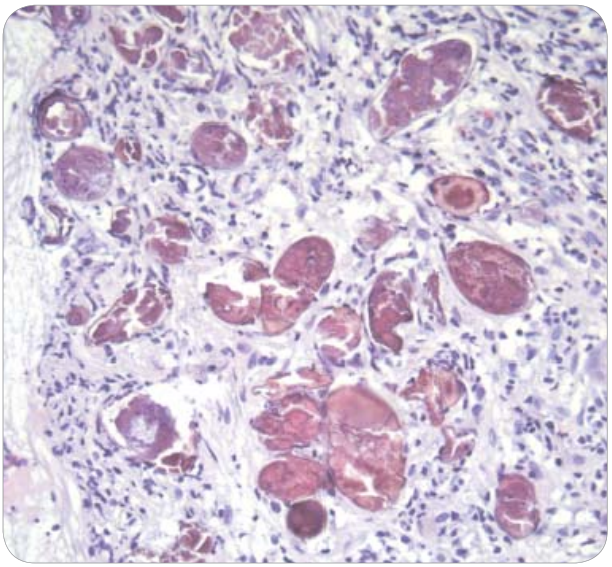
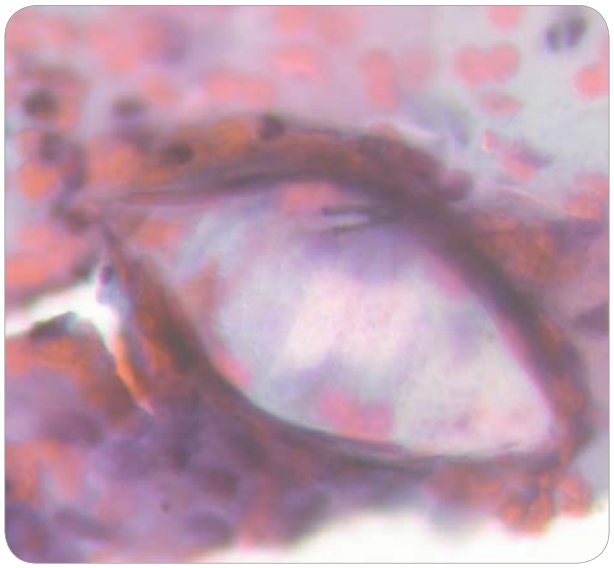
REPORT OF AN INFORMAL WORKING GROUP MEETING ON UROGENITAL SCHISTOSOMIASIS AND HIV TRANSMISSION

Geneva, Switzerland, 1–2 October 2009

World Health Organization





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Preface

Recent investigations have provided information about a possible association between urogenital schistosomiasis and human immunodeficiency virus (HIV) infection, but the available epidemiological evidence is not sufficient to make policy recommendations. The Bill & Melinda Gates Foundation convened a meeting in Seattle, Washington, United States of America, in April 2009 to review the existing evidence for an association between *Schistosoma haematobium* infection and risk for HIV infection among women and to determine whether there are short-term opportunities to gain additional knowledge rapidly. The Gates Foundation is considering multiple investments: for retrospective analysis of adults enrolled in a cohort of couples discordant for HIV infection to determine whether there is an association between HIV status and *S. haematobium* infection, and for an investigation of whether current schistosomiasis control programme recommendations are sufficient to prevent female urogenital schistosomiasis, by cross-sectional follow-up of previously treated women and a study of prospective treatment of schoolgirls.

WHO convened an informal working group to address: methodological issues, such as the diagnosis and clinical description of female genital schistosomiasis; opportunities for integrating new studies into existing large-scale schistosomiasis treatment programmes; and ethically acceptable designs for such prospective studies. Agreement on these guidelines opens the door for further investment in studies of the potential causal association between urogenital schistosomiasis and HIV infection.

1. Background

1.1 History and terminology

Genital schistosomiasis was first described by Theodor Bilharz, who observed eggs in seminal vesicles. In 1899, Madden published the first case report of schistosomiasis in the vagina of a girl (Madden, 1989), and in the same year Petrides described a case of uterine schistosomiasis (Petrides, 1899). Subsequently, numerous case reports and pathological studies were published, confirming the frequent involvement of female and male genital organs in *S. haematobium* infection. In 1989, the first community-based study was conducted to investigate the prevalence of vaginal schistosomiasis in Niger (Renaud et al., 1989).

Urogenital schistosomiasis is a persistent disease acquired primarily in childhood by exposure to *S. haematobium,* one of the two main schistosome species that are endemic in Africa. Approximately 120 million people in Africa are infected with *S. haematobium*, and they develop disease in the urinary or genital tract. The term **'**urogenital schistosomiasis' was coined to reflect the frequent co-existence of urinary and genital schistosomiasis in the same individual (Mühlens et al., 1942). Later, Gentilini et al. (1986) stated that "female genital schistosomiasis is clinically a frequent and anatomically a constant condition".

1.2 Female genital schistosomiasis

Female genital schistosomiasis can be defined as the presence of ova in the female reproductive organs or a characteristic clinical pathology (Feldmeier, Krantz, Poggensee, 1995; Kjetland et al., 2005). The clinical picture may consist of a range of signs and symptoms and may affect external and internal genital organs; however, *S. haematobium* ova can also be found in normal tissue. The possible clinical manifestations of female genital schistosomiasis are summarized in Table 1.

Table 1. Sequelae of female genital schistosomiasis

Organ affected	Sequel	Evidence
Vagina, vulva	Destruction of hymen or clitoris	Case report
	Vesico-vaginal fistula	Case report
	Contact bleeding	Case report
	Spontaneous bleeding	Case report
	Dyspareunia	Case report
	Increased susceptibility for sexually transmitted infections	Hypothesis
Cervix	Genito-pelvic discomfort	Community-based study
	Increased susceptibility for HPV and HIV infection*	Circumstantial evidence
Uterus	Miscarriage, premature labour	Anecdotal
Fallopian tubes	Ectopic pregnancy	Case report
	Infertility, subfecundity	Submitted for publication
Ovaries	Delayed puberty	Community-based study
	Infertility, subfecundity	
	Menstrual irregularities	Hypothesis

Placenta	Preterm delivery	Case report
	Small-for-date infant	Case series
Douglas space	Haemoperitoneum	Case report

* HPV, human papillomavirus; HIV, human immunodeficiency virus

1.3 Male genital schistosomiasis

Male genital schistosomiasis is defined by the presence of ova in semen or characteristic clinical pathology. Claude Barlow first demonstrated that schistosomiasis can cause haemospermia after he deliberately infected himself with *S. haematobium* cercariae in 1944 and then observed haemospermia and the presence of ova in his semen (Barlow and Meleney, 1944). The presence of eggs of *S. haematobium* was reported in more than 40% of semen samples from infected individuals in an endemic area (Leutscher et al., 2000a). The possible clinical implications of male genital schistosomiasis are summarized in Table 2.

