombolo kamakhalekhukhwini** / Cell |___|___|___| - |___|___|___|___|___|___|___|___|
11. **Inombolo yocingo lwasekhaya** / Landline |___|___|___|-|___|___|___|___|___|___|___|___|
12. Email: _____
13. **Kungani ubelapha isikhathi esiningi?** / Why are you here most of the time? _____
    _____
14. **Ubani okunakekelayo lapha? [igama]** / Who is looking after you here? [name] _____
    _____
15. **Igama lombheki /** Name of a guardian_____sex [M] [F]
16. **Ubuhlobo /** Relation (see below)[r_____
17. **Ngubani ongumninimuzi? /** Who owns the house?_____
18. **Ngubani oyinhloko yekhaya? /** Head of the household?_____
19. **Uyasebenza na? njenge?**/Are you working? As? Where ?_____

Person 2

Ingabe ikhona enye indawo ohlala kuyo ngezimpelasonto, amaholide noma ezinye izinsuku? Another place where you stay (eg weekends,holidays,other)? [**yebo**] [**cha**]
If no NEXT PG

21. **Ujwayele ukulala kangakanani lapho? ngezinye zezinsuku zesikole ngezimpelasonto amaholide okunye [chaza] /** / How often do you sleep there? [some school days] [weekends] [holidays] [other
22. **Ikheli lapho ovakashela khona** / Physical address _____
    _____
    _____
23. **Ikhelileposi /** Postal address_____
    _____
24. **Inombolo kamakhalekhukhwini** / Cell |___|___|___|-|___|___|___|___|___|___|___|___|
25. **Inombolo yocingo lwasekhaya** / Landline |___|___|___|-|___|___|___|___|___|___|___|___|
26. **Kungani uhlala lapha ngesinye isikhathi?** / Why do you stay here sometimes? _____
    _____
27. **Ubani okunakekelayo lapha? [igama] /** Who is looking after you here? (name) sex [M] [F]_____
28. **Ubuhlobo umama / umalume / umngani etc.** / Relation (see below) _____
29. **Ngubani ongumninimuzi? /** Who owns the house?_____

30. **Ngubani oyinhloko yekhaya?(ubuhlobo) /** Head of the household? (Relationship)

_____

Asisoze sanikeza omunye umuntu imininingwane yakho kodwa kungenzeka sifune ukukuthola. Singabuza bani? / We will never give information about you to anyone, however we may want to find you. Who should we ask?

Person 3

31. **Sicela usinike igama lomunye umuntu osondelene naye esingamthinta uma singakwazi ukukuthola ocingweni lakho. /** Please give the name of another close person we can contact if we can`t get hold of you

32. **Igama? /** Name _____ sex [M] [F]

33. **Ubuhlobo** / Relation (see below)_____

34. **Kungabe uhlala endaweni efanayo njengeyakho** / Does he / she live in your area? [yebo] [cha]

35. **Ikheli lapho ehlala khona** / Physical address *(may be skipped)*_____

_____

36.

37. **Inombolo kamakhalekhukhwini** / Cell |___|___|___|-|___|___|___|___|___|___|___|

38. **Inombolo yocingo lwasekhaya** / Landline |___|___|___|-|___|___|___|___|___|___|___|

Person 4

39. **Uma ungase ube nohambo noma usuke kulendawo, ubani ongaba nemininingwane yakho yokukuthinta [ngaphandle kwalena engenhla]?** / If you were to travel or move away, who in your class or in school would have your contact details [other than the above]? _____

40. **Igama** / Name_____ sex [M] [F]

41. **Ubuhlobo umama / umalume etc** / Relation (see below) _____

42. **Ikheli lapho ahlala khona** / Physical address *(may be skipped)*_____

_____

43. **Inombolo kamakhalekhukhwini** / Cell |___|___|___|-|___|___|___|___|___|___|___|

44. **Inombolo yocingo lwasekhaya** / Landline |___|___|___|-|___|___|___|___|___|___|___|

Relation: M = mother. F= father. G= Legal guardian. B = brother. S = sister. Gm = grandmother. Gf = grandfather. U = uncle. A = aunt. C = cousin. Sf = Stepfather. Sm = Stepmother. Ss = Stepsister. Sb =Stepbrother. FR- friend, H=housekeeper. Ch= Child (son/daughter of study subject). Y= Husband / boyfriend of study subject. O = other. Ni = Niece. Ne=Nephew. X=study subject

BILHARZIA IN YOUNG WOMEN OF KWAZULU-NATAL
THE EFFECT OF TREATMENT

Information and request for participation in the research project

We, the Universities of Oslo in Norway and KwaZulu-Natal, are doing research on Bilharzia
To find out if and how Bilharzia affects young women.
To investigate if treatment works better in the young.
We are also hoping that maybe younger women will be better protected from sexually transmitted diseases and also HIV after treatment.

