Epidemiological Context of Sexually Transmitted Infections in Zambia:

Determinants, aetiological agents and trends over time

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Dissertation for the degree philosophiae doctor (PhD)
at the University of Bergen

2012

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To my parents,

Esther Chikumbi and Mukula Robinson Makasa

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Acronyms and Abbreviations

ANC	Antenatal clinic
CI	Confidence Interval
DNA	Deoxyribonucleic acid
FSW	Female Sex Workers
GU	Genital Ulcer
GUD	Genital Ulcer Disease
HIV	Human Immuno-deficiency Virus
HSV	Herpes Simplex Virus
LDHMT	Lusaka District Health Management Team
LGV	Lymphogranuloma Venereum
MoH	Ministry of Health
MSM	Men who have Sex with Men
OR	Odds ratio
PCR	Polymerase Chain Reaction
STI	Sexually Transmitted Infection
WHO	World Health Organization
ZDHS	Zambia Demographic and Health Survey

Operational definitions

In this thesis STI has been used to mean classical STIs (excluding HIV) and HIV has been specified when referred to.

Abstract

Background: Sexually transmitted infections (STIs) remain a challenge mainly in developing countries, and in particular in sub-Saharan Africa, which also faces a serious HIV epidemic. Sexually Transmitted Infections can lead to serious complications such as infertility, spontaneous abortions, still births, cervical cancer and also enhance transmission of HIV. Control of STIs is thus important. Many STIs are curable, and prompt care seeking shortens the duration of an STI and prevents complications in the source patient as well as secondary spread of the infection. The World Health Organization recommends the use of syndromic guidelines for the management of STIs in countries with limited diagnostic facilities. A search of the literature showed gaps in current information on STIs in Zambia. This thesis focused on the causes of genital ulcer disease (GUD), its predictors, healthcare seeking and sexual behaviour among individuals with genital ulceration, and further examined syphilis trends in pregnant women compared with changes in the general population.

Methods: The thesis utilised data from the Antenatal Clinic (ANC) sentinel surveillance system, the Zambia Demographic and Health Surveys (ZDHS) and a cross sectional survey of 200 patients with GUD in Lusaka district. Both the ANC and ZDHS data comprised information from interviews as well as syphilis and HIV test results. In the GUD study, swabs from the genital ulcers were tested for *Treponema pallidum*, Herpes Simplex types 1 and 2, *Haemophilus ducreyi,* and *Chlamydia trachomatis* using polymerase chain reaction (PCR).

Results: The ANC surveys showed an overall significant decline in syphilis trends between 1994 and 2008 among urban and rural women. The decline was sharp irrespective of educational level, however, there were striking provincial variations noted. A comparison with the ZDHS 2001/2 and 2007 data also showed an overall reduction (though not significant), in syphilis prevalence among urban and rural men and women in the general population. The pathogens detected by PCR from the 200 patients with GUD in Lusaka were as follows: Herpes Simplex Virus type 2 (HSV-2) was detected in 28% of ulcers, *Treponema pallidum* in 11%; *Chlamydia trachomatis* in 3%; Herpes Simplex Virus type 1 in 0.5%, and *Haemophilus ducreyi* was not detected at all. Fifty five percent of the patients did not have any pathogens detected from their ulcers. The 2007 ZDHS showed a low prevalence of self-reported genital ulcers (GU) in the general population (3%). Important predictors for GU were age (25-39 years), being widowed/separated/divorced and having had a high number of lifetime sexual partners. No differences in care-seeking for GU were observed by age and gender, and more than half the respondents sought care from public health facilities. Among patients that presented with GUD in Lusaka, 57% reported sex after onset of symptoms and only 15% of these reported consistent condom use.

Conclusion: Syphilis declined by about 60% or more in rural and urban pregnant women between 1994 and 2008. The variations however that were noted at provincial level need to be studied further to understand the local context of the epidemics, and to guide STI prevention and control programmes in the different geographical settings. Detection of pathogens using PCR showed that HSV-2 was the commonest cause of GUD among patients with genital ulceration in primary health care clinics in Lusaka. The fact that *Haemophilus ducreyi* was not detected in any of the patients requires further studies. If the findings are validated, treatment guidelines for GUD need to be revised in Zambia. Since the majority of the respondents who reported symptoms of GU also reported having sex and only a minority had used a condom, there is need for awareness campaigns on the importance of abstinence or use of condoms when experiencing symptoms of GUs.