Table 2. Sequelae of male genital schistosomiasis

Findings	Evidence
Erectile dysfunction, painful ejaculation	Community-based study
Infertility	Community-based study
Acute orchitis	Case report
Chronic prostatitis	Case report
Vesiculitis	Ultrasound scan study
Cancer of testis	Anecdotal
Increased shedding of HIV	Hypothesis

2. Epidemiology, pathophysiology and pathology[1]

2.1 Frequency of female and male genital schistosomiasis

Female genital schistosomiasis is a common manifestation of infection with *S. haematobium*, even in lightly infected individuals with few eggs excreted in the urine (Leutscher et al., 1997). Egg granulomas and subsequent disease can occur anywhere in the genital tract. Hitherto, only a few population-based studies have provided data on the frequency of female genital schistosomiasis of the lower genital tract, and estimates for the upper genital tract do not exist (Table 3).

Table 3. Prevalence of female genital schistosomiasis in community-based studies

Country	Group	No.	Prevalence (95% CI)
Niger	Community	61	75 (65–87)
Malawi[a]	Outpatients	51	63 (48–76)
Madagascar	Community	36	33 (19–51)
Tanzania (United Republic of	Community	122	43 (39–56)
Tanzania (United Republic of)	Community	263	55 (48–61)

From Renaud et al., 1989; Kjetland et al., 1996; Leutscher et al., 1998; Poggensee et al., 2000; Kjetland et al., 2005 CI, confidence interval
[a]Only in women with urinary schistosomiasis

Post-mortem and systematic histopathological studies have shown the frequency of female genital schistosomiasis in selected settings. Autopsy studies have shown that schistosomiasis can affect any genital organ, the cervix being the commonest site, followed by the vagina and the Fallopian tubes (Table 4).

Table 4. Frequency of female genital organ involvement in post-mortem studies

Genital organ	Frequency of organ involvement (%)	
	All cases	Patients with heavy infection
Vagina	11–18	100
Cervix	24–87	100
Uterus	12–82	66
Fallopian tubes	7–63	33
Ovaries	11–67	33

From Charlewood et al., 1949; Gelfand, 1950; Youssef et al., 1970; Gelfand et al., 1971; Edington et al., 1975

[1] Based on a presentation by Professor H. Feldmeier at the meeting

Male genital organs are also frequently affected (Table 5).

Table 5. Frequency of male genital organ involvement in a post-mortem study in Zimbabwe

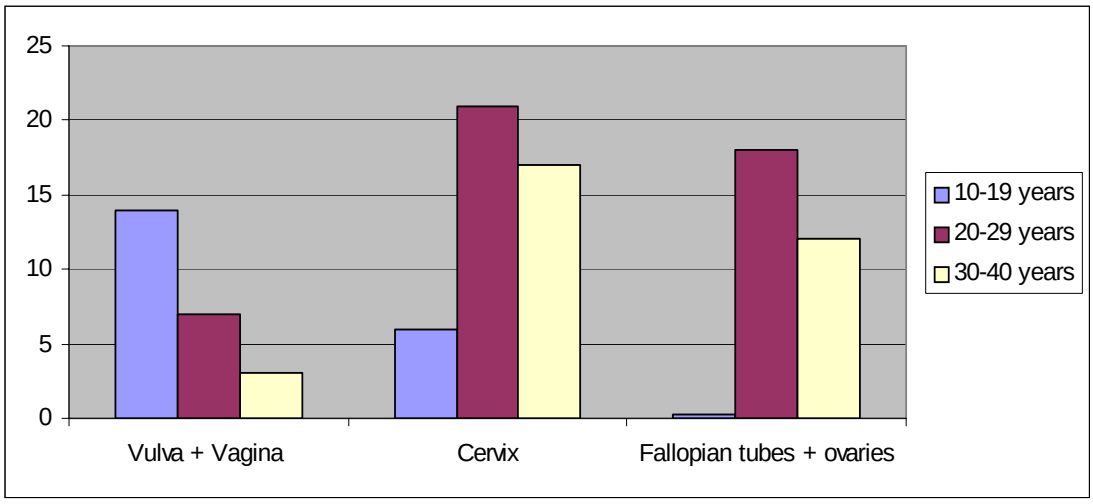
Organ	Frequency (%)	Eggs per gram
Bladder	56	129 000
Seminal vesicles	55	415 000
Vas deferens	40	3 700
Prostate	21	75

From Gelfand et al., 1970

2.2 Organ involvement according to age

Specimens are rarely taken from the cervix or vagina of girls or young women; however, an analysis of post-mortem and histopathological studies shows that the vulva and the vagina may be the organs most frequently affected during puberty and adolescence. Lesions have been seen to shift to the cervix, the ovaries and the Fallopian tubes with increasing age (Figure 1). Therefore, lesions may be present when a girl reaches sexual maturity, and the barrier function of the vagina and the cervical epithelium may already be impaired before sexual debut. Female genital schistosomiasis could therefore contribute significantly to the spread of agents of sexually transmitted infections in those countries where urogenital schistosomiasis is endemic (Feldmeier et al., 1995; Helling Giese et al., 1996; Poggensee, Feldmeier, Krantz, 1999).

Figure 1: Relation between age and genital organ involvement*.



*Sources: Charlewood et al. 1949, Edington et al. 1975, Gelfand 1950, Gelfand et al. 1971, Youssef et al 1970, Williams 1967, Bland and Gelfand 1970.

2.3 Association with sexually transmitted infections

Female and male genital schistosomiasis co-exist in areas endemic for *S. haematobium*. Co-infection with agents of sexually transmitted infections is also frequent in both females and males, as shown in a study in Madagascar, in which 35% of females and 17% of males with urogenital schistosomiasis had one or more sexually transmitted infections (Leutscher et al., 2008).

The presence of pathological alterations in the vagina or cervix before sexual debut and a relatively high prevalence of HIV infection in sexually active males support the hypothesis that female genital schistosomiasis might increase the transmission of HIV in the female population (Badawy, 1962; Diouf, Spay, Toure, 1973; Koller, 1975; Kjetland et al., 1996; Poggensee, Feldmeier, Krantz, 1999; Poggensee et al., 2000; Kjetland et al., 2006).

3. Association between female genital schistosomiasis and HIV infection[2]

A competition organized in 1992 by the International Development Research Centre and the WHO Special Programme for Research and Training in Tropical Diseases on Gender and Tropical Diseases stimulated scientific interest in schistosomiasis. Feldmeier, Krantz and Poggensee (1995) conducted an extensive literature review to explore the parasitological, clinical and epidemiological characteristics of female genital schistosomiasis and formulated the hypothesis that this disease facilitates the transmission of HIV and human papillomavirus (HPV) (Krantz I, Feldmeier H, 1994; Feldmeier, Krantz, Poggensee, 1994; Feldmeier, Krantz, Poggensee, 1995; Feldmeier, Krantz I, 1996; Feldmeier et al., 1998; Poggensee, Feldmeier, Krantz, 1999).

3.1 Biological plausibility

The biological plausibility of a causal association between female genital schistosomiasis and HIV infection is based on two concepts: impaired barrier function of the genital epithelium and deleterious effects of the characteristic granulomatous inflammation and, later, immunomodulation in schistosomiasis (see section 3.1.2).