Why you are being asked to participate
We know that treatment kills the Bilharzia worms and works for urinary disease. Previous research has shown that treatment did not work properly for genital damage in older adult women. Now we wish to test it in young adults. We ask you
- because you may have had contact with the parasite in the water
- because you are still young
- because we hope to protect you from genital damage from Bilharzia.

Your decision
Participation in the study is entirely voluntary. You do not have to give a reason if you do not wish to participate. Your treatment now or in the future will not be affected by your decision. You may also interrupt any investigation as you wish. Approximately 2000 young women may participate.
If you are pregnant you can not join the study, but you are welcome to participate in the study two months after giving birth.

Consequences for you
Participating in this study will mean that
- you will give urine, stool and have blood tests, and an interview will be done
- you will have gynaecological investigations for diseases. If there is reason to do more tests during the examination you will be asked for permission.
- you will be taken to the VIBE youth clinic by a female driver
- samples will be tested for cancer, infections and HIV, and you decide if you want the results or not
- you will receive treatment for Bilharzia as recommended by the World Health Organisation
- you might be asked to participate for a period up to 2 years.

Gynecological examination
You will have an ordinary gynaecological investigation to look for genital Bilharzia and other genital problems by experts. The procedure is uncomfortable and it is better if you relax. The examination will be done by a female doctor with a female nurse/assistant present in the room. The doctor will explain the procedure to you before the examination. You will be lying in a special chair with your legs spread apart. A speculum will then be inserted in your private parts, samples will be collected and findings will be documented.

Risks
The tablets for Bilharzia may have side effects; some will feel sick for a couple of days. Some may get a rash, diarrhoea, or vomit. People with many worms feel worse as the worms die.

Benefits
You will receive treatment for any disease we discover at no charge and be referred if necessary. Your Bilharzia worms will die and you will feel healthier

How your samples and personal data are taken care of
Samples will be analysed and stored *without* your name on it. This will be done both before and after treatment so that the investigators can see if the disease is better. The specimens will be investigated in the best laboratories *without* your name. Samples might be sent to other countries for analysis by experts. If you agree to participate in the study, you also give permission for this. All information will be stored securely. The South African ethical committee may come to check the work and will therefore have access to the data. The confidentiality will not be breached unless required by law (if someone is currently abusing, hurting or planning to kill or if called by court), or unless you give written permission.
The samples and information will only be used to study Bilharzia together with women's diseases, risks for cancer and HIV. It will not be used for other purposes. The Principal Investigators and the doctors of the study are formally responsible for the security. These have access to the address file. If important information is discovered during the study we will make sure that you are informed, if you wish.

Who approved the project
The project has been reviewed and approved by the KwaZulu-Natal Department of Health and ethical committees in both South Africa and Norway.

Economy
The study is financed by international research grants. There are no plans for collaborations with industry, nor plans for commercialisation. The researchers involved in the study have no personal financial gain in connection with this study.

Project Management/More information
If you have any questions regarding the study, please feel free to contact the Principal Investigator and project manager: Dr M Taylor 031 2604499 or 2661592.

Contact details in case you have problems. We will also contact the person below in case of adverse events: Medical Research Administration
tel.: (031) 260 4495; fax: (031) 260 4410; e-mail: ethicsmed@nu.ac.za.
The participants of the study: Dr M Taylor, Dr J Kvasvig 031 260-4499
The Department of Public Health Medicine at the University of KwaZulu-Natal
Dr. Eyrun Kjetland, Department of Infectious diseases, University of Oslo, Norway
Dr Elisabeth Kleppa 079 194-2652

Your rights
If you agree to participate in the study, you have the right to access all personal information we have registered about you. You have the right to correct any faulty information. You may at any time withdraw from the study. If consent is withdrawn, you may request material/ information to be destroyed/ deleted.

Consent to participate in the research project

You have been informed about the study by _____

Participation in the study is based on voluntary, informed consent. You are free to ask for any additional information. If you, after having received all the information you deem necessary, wish to participate in the study, you must sign this consent form

I, _____(name in capital letters), confirm that I have received written information about the study and have had the opportunity to ask for additional information, and that I will participate in the project.