Original papers

The thesis is based on the following papers:

I. Makasa M, Fylkesnes K, Michelo C, Kayeyi N, Chirwa B, Sandoy I. *Declining syphilis trends in concurrence with HIV declines among pregnant women in Zambia: observations over 14 years of national surveillance.* Sexually Transmitted Diseases, 2012. **39**(3): p. 173-181

II. Makasa M, Fylkesnes K, Sandoy I. *Risk factors, healthcare-seeking and sexual behaviour among patients with genital ulcers in Zambia.* BMC Public Health, 2012. **12**(1): p. 407

II. Makasa M, Buve A, Sandoy I. *Etiologic pattern of genital ulcers in Lusaka, Zambia: Has chancroid been eliminated?* Sexually Transmitted Diseases, 2012. **39(**10): p.787-791

Acknowledgements

I wish to thank the Zambian government, through Ministry of Health for according me time away from work to pursue my studies in Norway, without which, this would not have been possible. I further wish to thank the Centre for International Health, University of Bergen, for giving me the opportunity to study in Norway. Indeed the time spent there shall forever be memorable. My gratitude also goes to the Norwegian government who funded my studies through the Statens Lånekasse quota funding. I am also grateful to the University of Zambia`s School of Medicine, Department of Public Health through who`s collaboration the training was made possible.

I am grateful to my supervisors, Ingvild F. Sandoy and Knut Fylkesnes who mentored me throughout the period of study. Special thanks to Ingvild for your thoroughness and great attention to detail. This being a long and windy journey with its ups and downs, I am thankful for the inspiration that you gave and the encouragement to keep going. It would be a great omission not to mention the overall leadership and direction provided by Knut when I side- tracked and the push when I slackened. I feel indebted to both of you, for your understanding when I needed to be back home in Zambia. Though there were challenges of being away, your flexibility enabled me to mix my roles of being a family person and a student.

I also wish to thank all my lecturers and administrative staff at the Centre for International Health for providing a conducive academic environment.

The time spent in Norway would not have been so memorable without the love, care and support of my Zambian "family" in Norway; Rachael and Nkomba Kayeyi, Florence Mjieldheim, Selia Phiri, Chola Nakazwe, Linda Kampata, Peter Chipimo, Lumbwe and Lister Chola, and Yvonne Topham. I also thankful to all colleagues and friends; Ferda Gulcan, Alemnesh Mirkuzie, Sally El Tayeb, Elisabeth Shayo, Juliet Babirye, Robert Byamugisha, Joseph Rujumba, Hawa Mbawalla, Catherine Kahabuka, Blandina Mmbaga, Gloria Sakwari, Gunn Moss and entire class of 2008/9. I am also grateful to Brit-Helene and Tom Sunde, Lulu Kgalemang, Grace Nassiwa, Slawek Platek, Wisdom Onu and other members of the Bergen Adventist church for the spiritual encouragement.

I further extend my appreciation to Mr. Jason Songwe for reading the thesis and for the valuable comments provided.
Special thanks to all the respondents and data collectors of the ANC and ZDHS surveys well as the patients that participated in the GUD study in Lusaka and the research assistants. My gratitude also goes to the Ministry of Health, Ministry of Finance, TDRC, UTH virology Laboratory, CSO, University of Zambia, and all the implementors and financiers of the ANC and ZDHS surveys. Sincere thanks to the KS laboratory managers and staff at UTH, for allowing us to use their facilities.

My heartfelt gratitude to Laston, for the support you gave me throughout the period of my studies and for the encouragement, and the push to carry on, which you did in a subtle way, as always, when I appeared to lapse, and indeed for your understanding especially when I had to shuttle between Zambia and Norway; for taking up the role of both mother and father despite your busy schedule and for doing it so well and indeed for reading the thesis and providing valuable comments. I shall forever be indebted to you! Our children, Chitambo, Mpundu, Mwelwa who were inconvenienced by my absence and many times did not understand why. I hope it will all make sense to you one day. My siblings Mukula, Mwenya, Musonda and Mutuna for the moral support and for filling in the void and standing by, for the children, words cannot express my gratitude. And yet another sibling, Mwape for the long hours we shared encouraging one another, and for lending your ear. Your support kept me going!

Above all, I am grateful to the Almighty God, who has seen me through life's journey and in my academic life and for His strength to take this stride; for ……"*the race is neither to the swift, nor the battle to the strong ... but time and chance happeneth to them all". (Eccl 9:11).* Through Him. May the honour and glory be unto Thee.