3.1.1 Impaired barrier function

Genital schistosomiasis is thought to increase susceptibility to HIV infection because of the breaks in the vaginal and cervical epithelium caused by thinning, erosion, inflammation or ulceration. Breaks in the integrity of the epithelial barrier, due to either trauma or ulcerative sexually transmitted infections, are associated with an increased risk for HIV infection (Rebbapragada et al., 2007; Rebbapragada et al. 2008; Kaul et al., 2008). Kjetland et al. (2006) showed that women with laboratory-proven genital schistosomiasis had a higher risk for HIV-1 infection than women without genital schistosomiasis.

Schistosomiasis of the cervix is characterized by sandy patches and neovascularization (Kjetland et al., 2005). The latter finding is important, as neovascularization of the epithelium could provide direct access to the systemic circulation for HIV (Greenhead et al., 2000). The same holds true for contact bleeding during sexual intercourse. Feldmeier, Krantz and Poggensee (1994) suggested that HIV in semen might have easier access to deeper genital cell layers in women with genital schistosomiasis because of the friable, eroded epithelium or through broken vessels during coitus, creating direct points of contact between HIV and the receptive cells in genital tissue. These pathological alterations may also increase the transmission of HIV from infected women to healthy men. Alterations in the epithelium of the cervix may also facilitate infection with and propagation of HPV (Krantz, Feldmeier, 1996; Feldmeier et al., 1997). Petry et al. (2003) suggested in a case report that the presence of urogenital schistosomiasis is associated with an increased risk for infection with high-risk HPV genotypes.

[2] Based on a presentation by Dr Gabriele Poggensee at the meeting

3.1.2 Modulation of immune response

There is increasing evidence that schistosomiasis modulates the transmission of HIV infection in several deleterious way (Poggensee et al., 2000; Poggensee et al., 2001). First, immune cells in the genital lesion or in adjacent areas may offer an alternative route for HIV infection (Fincham, Markus, Adams, 2003; Sheffield et al., 2007). In fact, immunologically activated areas in the genitals may provide easy points of entry for HIV to attach to susceptible cells (Wald, 2002; Rebbapragada et al., 2008; Wira, Fahey, 2008). Secondly, schistosome ova elicit a complex cellular and humoral immune response. The granulomata that form around an egg, with a diameter 5–10 times larger than that of the ovum, are composed of activated lymphocytes, macrophages, epithelioid cells and Langerhans giant cells, cell types that express CD4 cells, the receptors used by HIV-1 to enter the host cell (Harms, Feldmeier, 2002). These cells are abundant within egg granulomas and adjacent areas. (Helling-Giese et al., 1996). Thirdly, eosinophils may sometimes represent almost 50% of the granuloma cells (Helling-Giese et al., 1996; Weinstock et al., 1999), and these cells also express CD4+ receptors (Weiss, 2002). Fourthly, patients with active schistosomiasis have higher cell surface densities of CCR5 and CXCR4 HIV receptors on CD4 T cells and monocytes in peripheral blood samples (Secor et al., 2003). Cells with higher levels of these receptors are more susceptible to HIV infection (Weinstock et al., 1999; Ganley-Leal et al., 2006). In addition, more HIV co-receptors (CCR5) have been found on monocytic cells recruited to genital lesions than in nearby healthy tissue (Sheffield et al., 2007). Taken together, the immunological characteristics of schistosomiasis in general and the clinicopathological characteristics of female genital schistosomiasis in particular, make infection of women with HIV and rapid propagation of the infection likely.

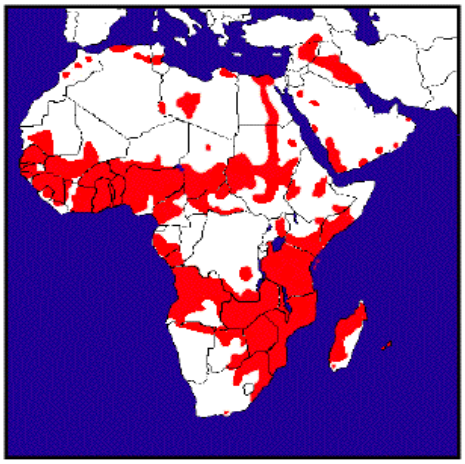
3.2 Epidemiological evidence

3.2.1 Spatial overlap

Almost 85% of people infected with schistosomes live in sub-Saharan Africa, the geographical region most heavily affected by the HIV epidemic (68% of all people infected with HIV worldwide and 76% of AIDS deaths occur in this region) (UNAIDS, 2007). Several studies have shown an unexplained gender ratio of HIV-1 infection that disfavours rural women, with a prevalence of HIV infection in young women up to eight times higher than in young men in some rural settings (UNAIDS, 2008). Figure 2 shows a disturbing overlap of areas endemic for *S. haematobium* and areas with a high prevalence of HIV-1 infection in females.

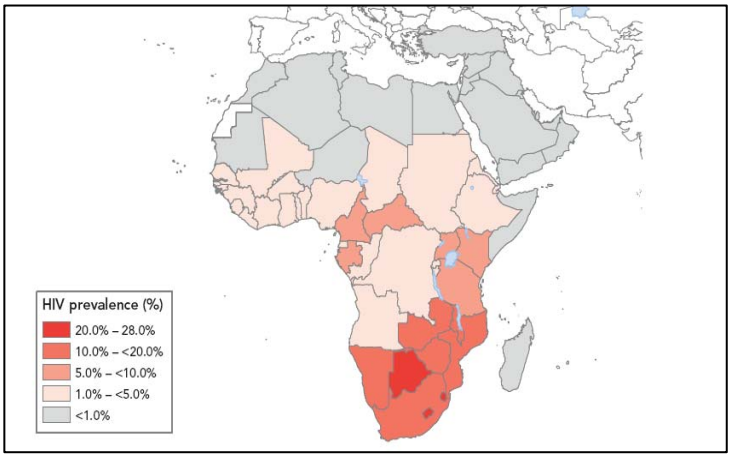
Figure 2: Geographical overlap of SCH and HIV distribution

Urogenital schistosomiasis



From King, 2001

HIV prevalence (%) in males and females aged 15–49 years in Africa, 2007



From UNAIDS, 2008

3.2.2 Temporal association

Infection with *S. haematobium* is usually a rural phenomenon. In the early phases of the HIV epidemic, the greatest increase in prevalence of HIV infection occurred in the Great Lakes region of sub-Saharan Africa, in countries such as the Central African Republic, Kenya, Malawi and Uganda. In this region, the prevalence of *S. haematobium* in the study population was up to 70% (Barongo et al., 1992), and the prevalence of HIV infection in these areas was calculated to be 1.2–1.7 times higher in women than in men during the early phase of the epidemic (Feldmeier et al., 1994).

3.2.3 Other epidemiological evidence

In sub-Saharan Africa, the characteristic age-dependent prevalence curve of *S. haematobium* infection and the age-specific distribution of HIV infection in women follow a similar pattern (Table 6, below). The prevalence of HIV infection peaks earlier in rural women.