Signature_____Date_____
(Signed by the project participant)   (Dated by the project participant)

If you would like information about the study as we go along, how do you wish to be contacted?
_____
_____
_____
_____
_____
_____
_____
_____
_____

UNIVERSITETET I OSLO

DET MEDISINSKE FAKULTET

Dr. Eyrun F. Kjetland
Infeksjonsmedisinsk avdeling
Ullevål universitetssykehus
Internpost

Regional komité for medisinsk forskningsetikk
Øst- Norge (REK Øst)
Postboks 1130 Blindern
NO-0318 Oslo

Telefon: : 228 44 667
Telefaks: 228 44 661
E-post: i.s.nyquist@medisin.uio.no
Nettadresse: www.etikkom.no

Dato: 17. september 2007
Deres ref.:
Vår ref.: 469-07066a 1.2007.535
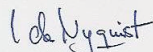
Schistosomiasis in young women and girls of KwaZulu-Natal,
manifestations, effect of treatment, association with HIV

It is hereby confirmed that the study "Schistosomiasis in young women and girls of KwaZulu-Natal, manifestations, effect of treatment, association with HIV" was approved by the Regional Committee for Medical Research Ethics of Eastern Norway September 7th 2007.

Med vennlig hilsen

Knut Engedal (sign.)
professor dr.med.
leder

Ida Nyquist
sekretær

20 February 2009

Prof. M Taylor
Department of Public Health Medicine
Nelson R Mandela School of Medicine
University of KwaZulu-Natal

PROTOCOL: Schistosomiasis in young women and girls of KwaZulu-Natal, manifestations, effect of treatment, association with HIV. Professor Myra Taylor. Department of Public Health Medicine. Ref: BF029/07.

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application.

The study was approved by a quorate meeting of BREC on 10 April 2007 pending appropriate responses to queries raised. Your responses dated 04 February 2009 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from **today; 20 February 2009.**

This approval is valid for one year from **(today's date, 20 February 2009).** To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.
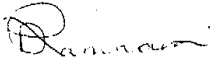
Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2004), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/ResearchEthics11415.aspx.
BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

pp **PROFESSOR D R WASSENAAR**
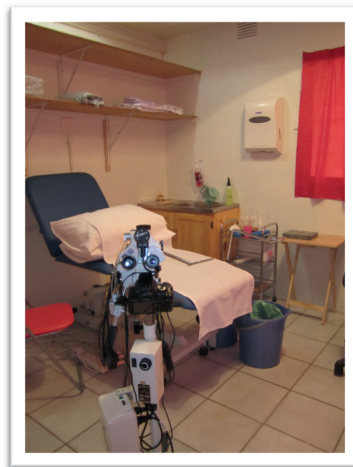**Chair: Biomedical Research Ethics Committee**

Photographs from the project site in KwaZulu-Natal.
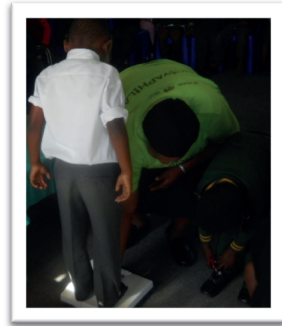

1. The project was situated in rural KwaZulu-Natal.

2. Building the research clinic in Ugu district.

3. Clinical examination room with colposcope.

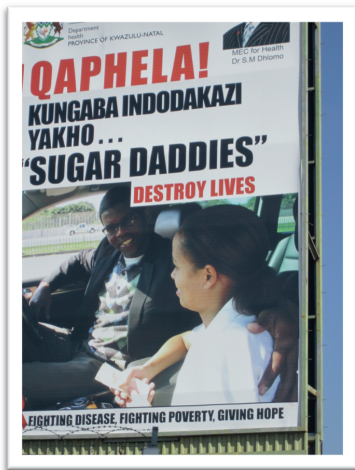4. Fetching study participants from high schools.
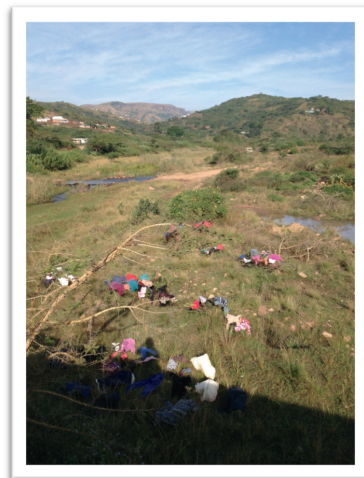

5. Mass treatment with praziquantel.

6. Urine samples were collected.

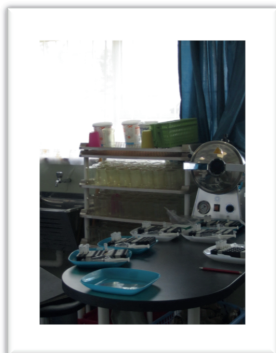7. A typical high school during a school break.

8. Poster warning young girls against "sugar daddies" at the South African border.

9. Laundry by the river. This may be a possible transmission site for *S. haematobium*.

10. Poster from the Department of Health with information about schistosomiasis.

11. Autoclaving and preparing equipment for gynaecological examinations.

12. Urine microscopy at the research clinic.

13. Sipho Zulu running the flow cytometry at the University of KwaZulu-Natal, Durban.

Photographs: Ilse van de Koppel and Elisabeth Kleppa