Introduction

The burden of sexually transmitted infections

Sexually transmitted infections (STIs) are a public health problem globally [1, 2]. About 450 million new infections of the four main curable STIs are reported annually. These include chlamydia, gonorrhoea, syphilis and trichomoniasis [3]. Transmission occurs sexually, but can also occur vertically, during pregnancy from mother to child and through blood products or tissue transfer. There are over 30 pathogens that can be transmitted sexually. These include bacteria, viruses, and parasites [2, 4]. Sexually Transmitted Infections can be broadly classified into curable and non-curable infections. Among the non-curable are the viral infections caused by Human Immunodeficiency Virus (HIV), Herpes Simplex Virus types 1 and 2 (HSV-1 and HSV-2) and Human Papilloma Virus. Some of the causes of the curable STIs include *Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoea, Treponema pallidum, Haemophilus ducreyi* and *Lymphogranuloma venereum* [2]. Many other organisms that are not deemed sexually transmitted have also been documented to be at least occasionally transmitted through that route, such as *Neisseria meningitidis*, *Haemophilus parainfluenzae* and Epstein-Barr virus. Sexually transmitted infections can present as asymptomatic infection or as overt disease which may manifest as genital ulceration, discharge or inguinal swelling. Both symptomatic and asymptomatic disease can lead to chronic infections and delayed consequences such as infertility, ectopic pregnancy, cancer of the cervix, and untimely death of infants and adults (See Table 1).

Table 1: Some complications due to STIs

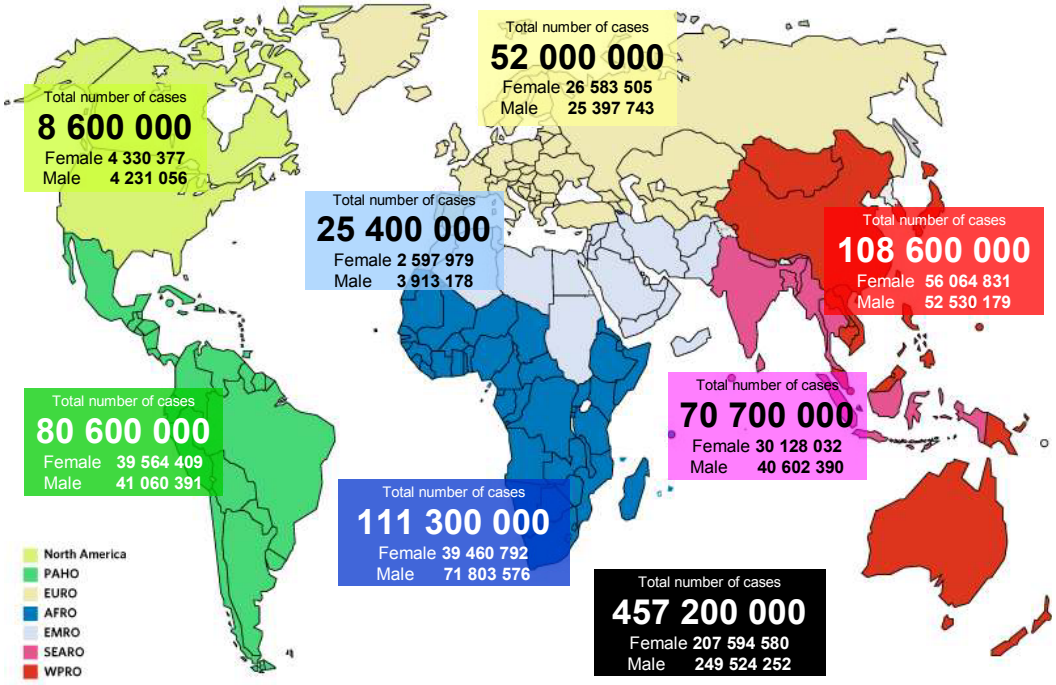
In adults	In children
Pelvic inflammatory disease	Congenital syphilis
Ectopic pregnancy	Pneumonia
Infertility	Prematurity; Low birth weight
Cervical cancer	Blindness
Spontaneous abortions	Still birth

Source: WHO, 2001[5]

Complications due to STIs rank among the top ten causes of morbidity among patients seeking care from health facilities in most developing countries, and drain both national and household income [2]. The complications are particularly bad among women. After pregnancy-related causes, STIs, excluding HIV infection, are the second leading cause of loss of healthy life years in women [1, 6]. Other consequences of STIs include social costs such as conflicts between sexual partners and domestic violence [2, 7]. These infections are also associated with stigma, which negatively affects healthcare seeking. Due to stigma and many other reasons, studies in Africa show that some patients seek care outside the public sector where data reporting is poor or non-existent [7, 8]. Hence the real disease burden may be bigger than what official estimates indicate.

An estimated 75-85% of STIs occur in developing countries [9]. Sexually Transmitted Infections mostly affect young adults (15-35 years), poor populations and urban dwellers [5, 10]. Migrant work, situations that displace populations such as war, low socio-economic status and poor access to health care contribute to the high burden of STIs [9, 10]. Figure 1 below illustrates the estimated number of incident cases of the four main curable STIs by region in 2005.