Table 6. Frequencies of urinary schistosomiasis and HIV infection in sub-Saharan Africa

Age group (years)	Frequency of urinary schistosomiasis (%)		Frequency of HIV infection (%)	
	Male	Female	Male	Female
0–4	51	50	3 (2–14 years)	2 (2–14 years)
5–9	60	40		
10–15	80	78		
16–25	52	78	2 (15–19 years) 5 (20–24 years)	7 (15–19 years) 21 (20–24 years)
25–40	58	59	16 (25–29 years)	33 (25–29 years)

From Hatz et al. 1990 (United Republic of Tanzania)

3. 3 Knowledge gaps

Despite the significant amount of information that has been generated over the past 15 years with respect to a putative cause–effect association between female genital schistosomiasis and HIV infection, the epidemiological and biological evidence remains inadequate.

3.3.1 Link between female genital schistosomiasis and acquisition of HIV infection

If genital schistosomiasis is a risk factor for infection of women with HIV at a population level, it interacts with other risk factors, such as the presence of genital ulcer disease, age at sexual debut and number of partners. Thus, the attributable risk of female genital schistosomiasis for infection with HIV may vary from setting to setting. Studies of a cause–effect relationship must take the broad range of potential confounders into consideration.

3.3.2 Link between schistosomiasis and progression of HIV infection

Follow-up of a cohort of HIV-1-seropositive adults infected with *S. mansoni* infection did not show that the helminth infection caused more rapid progression of HIV infection to AIDS as compared

with people without schistosomiasis (Brown et al., 2004). Another study also failed to show that treating schistosomiasis is beneficial in the management of disease associated with HIV-1 infection (Brown et al., 2005). Because of methodological constraints, however, these studies did not allow a definitive conclusion about the relationship studied. In other studies, treatment of schistosomiasis was beneficial to patients with HIV-1 infection (Kallestrup et al., 2005). A systematic review of observational studies and one randomized study showed that there may be a potential benefit of anthelminthic treatment in HIV co-infected individuals, although more studies are needed (Walson, John-Stewart, 2007). It should be noted, however, that almost all the studies of immunological alterations occurring during chronic schistosomiasis in humans involved *S. mansoni* and not *S. haematobium* schistosomiasis.

3.4 Methodological challenges[3]

The possible association between genital schistosomiasis and HIV infection is complex. The risk factors for genital schistosomiasis are unknown, the history of granuloma formation and resolution in genital tissue is poorly understood, and reliable disease markers are not available. Multidisciplinary efforts are therefore required to generate conclusive epidemiological, clinical, virological and parasitological evidence to evaluate the cause–effect relationship between the two disease entities.

Randomized controlled trials in which women with known genital schistosomiasis and no HIV infection were allocated to different cohorts would be the most rigorous way of establishing a cause–effect relationship. Given the life-long complications that may result from withholding treatment from people with schistosomiasis or HIV infection, however, it would be unethical and therefore impossible to conduct such studies. Hence, alternative study designs are needed that have sufficient power to prove or refute the hypothesis of an association between urogenital schistosomiasis and HIV infection.

In view of the methodological challenges and the many possible confounders, future studies should be conducted with standardized tools. The specificity and sensitivity of diagnostic approaches have to be known. The same applies for development of a clinical algorithm. The diagnostic gold standard must include an ethical component: the best diagnostic method, searching for eggs in genital tissue by biopsy, temporarily places women at an unnecessary risk for sexually transmitted and HIV-1 infections. Laboratory techniques with polymerase chain reaction (PCR), e.g. for detecting parasite DNA (in lavage specimens, Pap smears, blood spots, urine), require sophisticated laboratory infrastructure, are expensive and are therefore difficult to perform in areas endemic for *S. haematobium*. Other diseases that may mimic schistosomiasis-associated lesions, including precancerous and cancerous alterations, must be considered and ruled out as differential diagnoses.

Data on several populations in Africa show substantial differences in the prevalence of HIV infection between females of the same age group, presumably as a result of behavioural changes over time at the community level (e.g. delayed sexual debut, fewer concurrent partnerships). Thus, the risks for HIV infection of girls aged 16–20 years at the beginning of a longitudinal study on genital schistosomiasis and HIV infection might be different from those that girls aged 12–14 years would encounter when they are 16–20 years old. Hence, differences in the prevalence of HIV infection between older and younger girls during such a study might reflect changes in risk behaviour rather than an effect of mass treatment with praziquantel in previous years. Any study design must take such confounders into consideration.

[3] Based on a presentation by Dr Birgitte Vennervald at the meeting

3.5 Ethical issues[4]

Research on the association between female genital schistosomiasis and HIV infection raises delicate and difficult ethical issues, which must be dealt with delicately and with great circumspection. Colposcopy is unacceptable in virgins and may not be acceptable even in sexually active adolescents and women in certain cultural settings. Biopsies may temporarily expose women to a risk for infection with sexually transmitted agents, including HIV, if their partners do not accept the use of condoms after the examination.

While studies of the association between schistosomiasis and HIV infection may have large benefits for women with genital schistosomiasis, great care should be taken to ensure that the research is based on a sensitive balance of the risks and benefits for the populations in which the studies are conducted. These studies must also seek to ensure adherence to the basic ethical principles of autonomy, beneficence, equity and non-malificence, which are considered to apply across cultures. Annex I summarizes some of the ethical issues that should be considered in designing studies on female genital schistosomiasis and the association with sexually transmitted infections, particularly HIV.

4. Diagnosis[5]

4.1 Overview

Schistosomiasis in the reproductive tract is usually diagnosed by incidental observation of schistosome eggs in histological sections made from biopsy samples taken during gynaecological examination or laparoscopy (Williams and Keen, 1967; Bland, Gelfand, 1970). *S. haematobium* ova have, however, also been found in tissue that may seem normal. Moreover, ova located in clusters are sometimes missed behind typical lesions unless several histological sections are done. This is a matter of concern, as female genital schistosomiasis is a common disease in endemic areas, and untreated genital schistosomiasis may be a significant health hazard for affected women.

Six categories of approach are available for the diagnosis of schistosomiasis in the reproductive tract: (i) the syndromic approach, (ii) macroscopically observable alterations of the genital epithelium, (iii) colposcopy, (iv) demonstration of eggs, (v) immunological and molecular disease markers presumably released during the development of egg-related lesions, and (vi) ultrasound.

4.1.1 Syndromic approach

Female genital schistosomiasis has been suggested to cause a wide range of genital symptoms and signs; however, most reports are case reports, and in only two studies was there comprehensive control for sexually transmitted diseases (Harouny, Pedersen, 1988; Ville et al., 1991; Leutscher et al., 1997; Kjetland et al., 2008). Furthermore, symptoms such as dyspareunia, intermenstrual bleeding and bloody vaginal charge are not specific for genital schistosomiasis and occur in many other infectious and non-infectious diseases of the female genital tract (Leutscher et al., 1997; Poggensee et al., 2000; Kjetland et al., 2008).

4.1.2 Clinical diagnosis

Lesions associated with genital schistosomiasis may mimic a host of infections and premalignant or malignant conditions occurring in the vagina or cervix. It is therefore crucial to identify alterations that are pathognomic. Sandy patches and neovascularization are considered to be strongly associated with the presence of eggs in the stroma or epithelial tissue (Poggensee et al., 2000; Kjetland et al., 2005), but it is not known whether these alterations are specific for genital schistosomiasis.

[4] Based on a presentation by Dr Patricia Ndhlovu at the meeting
[5] Based on a presentation by Professor H. Feldmeier at the meeting

4.1.3 Colposcopic diagnosis

Colposcopy gives the same findings as clinical diagnosis, except that more subtle findings may become visible and the application of acetoacetic acid may assist in differential diagnosis (Kjetland et al., 2005).

4.1.4 Microscopic diagnosis

The most sensitive technique for parasitological diagnosis remains the quantitative compressed biopsy technique, in which a biopsy is taken from a suspected lesion and the tissue is compressed between two glass slides (Kjetland et al., 1996; Poggensee et al., 2001). This technique is more sensitive than histological sections made from a biopsy sample, and its specificity is 100%, as eggs cannot be mistaken for artefacts (Poggensee et al., 2001). If malignant-looking lesions are found, it is advisable to take several biopsy samples to exclude malignancy (Petry et al., 2003).

Two other diagnostic methods have been tested: examination of routinely obtained cervical Pap smears and wet examination of scrapings of the epithelium of the vulva or the vagina. The sensitivity of Pap smears is extremely low and cannot be recommended (Table 7). Scraping the epithelium with a spatula and wet smears are valid supplements (Swart and van der Merwe, 1987; Savioli, Gabrielli, Neve, 1990).

Table 7. Diagnosis of female genital schistosomiasis by wet crushed biopsy, histological sectioning and Pap smear in Malawi, 1994

Organ biopsied	Eggs detected by			
	QCBT	Histological sectioning	QCBT and histological sectioning	Pap smear
Cervix	49%	44%	65%	4%
Vagina	36%	50%	58%	
Vulva	50%	50%	50%	

From Kjetland et al., 1996; Feldmeier et al., 2001
QCBT, quantitative compressed biopsy technique

Examination of urine for the presence of eggs does not exclude female genital schistosomiasis nor prove its occurrence. In fact, this condition has frequently been reported in women with scanty or even no egg excretion in urine (Camain, 1953; Bland and Gelfand, 1970; Gloor et al., 1979; Vass and Lucey, 1982; Attili, Hira, Dube, 1983; Goldsmith et al., 1993). Even if a large volume of urine is filtered through a polycarbonate membrane and filtration is repeated on 3 consecutive days, 23% of women with genital schistosomiasis would falsely be classified as negative (Poggensee et al., 1998).

4.1.5 Immunological disease markers and PCR

Various putatively useful molecular markers have been suggested, but only a few have been validated (Poggensee et al., 1996; Table 8). Studies with eosinophil cationic protein, a cytotoxic granule protein released by activated eosinophils, as a diagnostic marker have had mixed results (Poggensee et al., 1996; Leutscher et al., 2000b; Midzi et al., 2003). The usefulness of *S. haematobium* egg antigen or PCR for detecting schistosome DNA in vaginal fluid should be investigated (Kjetland et al., 2009). With a dip-stick-like technique, egg antigen could be detected in elutions of sanitary pads designed for this purpose, allowing diagnosis at primary health care level. Similar samples could be used for PCR analysis.

Table 8. Immunological markers studied in female genital schistosomiasis

Marker	Determined in	Positive	Negative	*p*
Eosinophil cationic protein (semiquantitatively on a scale of 0–3)	histological sections by immuno-histochemistry	1.0 (1–2)	0 (0–0.5)	0.01
Eosinophil cationic protein (ng/ml)	lavage	134.0 (39–535)	24.3 (16.6.–39.9)	0.03
Neopterin (nmol/l)	lavage	11.3 (5.2–17.4)	3.8 (3.4–7.3)	NS
Total immunoglobulin A (mg/l)	swab eluate	7.0 (4.0–9.5)	6.3 (2.9–12.3)	NS

From Poggensee et al., 1996. Data are medians and 95% confidence intervals; NS, not significant

4.1.6 Ultrasound

Ultrasound is a well-established, non-invasive method in gynaecological and obstetrics practice all over the world. It has great advantages in terms of safety, reliability, acceptability and relative economy. Ultrasound studies in women excreting eggs in urine have shown a number of nonspecific findings in the presence of urogenital schistosomiasis (Richter et al., 1995), but it is not known whether these alterations specifically reflect schistosomiasis of the upper reproductive tract (Richter et al., 1995; Helling-Giese et al., 1996; Leutscher et al., 1998). As transvaginal ultrasound is the method of choice for examination of genital organs of the upper reproductive tract, the true prevalence of lesions of the upper genital organs in women with schistosomiasis may be underestimated by transabdominal ultrasound. Use of recently developed high-resolution transvaginal probes may make it possible to detect relevant lesions in genital organs.

4.2 Knowledge gaps

Reliable diagnostic tools are not available. The sensitivity and specificity of colposcopic findings and of markers present in vaginal fluid are not known. Use of a syndromic approach with a clinical algorithm (with or without a simple laboratory test) must be explored further (Kjetland, 2008) .

4.2.1 Validity of available diagnostic techniques

Use of urinanalysis reagent sticks to detect haematuria failed to identify more than half of women with ova in genital tissue (Feldmeier and Krantz, 1993; Poggensee, Feldmeier, Krantz, 1999). Pap smears have a very low sensitivity (Feldmeier et al., 2001) and cannot substitute for the quantitative fresh compressed biopsy technique. Whether the presence of grainy sandy patches—alterations considered to be characteristic of schistosomiasis in the cervix—are actually pathognomic for ova in tissue remains to be demonstrated. Nonspecific findings such as neovascularization, homogeneous yellow patches and other mucosal signs should be explored as co-indicators of genital schistosomiasis (Kjetland, 2005).

4.2.2 Validity of a clinical algorithm

Most cases of female genital schistosomiasis occur in areas where diagnostic tools for gynaecological diseases are unavailable (Kjetland et al., 2005). Given the nature of the symptoms and signs, women are likely to approach a clinic for sexually transmitted infections first. Yet, genital schistosomiasis is not on the list of differential diagnoses of sexually transmitted infections in most endemic countries (Lessing, 1998). Therefore, women with genital schistosomiasis are often

misclassified as having a sexually transmitted infection, and, by consequence, these patients receive unnecessary, potentially harmful treatment.

The syndromic management of vaginal discharge and genital ulcer disease is an effective strategy in the prevention of HIV infection in developing countries (O'Farrell, 1999; Wilkinson et al., 1999). A similar approach could be used for female genital schistosomiasis. As a prerequisite, a clinical algorithm for this disease, in which the commonest sexually transmitted infections are considered as differential diagnoses, should be developed and tested. Presumably, its validity would be increased if the algorithm was extended by a simple laboratory parameter, e.g. detection of eosinophilic cationic protein in vaginal fluid with a dip-stick.