Figure 1: Global estimated total of selected STIs, (incident cases), 2005



Source: WHO, 2005 [11]

Despite the risk posed by these infections, STIs are poorly addressed in many settings, especially in developing countries where the need is greater [7, 12]. Knowledge of the potential role of STIs in facilitating HIV transmission and acquisition and the evidence that STI control interventions can reduce the spread of HIV has, however, brought renewed interest in the subject, since the 1990s [13-17]. In some African countries a reduction in bacterial STIs has been reported, and the reduction has in part been attributed to successful antibiotic treatment combined with reductions in sexual risk behaviour [13, 18, 19].

Genital ulcer diseases

Genital ulceration is described as discontinuity of the epithelial or mucosal surfaces of the genital or peri-anal area [20, 21]. The most common pathogens found to be responsible for causing genital ulcer disease (GUD) in Africa include Herpes simplex virus type 2 (HSV-2), *Treponema pallidum* and *Haemphilus ducreyi* [22-24]. Other pathogens that cause genital ulcers include *Chlamydia trachomatis* (serovars L1-L3), *Klebsiella granulomatis* and Herpes simplex virus type 1 (HSV-1) [22-28]. Some of the sequelae associated with the pathogens causing GUD include chronic infection, prematurity and low birth weight, spontaneous abortions, still-births and congenital infection [29]. Of particular importance is their association with increased risk of HIV acquisition and transmission [30]. A reduction in bacterial STIs has been observed in some African countries, such as Botswana and South Africa. In Botswana, a decline of GUD due to chancroid was shown, among STI clinic patients from 25% in 1993 to 1% in 2002, whereas the proportion of ulcers due to HSV-2 increased from 23% in 1993 to 58% in 2002. Diagnosis was based on PCR [31, 32]. Synopses of the most common GUD-causing pathogens are presented below.

Box 1: Main genital ulcer causing pathogens, their disease and manifestations

Pathogen	**Clinical manifestations**	**Picture illustration**
Herpes simplex virus type 2	Genital herpes • Both sexes: genital vesicular lesions and ulcerations. • Neonates: neonatal herpes, i.e. affecting skin, eyes, mouth, central nervous system or internal organs.	 §
Treponema pallidum	Syphilis • Both sexes: primary ulcer (chancre) with local lymph-adenopathy, skin rashes, condylomata lata (warts), bone, cardiovascular and neurological damage. • Women: abortion, stillbirth, premature delivery. • Neonates: Stillbirth, congenital syphilis.	 *
Haemophilus ducreyi	Chancroid • Both sexes: painful genital ulcers, may be accompanied by bubo.	 *
Chlamydia trachomatis (Strains L1-L3)	Lymphogranuloma venereum • Both sexes: genital ulcer, inguinal swelling (bubo), proctitis, genital elephantiasis, extensive genital destruction.	 §
Klebsiella (calymmatobecterium) granulomatis	Granuloma inguinale • Both sexes: nodular swellings and ulcerative lesions of the inguinal and ano-genital regions.	 §

*Source: Adapted from WHO, 2007 [2];Pictures:§ SADC, 2010 [33]; & *Dr D. Mabey, LSHTM*

Herpes Simplex Virus

Genital herpes is commonly caused by HSV-2 and is characterised by multiple vesicular and ulcerative lesions on the mucosal surfaces or skin in the ano-genital area. In people with compromised immunity and in newborns, infection with HSV-2 can lead to severe disease [34, 35]. The virus causes life-long infection. It remains in the ganglia during the latency phase and causes active infection when reactivated. Recurrences are characteristic of HSV-2 and are frequent in the first 3 years after primary infection, but diminish over time [36]. Herpes simplex virus type 1 commonly causes oro-pharyngeal herpes. However, it has been established that either one of the two viruses can cause oro-pharyngeal or genital herpes with indistinguishable lesions, mostly due to orogenital sexual contact [28, 35]. Worldwide, HSV-2 is the commonest cause of GUD, with sero-prevalence ranging from 21% in the United States, 40% in Europe and up to 80% in sub-Saharan Africa. Sero-prevalence estimates of HSV-2 infection increase with increasing age above 12 years, and plateaus after the age of 40. Among individuals who have never had sexual intercourse, HSV-2 is almost non-existent [37, 38]. Currently there is no cure for HSV-2, but antiviral drugs such as acyclovir can prevent recurrences, alleviate symptoms, reduce the duration of ulcers, reduce HSV-2 shedding and reduce the probability of secondary infection. Individuals who are HIV positive more often have a need for this treatment as they are at higher risk of recurrences and shedding of HSV-2 for a longer duration and suffer from more severe symptoms. [39].