5. Treatment

5.1 Overview

The widespread lack of awareness of genital schistosomiasis leads to misdiagnosis and, therefore, false and ineffective therapy (Swai et al., 2006). As female genital schistosomiasis is rarely diagnosed correctly, knowledge about the effect of treatment is also scanty. Schistosomiasis-induced lesions of the ovaries and Fallopian tubes, but also of the cervix, have been treated surgically by ovarectomy or hysterectomy, which are debilitating, irreversible operations (Camain, 1953; Charlewood et al., 1949). While such approaches may be justified in severe symptomatology or during laparoscopy, ovarial or tubal masses can resemble malignancy, and rapid-section histopathology cannot be performed (Boers et al., 2003). Aggressive surgery is usually not warranted, neither for lesions in the upper reproductive tract nor for manifestations of female genital schistosomiasis in the lower reproductive tract.

Only one community-based study has addressed the effect of praziquantel on genital lesions (Kjetland, 2006). After 3 and 12 months, genital lesions were refractory to treatment, while the effect on urinary ova excretion was good. Similarly, in a study in which cystoscopy was performed repeatedly in returned travelers to explore the effect of praziquantel on sandy patches in the urinary tract, the lesions remained (Silva, 2006).

A number of case reports have shown regression or disappearance of lesions after treatment (Richter et al., 1996); however, most of the patients were not seen after nonsurgical treatment. It is not known how long it takes for macroscopically visible lesions in the cervix, vagina or vulva to disappear completely. A study in Zimbabwe of 25 selected cases with severe lesions indicated that lesions may remain in the same site after several rounds of treatment, even after 2 years (Kjetland, 2006). Presumably, healing depends not only on the topographic site at which the lesions have developed, but also on the histopathological characteristics of the lesion. Inflammation and erosion of the cervix, for example, may resolve relatively rapidly after treatment with praziquantel, whereas fibrosis (when an egg granuloma is replaced by a fibrotic scar) may take longer to resolve or may not heal at all. This conclusion is supported by the findings of Kjetland et al. (2006), who observed that sandy patches persisted more than 12 months after treatment with three doses of praziquantel (40 mg/kg body weight at baseline, then two doses of 30 mg/kg body weight given 5 hours apart at 3 months).

The rationale for using praziquantel is that, although the drug acts only against schistosomulae and mature worms, elimination of the latter prevents further egg deposition and thus further disease. This is analagous to the finding that granulomatous lesions in the urinary tract involute after a single dose of praziquantel, as assessed by ultrasonography. As the maximal regression of late symptoms such as hydronephrosis may take 3–4 years (King et al., 1992), however, it can be assumed that this holds true for genital lesions as well. Complete resolution of egg-induced disease depends on the number of worms present and the efficacy of praziquantel, and multiple doses may be needed for women with severe genital disease. Exploration into chemotherapeutic agents to treat adult women with genital schistosomiasis is needed (Ouma et al., 2005; Kjetland, 2008).

The development of gross, irreversible disease related to egg-induced tissue fibrosis in adulthood can be effectively prevented by early, regular treatment in childhood (Ouma et al., 2005; Kjetland et al., 2008b). Three treatments with praziquantel during primary school age reduce bladder disease at a later age to almost zero (Ouma et al., 2005). Even a single treatment in childhood prevents half the cases of female genital schistosomiasis (Kjetland, 2008). Praziquantel is also safe in pregnancy and can therefore be given to girls and women of childbearing age (WHO, 2003).

5.2 Knowledge gaps

Data are urgently needed to verify which type of genital disease reverses after treatment with praziquantel, how long this takes and whether healing depends on the regimen of praziquantel (dose, repetition, intervals between repetitions). Most travellers returning from endemic countries have light infections, and numerous case reports in nonendemic settings indicate successful treatment in the large majority of cases, although not all (Herwaldt et al., 1995; Boers et al., 2003; Lawn et al., 2003; Silva et al., 2005; Alonso et al., 2006). Treatment failure has been reported in up to 15% of nonimmune patients, indicating that the efficacy of praziquantel may depend on yet unknown variables (Duus et al., 2009).

6. Recommendations

There is a convincing biological plausibility for a link between female genital schistosomiasis and the acquisition of HIV. The working group discussed the recent epidemiological evidence for a cause–effect relationship between the two infections and made the following recommendations:

6.1 Terminology

Infection with *S. haematobium* should be called ‘urogenital schistosomiasis’ in the English literature, in analogy to ‘bilharziose urogenitale’, a term that has remained in use in the French scientific literature. This term recognizes the fact that schistosomiasis haematobia affect both the genital and the urinary tracts.

6.2 Diagnosis

The current diagnostic tools are either inappropriate or their validity is not known. Therefore, a series of studies is proposed.

6.2.1 Colposcopy validation study

The working group agreed that a standard case definition is critical to improving recognition of genital schistosomiasis during colposcopy. The design of a study for validating colposcopy was drafted. The objective is to determine the specificity of macroscopic alterations considered to be characteristic for genital schistosomiasis of the cervix and the vagina. Wet crushed biopsies of suspected lesions of the cervix will be used as a reference, as this method is considered to be the most specific for detecting ova in the lower reproductive tract. The group recognized that ova can occur at any site in the lower reproductive tract, even in the absence of colposcopically visible disease, and that therefore the diagnostic potential of a single biopsy is limited.

The prerequisites for the validation study are a high prevalence of genital schistosomiasis in the female population (as indicated by a high prevalence of egg excretion in urine), in order to identify and examine a large number of women suitable for the study in a short time; and a low risk for HIV

transmission in the study population. Previous experience with studies of female or male genital schistosomiasis at the field sites would be preferable.

The study requires standardization of colposcopy and diagnostic procedures, standardization of the descriptions of alterations in the vulva, vagina and cervix and review of the findings by external experts from digitalized video and photo documentation. Madagascar, Niger and Zambia were suggested as potential study sites.

6.2.2 Classification of the clinical pathology of the cervix

The working group suggested that a classification of the clinical pathology of the cervix could be attempted during a preparatory training course on colposcopy, which could be used to set a score reflecting the severity of genital schistosomiasis in a patient.

6.2.3 Validation of a clinical algorithm

A history of water contact, spontaneous or contact bleeding and genito-pelvic discomfort are frequent findings in patients with genital schistosomiasis but are also common in women of reproductive age. The combination of self-reported genital itch, yellow discharge and current weekly or childhood contact with water bodies allowed identification of 80% of cases of female genital schistosomiasis in one study (Kjetland, 2008). The working group suggested that a clinical algorithm be devised and its diagnostic validity determined with colposcopy and biopsy as the references. To determine the specificity of the algorithm, the status of the women with regard to sexually transmitted infections in the area must be determined.

6.2.4 Validation of putative laboratory disease markers

Schistosomiasis is usually diagnosed by parasitological (microscopic detection of eggs) or immunological methods (presence of specific antibodies or circulating schistosomic antigens). The demonstration of eggs in urine or faeces confirms the presence of adult worms, but failure to detect eggs, even after repeated examinations of urine or stool samples, does not exclude active schistosomiasis.

Antibody detection cannot reliably differentiate between past and present infection, and the antibody concentration does not correlate with the number of worms present. The detection of circulating parasite antigens (such as anodic and cathodic antigen) confirms the presence of an active infection, reflects the number of worms present and is useful for assessing the efficacy of treatment. The immunological methods currently in use, however, require skilled personnel and well-equipped laboratories, as a dip-stick method is not available. The performance of a commercially available point-of-care wicking assay for circulating cathodic antigen in urine has been uneven. Trials are being sponsored by the Schistosomiasis Consortium for Operational Research and Evaluation to determine the performance characteristics of this test in areas endemic for *S. mansoni* and in areas of low prevalence of *S. haematobium*. *Schistosoma* real-time PCR may also be a candidate for use in diagnosis in research and evaluation projects, especially in young patients (Kjetland, 2009).

As a granuloma develops, it increases in size and eventually transforms into a fibrotic scar. This is mirrored by a complex pattern of immunological responses, some of which can be measured from cytokines and other molecules released into adjacent tissue and body fluids. Eosinophilic cationic protein (or other markers of eosinophilic activation), interleukin-5 and other pro-inflammatory cytokines have been detected in vaginal lavage fluid of patients with genital schistosomiasis

(Poggensee et al., 1996; Leutscher et al., 2000b). The m-RNA of such cytokines could be sought in cellular material obtained with a self-administered swab or cytobrush from the vagina.

To determine the specificity of a laboratory marker, the infection status of women with regard to sexually transmitted infections will be determined.

6.3 Studies of the association between female genital schistosomiasis and risk for HIV infection

6.3.1 Opportunities

Studies in ongoing control programmes for either schistosomiasis or HIV infection could provide short-to-medium-term opportunities for studying a cause–effect relationship between female genital schistosomiasis and the risk for HIV infection. The main drawback of this approach is that such studies might not provide conclusive evidence, as they were not designed to explore a causal relationship between the two conditions. In particular, the finding of negative results in a study would not allow exclusion of such a relationship. A study in Lusaka, Zambia, was considered to provide a short-term opportunity.

In this study, a retrospective analysis is being conducted of adults enrolled in a cohort of couples discordant for HIV infection to determine whether there is a relationship between HIV status and *S. haematobium* seropositivity. Two methods are being used: plasma samples are screened for schistosome infection, with identification of the schistosome species in those that are positive, and univariate and multivariate analyses are being conducted to determine the relationship between schistosomiasis and HIV-1 infection in this cohort.

6.3.2 New studies

After a meeting with the Bill & Melinda Gates Foundation in April 2009, two studies were designed and submitted for funding.

A study in Kenya involves a cross-sectional follow-up of women aged 20–49 years to determine the prevalence of genital schistosomiasis among those who were previously treated for schistosomiasis and those who were not. The analysis will be stratified by the number of treatments received (25% received one treatment, 20% received two treatments and 55% were treated three or more times) to determine the effect of multiple treatments on the development of genital schistosomiasis. HIV testing and counselling will be offered, and the results will be used to assess the potential association with genital schistosomiasis in previously treated and untreated women.

The goal of a prospective treatment study in South Africa is to determine whether early, regular, annual mass treatment of schoolgirls with praziquantel according to the WHO preventive chemotherapy guidelines will prevent the development of genital schistosomiasis in young women. Cohorts of different age groups of girls are recruited, which differ in a stepwise way, such that the age at admission to the cohort will increase as the number of annual treatments received decreases: younger girls will receive more annual treatments than girls recruited at older ages. When the girls leave school and become sexually active (around 16–20 years), they will be examined for the presence of genital schistosomiasis. Older girls in the participating schools, aged 16–20 years, who are sexually active and have received no antischistosomal treatment, serve as internal controls. In the last year of the study, untreated 16–20-year-old controls will be recruited as external controls. The prevalence of genital schistosomiasis in the internal and external controls will be compared with that in girls aged 16–20 years who received consecutive rounds of praziquantel. The design is based on a random sample from 530 schools in Ugu District, with approximately 120 girls in each

age group. When the girls are examined for genital schistosomiasis, their HIV status will be determined, if additional funding becomes available.

6.3.3 The 'ideal study'

This study was designed to test the hypothesis that early, regular treatment of girls with praziquantel in childhood prevents the development of genital schistosomiasis in puberty or adolescence, with a consequent reduced occurrence of HIV infection in women after sexual debut. The design of the study is complex, and conclusive results can be expected only after 6–8 years. The outcome measure is the incidence of HIV infection in cohorts of girls who have received defined but different schedules of praziquantel, e.g. one treatment at baseline versus two or three treatments a year for 4–6 years, and in whom the presence or absence of genital schistosomiasis and HIV status will be determined after sexual debut. Provided the study is performed in an appropriate setting, the attributable risk for female genital schistosomiasis in the acquisition of HIV infection after sexual debut could be determined. The study could be shortened if it is implemented in on-going schistosomiasis control programmes (3–4 years), in which the appropriate cohorts can be identified retrospectively.

6.3.4 Mathematical modelling

Mathematical modelling of the dynamics between female and male genital schistosomiasis and HIV infection is proposed to define the epidemiological framework that it is most likely to demonstrate a cause–effect relationship between female genital schistosomiasis and the acquisition of HIV infection at the population level. In addition, it will be useful for designing future studies and for maximizing the power of such studies to detect a causal association between urogenital schistosomiasis and HIV infection.

6.4 Capacity-building

The working group concluded that gynaecologists and clinicians involved in future studies should follow a standardized procedure for gynaecological examinations and use a standardized description of the gynaecological pathology identified. This will require training. It is therefore proposed to conduct a training workshop in colposcopy, linked to computerized digital imaging, in areas with a high prevalence of female genital schistosomiasis.

6.5 Ethical issues for research protocols

Planned and future studies must follow established ethical principles, which apply across cultures. Great care should be taken to ensure that the research achieves the sensitive balance between the risks and the benefits for the populations in which the study is conducted. Study designs must account for the unacceptability of and the risks associated with certain gynaecological examinations in some cultural settings.

7. Public health recommendations

In view of the co-endemicity of HIV/AIDS and schistosomiasis haematobia, an immediate effect on the health of millions of girls and young women could presumably be achieved if antischistosomal treatment and HIV prevention were integrated. In global efforts to expand access to antiretroviral drugs in areas endemic for urogenital schistosomiasis, organizations and governments have an ethical obligation to provide access to praziquantel in parallel. Operational research should be encouraged wherever schistosomiasis control programmes and longitudinal HIV seroconversion

studies are implemented in the same area, so as to generate additional scientific evidence on the association between female genital schistosomiasis and the risk for HIV infection. In view of the time required to accumulate definitive evidence of a cause–effect relationship, the working group made the following recommendations.