Treponema pallidum

Treponema pallidum (*T. pallidum*) is a bacterium that belongs to the spirochaetaceae family. It causes syphilis, and if left untreated can remain a life-long infection. Syphilis remains a global challenge with an estimated 11 million people infected each year [3]. Prevalence estimates are highest in Africa, ranging from 3 to 4% in adults, compared to 0 to 1% in other regions [29]. *T. pallidum* can be transmitted sexually, vertically through the placenta of a pregnant woman to her fetus, or through blood transfusion or tissue transplant [40]. Syphilis is characterised by short symptomatic and long asymptomatic phases, and is divided into the early, infectious and late non-infectious stages of disease, as illustrated in Table 2 [34]. The incubation period ranges from 14 to 28 days. About 3-weeks after primary infection, a painless single ulcer, also known as a chancre, usually appears at the site of infection. The chancre resolves 2-6 weeks after its appearance, even

without treatment, and about 2 months later, secondary syphilis develops, characterised by a rash on the trunk and limbs. If untreated, the disease becomes latent and the patient can develop tertiary syphilis, a condition rarely seen now. Tertiary syphilis can present in three forms: benign tertiary syphilis, cardiovascular syphilis and neurosyphilis. Benign tertiary syphilis presents with chronic granulomatous lesions and mainly affects the skin, mucous membranes, bones or muscles. Aortitis is the commonest complication of cardiovascular syphilis, while late involvement of the central nervous system causes meningovascular disease. Co-existence of neuro- and cardiovascular syphilis is referred to as Quartenary [41]. Syphilis can lead to spontaneous abortions, premature births, low birth weight, perinatal deaths, and congenital syphilis [5]. Early syphilis causes stillbirths in 25% of infected pregnant women and neonatal deaths occur in 14% of children born to infected mothers if the infection is not treated (giving an overall perinatal mortality due to syphilis of about 40%) [42]. Early congenital syphilis, which occurs in the first two years of life, may manifest with snuffles, rash, enlarged liver and spleen, anaemia, jaundice and pseudo-paralysis. Late congenital syphilis, occurs after 2 years of life and affects neurological and musculoskeletal systems and can lead to developmental problems and death [43]. Syphilis is treated using penicillins. Doxycycline can be used in pregnancy or in persons allergic to penicillins [41]. Globally, the sero-prevalence among pregnant women ranges from 0.02 to 8% [44]. Syphilis in adults has been under control from the 1970s, until the 1980s in America, and the 1990s in China and Europe, when resurgences occurred, mostly affecting minority sub-populations such as men who have sex with men (MSM), female sex workers (FSW), and migrant groups [45-48].

Table 2: Classification of syphilis

Stage	Manifestations	Time
Early syphilis		
Primary	Chancre	~3 weeks after infection
Secondary	Rash on trunk and limbs	6-8 weeks after chancre resolves
Latent	Asymptomatic	≤2 years after infection
Late syphilis		
Latent	Asymptomatic	>2 years after infection
Tertiary	1. Benign tertiary: Chronic granulomatous lesions (gumma) of the skin, mucosa, bones, muscles, viscera	3-10 years after infection
	2. Cardiovascular: Aortitic incompetence, angina, aortic aneurysma	Up to 20 years after infection
	3. Neurosyphilis: Tabes dorsalis, general paralysis, psychosis	Up to 20 years after infection

Source: Adapted from Boon et al, 2006, & Egglestone, 2000 [41, 49]

Haemophilus ducreyi

Haemophilus ducreyi (*H. ducreyi*) is a gram negative bacterium that causes chancroid. It has a short incubation period of 3-5 days. The clinical manifestations include painful genital ulceration with soft irregular borders (soft chancres), and enlargement of the inguinal lymph nodes on one or both sides in about 60% of the cases. The nodes become tender and later suppuration and formation of abscesses (buboes) occurs [34, 50, 51]. Chancroid affects the poorest regions with the weakest public health infrastructure, such as Asia, Africa and the Caribbean [51]. Declines in chancroid have been documented in Africa and in other parts of the world [27, 31, 32], and this is attributed to effective preventive and control programmes, including the use of antibiotics. The feasibility of chancroid eradication is well documented. Asymptomatic carriage of *H. ducreyi* is rare [52], and since it usually presents as an overt and very painful disease, patients are likely to

seek care to relieve the symptoms. A short incubation period and severe clinical symptoms, prompting one to seek care, shortens the infectivity period. Thus for the disease to remain sustained or increase in a population, it requires a high partner change rate. This makes it less of a problem in the general population, and explains why chancroid tends to be concentrated among high-risk sub-populations such as sex-workers. Further, *H. ducreyi* has human beings as the sole reservoir, and affordable and effective single-dose drugs such as Azithromycin, are readily available. Lastly, even simple interventions such as washing with water and soap have been shown to reduce transmission [51, 53-55].