7.1 As there is sufficient evidence to assume that regular treatment with praziquantel prevents the development of female genital schistosomiasis, the policy of regularly treating schoolgirls with praziquantel should be reinforced and extended, with programmes for preventing HIV and other sexually transmitted infections.

7.2 Pre-pubertal and pubertal girls in rural Africa are at particularly high risk for genital schistosomiasis, and regular treatment with praziquantel should be ensured. Boys should be treated concurrently to prevent development of male genital schistosomiasis.

7.3 As treatment with praziquantel is recommended during pregnancy in endemic areas, adolescent girls should not be excluded from control programmes. Treatment with praziquantel should be administered to women of child-bearing age and to pregnant women wherever schistosomiasis is diagnosed.

8. Conclusions

The HIV/AIDS pandemic has become part of the contemporary global landscape, particularly in sub-Saharan Africa. This region also remains heavily affected by schistosomiasis, including urogenital schistosomiasis. The literature supports the hypothesis that the public health advantages of anthelminthic treatment go beyond the simple benefits of curing schistosomiasis. The working group assumed that regular treatment of children with praziquantel contributes to reducing HIV transmission in areas endemic for *S. haematobium*, in addition to the many other benefits of such control programmes for poor communities in sub-Saharan Africa.

A possible link between female genital schistosomiasis and HIV acquisition is convincingly biologically plausible. The working group discussed the epidemiological association and recommended that more studies be carried out in the short and medium term to assess the extent to which systematic mass treatment with praziquantel will contribute to reducing HIV acquisition in women. It also proposed studies on the diagnosis and treatment of female genital schistosomiasis and suggested opportunities for evaluating the impact of schistosomiasis control programmes on the prevention of HIV infection in young women. Regardless of the presumptive causal association with HIV infection, urogenital schistosomiasis is a disabling disease by itself, and it should be prevented with the currently available means. Resolution 54.19 of the World Health Assembly, WHO's governing body, recommends that Member States regularly treat all at-risk school-aged children with single-dose drugs against schistosomiasis and soil-transmitted helminth infections (WHO, 2001). As of 2008, this treatment, which costs approximately US$ 0.30 per person, reached only 7.7 % of those who need it (WHO, 2010). The policy of regularly treating school-age children with praziquantel should be reinforced and extended, with programmes for preventing HIV and other sexually transmitted infections.

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Annex I. Ethical issues

The Helsinki Declaration will be followed, and all collaborators will have passed recent examinations in good clinical practice. Ethical permission will be sought from the national committee and from the committees in the collaborating countries. Researchers investigating the association between schistosomiasis and HIV infection have the obligation to work together to achieve high ethical standards. The concerns in schistosomiasis–HIV research that must be taken into consideration when studies are planned are as described below.

Colposcopy and transvaginal ultrasound are unacceptable for virgins. Colposcopy and transvaginal ultrasound may not be acceptable for adolescent or sexually active women in certain cultural settings.

Biopsies expose women to a risk for infection with sexually transmitted agents, including HIV, if their partners do not use condoms after the examination..

Biopsy samples should be taken from a representative lesion. If the biopsied area starts to bleed, coagulant must be applied locally and the woman monitored until bleeding is arrested. Sexual abstinence or use of condoms is required for 2 weeks after biopsy. This implies that partners must be cooperative. Biopsies should not be done in couples discordant for HIV infection.

If HIV testing is performed, adequate pre- and post-test counselling must be provided. If a person is HIV positive, CD4 counts must be ensured, and, if applicable, treatment must be provided, either directly or by referral to specialized institutions. National or ideal requirements will be followed.

Any additional gynaecological condition detected during the examination which requires differential diagnosis or treatment must be cared for appropriately. The costs of treatment and further diagnosis are the responsibility of the study.

Women who participate in a study of genital schistosomiasis must have a clear benefit, such as screening for cervical cancer, treatment of other genital abnormalities, free treatment for any sexually transmitted infections detected and tetanus vaccination.

If a study of female genital schistosomiasis is added to an existing study or extended for a different purpose, additional ethical clearance is needed, even if only already existing samples are examined.

Not only must ethical clearance for new studies be obtained from a legal–ethical body, but consent must be obtained from each woman, local authorities such as village leaders, and representatives of women associations. In some settings, informed consent must also be obtained from the husband or partner.S

Further consent is required for the use of new tests that may be developed during the studies.

The results of laboratory examinations must be conveyed to patients within a relevant time.

Annex II. Research subgroups and timelines

Three subgroups were created to develop study protocols and provide technical support.

Subgroup I: Case validation study

Group leader: Evan Secor

Members: Susan Allen, Abdoulaye Diarra, Hermann Feldmeier, Amadou Garba, Peter Leutscher, Eyrun Kjetland, Gabriele Poggensee, Erin Shutes and Birgitte Jyding Vennervald

Subgroup II: Short- to medium-term 'short cut' studies to generate evidence rapidly for areas in which widescale schistosomiasis treatment has been given.

Subgroup II A: Schistosomiasis studies

Group leader: Amadou Garba, Schistosomiasis Control Initiative

Members: Erin Shutes, Charles-Emile Ramarokoto, Abdoulaye Diarra, Wendy E. Harrison, Lynsey Blair, Wendy Harrison, Antonio Montresor, Olivia Andan

Subgroup II B: HIV/AIDS studies

Group leader: Professor Mabey to suggest a team leader from LSHTM

Members: Suzan Allen, Erin Shutes, others to be determined

Sub-group III: New prospective study: the 'ideal' study

Group leader: Hermann Feldmeier

Members: Gabriele Poggensee, Peter Leutscher, Patricia Ndhlovu

Timeframe

Short term:

By November 2009, diagnosis study designed and budget estimated (Focal points: Dr Evan Secor and Professor Hermann Feldmeier)

By March 2010, training workshop on colposcopy linked to computerized digital imaging (Focal points: Dr Birgitte Jyding Vennervald and Dr Eryun Kjetland)

By May 2010, mathematical modelling of the dynamics between female and male genital schistosomiasis and HIV infection performed (Focal points: Dr Eyrun Kjetland, Professor Niels Christian Stenseth)

By September 2010, diagnostic study completed (Focal points: Dr Peter Leutscher and Dr Charles-Emile Ramarokoto, if study is performed in Madgascar)

By December 2012, evaluation of clinical algorithm and of laboratory disease markers (Focal points: Dr Birgitte Jyding Vennervald, Dr Eyrun Kjetland)

Medium to long term:

By January 2011, start of 'ideal' study (Focal points: Professor Hermann Feldmeier and Dr Gabriele Poggensee)

By September 2011, analysis of Kenya and Zambia studies

By September 2014 (female genital schistosomiasis) and 2016 (HIV), analysis of the South Africa study (Focal points: Dr Eyrun Kjetland, Professor Myra Taylor, Professor Svein G. Gundersen)

By 2015–2016, analysis of the 'ideal' study

Annex III. List of participants

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