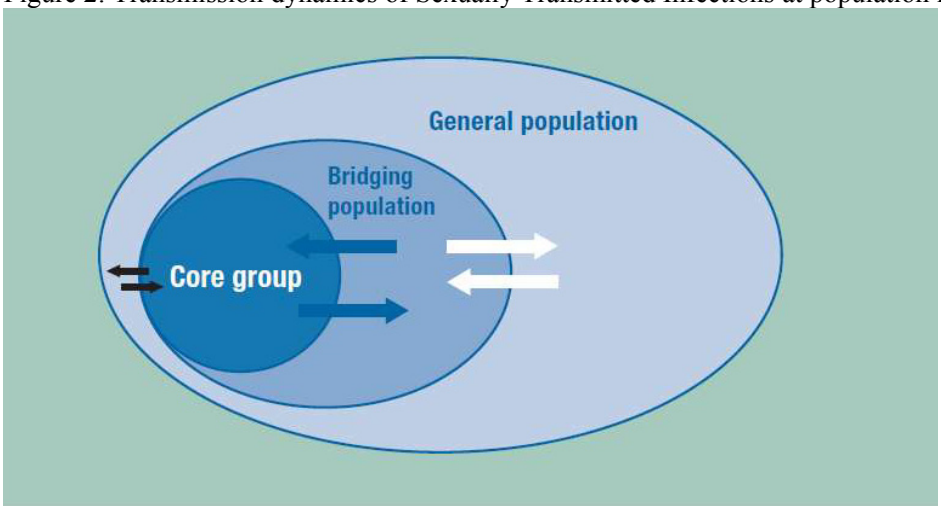
Lymphogranuloma venereum

Lymphogranuloma venereum (LGV) is caused by *Chlamydia trachomatis*, serovars L1 to L3. It is sporadic in countries with temperate climates and endemic in tropical countries [34]. Clinically, it is difficult to distinguish LGV from chancroid [56]. The incubation period is three to thirty days, and it presents in three stages. In the primary stage, a painless papule at the site of infection develops. The ulcer is self-limiting and may pass unnoticed by the patient, or may not develop at all. Within one to four weeks of the appearance of the primary lesion, regional lymph nodes enlarge, become matted and tender and form abscesses. Involvement of the rectum is more common in women and MSM. Lymphatic drainage of the vulva and cervix goes to the retroperitoneal lymph nodes, thus explaining rectal involvement in women [34, 56, 57]. Chronic inflammatory lesions in the tertiary stage lead to fibrosis. Consequently this can lead to genital elephantiasis, strictures and fistulae in the rectum, infertility and widespread destruction of the external genitalia [57, 58]. Lymphogranuloma venereum is endemic in Africa, Asia and the Caribbean, and changing sexual behaviour, increasing migratory patterns, sex tourism and easy travel, point to the possibility of sporadic cases in other parts of the world. Outbreaks of LGV in MSM have been reported in Europe and America since 2004 [58, 59].

The epidemiological context of STIs

The concept “epidemiological context” has been used to understand HIV dynamics and the impact of interventions applied in a given population, but may also be employed to understand other STIs. It is referred to as the present state of the behavioural and biological factors that influence transmission dynamics of a disease and what the impact is for the applied intervention. For instance, the epidemiological context of STIs is affected by the distribution of risk factors, primary transmission modes, sexual behaviour, and incidence in different population subgroups. Other factors include the HIV prevalence in the target population, and sexual mixing between high-risk groups and other population groups [60]. Garnett describes epidemics of sexually transmitted infections as being dynamic processes with changing patterns of transmission. Five typical phases are described: phase I is the invasion period of the host population; phase II is a hyper-endemic phase, where control measures are deficient; phase III is a decline phase when control measures start to have an effect; phase IV is an endemic phase or a steady state when control measures are in place, and the finally, phase V is the elimination stage [61]. These phases can be used to explain why the epidemiology of STIs varies in different settings, but is also dynamic over time within a given population [2, 60-62]. In the early phases of an epidemic, the transmission and the prevalence of STIs are likely to be high in risk groups such as FSW. A bridging population, e.g. clients of FSWs who also have other partners, are a sexual link between the core group and the general population [2, 63-65]. The relative importance of high-risk groups is reduced in the hyper-endemic phase. Hence control programmes need to be tailored towards the transmission dynamics in a specific setting.

Figure 2: Transmission dynamics of Sexually Transmitted Infections at population level

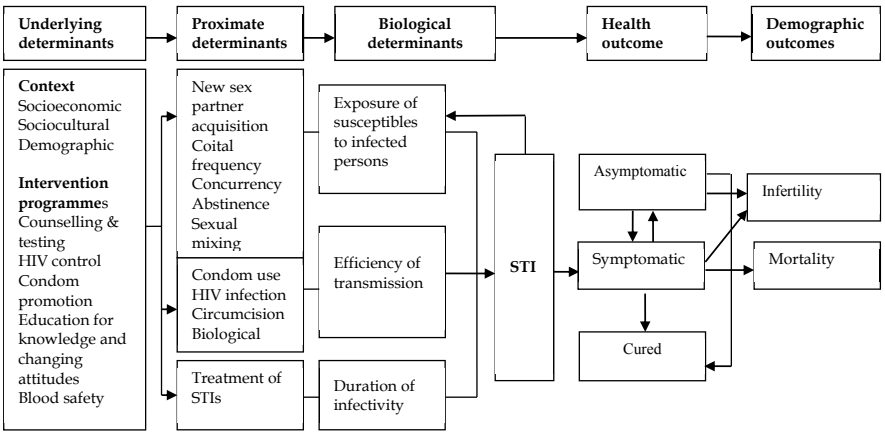


Source: WHO, 2007 [2]

The proximate determinants framework

Conceptual frameworks provide guidance for analysis and can aid interpretation of hierarchical inter-relationships between variables [33]. One such framework is that of the proximate-determinants, which was originally developed for studies of fertility and child health. The framework identifies a set of proximate determinants which affect biological determinants of transmission of HIV or an STI. The proximate determinants have both behavioural and biological components. The framework illustrates sequentially, in a simplified way, how, the underlying determinants, through proximate determinants, lead to exposure to infection, followed by disease, recovery (in the case of STIs), chronic infection or death [66, 67]. The transmission of STIs, is determined by the infectivity of the pathogen, which is the probability of infection in case of exposure (β); the rate of exposure of susceptible individuals to infected persons (C); and the duration of infectiousness (D) [61, 63, 67]. This is expressed as an equation; $R_o = \beta * C * D$, where R_o is known as the reproductive rate. When the reproductive rate is less than 1 there is a reduction in an epidemic, while R_o equal to one implies that there is a stable epidemic. A reproductive rate greater than one means increased spread of an epidemic [63]. Figure 3 shows the adapted proximate determinants framework for STIs and defines HIV as a proximate determinant, illustrating its role in the transmission of and/or acquisition of STIs [66].

Figure 3: Proximate determinants framework for factors affecting the risk of transmission of STIs.



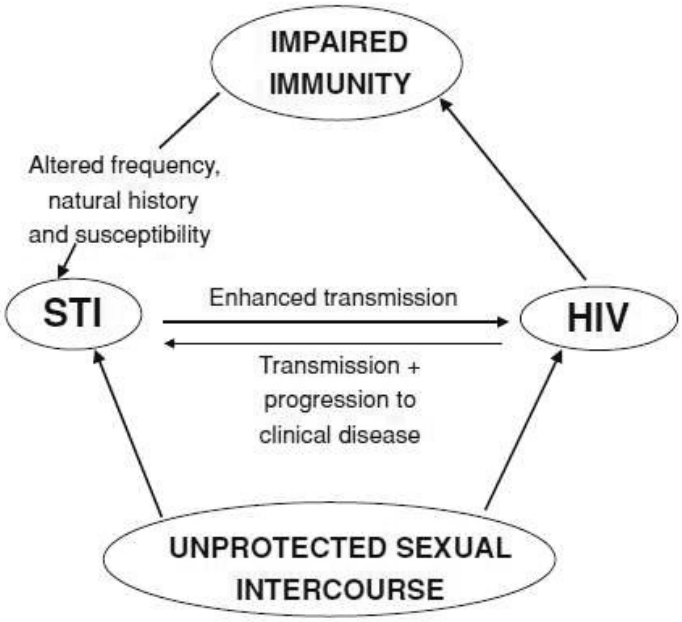
Source: Adapted from Boerma J and Weir S JID 2005 [66]

STIs and HIV

Sexually Transmitted Infections and HIV have an "epidemiological synergy" as they share a common mode of transmission and are driven by the same sexual behaviours [17]. As illustrated in Figure 4, the interaction is bi-directional and each alters the clinical manifestations and progression of the other and reciprocally enhances the transmission of the other [13, 68]. Biologically the presence of an STI leads to infiltration of inflammatory cells to the genital area, increasing the susceptibility to HIV infection. Pathogens that cause genital ulcers target the epithelial cells and cause necrosis to the surface cells, exposing the immune cells present in the sub-epithelium to HIV-infected genital secretions [69, 70]. Increased infectiousness to HIV in a person co-infected with HIV and an STI occurs as a result of increased viral shedding and higher frequency of bleeding in the genital tract [13]. Increased viral shedding has been shown in people co-infected with HIV and bacterial STIs, whereas treatment with antibiotics reduces viral shedding in genital secretions [71, 72]. The presence of a non-ulcerative STI increases the probability of HIV transmission by 2 to 4-fold within a partnership [13, 14, 30, 72], while with an

ulcer the risk increases by 5 to 10-fold. Concurrent STIs increase the probability of HIV transmission through an additive or multiplicative effect. Multiplicative effects may occur when co-factors have different interaction mechanisms [73].

Figure 4: Relationships between STIs and HIV



Source: British medical bulletin, 2001[17]

STI interventions may have an effect on HIV incidence, depending on the epidemiological context of both STIs and HIV. Trials that have tested the effect of such interventions in Tanzania and Uganda, have yielded varying results. A randomised community trial conducted in the early 1990s in Mwanza, Tanzania, tested the hypothesis that improved STI services integrated at primary health care level can reduce HIV transmission at population level. Six paired communities were randomly allocated into intervention and control sites. Improved STI services were provided at health facilities within the intervention communities, which included training of staff, provision of drugs and community campaigns promoting healthcare seeking. Follow up after 2 years showed that HIV incidence was 38 % lower in the intervention than in the comparison

communities. The researchers concluded that an effective STI control programme can reduce the incidence of HIV infection [74, 75]. Another study conducted in 1994-98 in Rakai, Uganda, set out to test the hypothesis that intensive STI control through home-based mass treatment with antibiotics would result in lower HIV incidence in comparison to non-intervention sites. Five of ten community clusters were randomly assigned to the intervention. Participants were visited every 10 months for an interview, specimen collection for HIV and STI tests, and received mass treatment with antibiotics (intervention) or vitamins and anti-helminthics (control). After 20 months the results showed no difference in HIV incidence in the intervention and control communities [76]. Reviews of the two landmark studies suggest that the findings of these studies are in fact not contradictory, but rather consistent and complementary. The diverging findings could be attributed to the differences in research questions, methodologies and epidemiological context, particularly the stage of the HIV epidemic and STI prevalence and incidence. In the case of Tanzania, the HIV prevalence at the time of the study was 4%, with less than 10% of ulcers being due to HSV-2. The Ugandan communities already had a mature generalised HIV epidemic with a prevalence of 16%, and 43% of genital ulcers were due to HSV-2 infection. The district programme in Tanzania ensured continuously available STI services that were probably more efficient than intermittent mass treatment. In addition, bacterial STIs that could be treated with antibiotics were more prevalent in Tanzania [1]. Whereas a significant reduction in HIV incidence was observed in the Mwanza trial in Tanzania where most of the STIs were curable, no effect was seen in the Rakai and Masaka trials in Uganda [74, 76, 77]. Findings from simulation studies based on data from the three trials suggested that low rates of curable STIs and risky sexual behaviours, which were secondary to the advanced HIV epidemic in Uganda, may explain the low impact of STI treatment on HIV incidence in Rakai and Masaka, reaffirming the importance of the local contextual determinants of the two diseases.

Control of STIs

There are many determinants of STIs and so are the approaches to control them. Interventions for control mainly focus on behaviour change and condom promotion activities. Appropriate and effective interventions need to be assessed through research. Successful and evidence-based interventions are usually taken as models and replicated in other settings with similar

epidemiological and socioeconomic profiles [2, 60]. Community or individual approaches can be applied as strategies for STI control. Strategies that target individuals include screening, case management and partner notification. Individual interventions can be targeted at high risk individuals such as attendees of STI clinics, ANC, voluntary counselling and testing centres. Community strategies include information, education and communication campaigns and mass treatment [17]. Community interventions are able to reach a large population, but may deliver a less intensive dose of the intervention to individual members of the community [78]. A population approach would be appropriate in an early epidemic phase when it is difficult to identify individuals at risk. When sexual mixing patterns are known, targeting is appropriate, while a combination of the two can be adopted when the epidemic involves the general population [17]. Who constitute high risk and vulnerable populations for STIs vary in different settings, hence the need to identify them before appropriate interventions are designed [2].

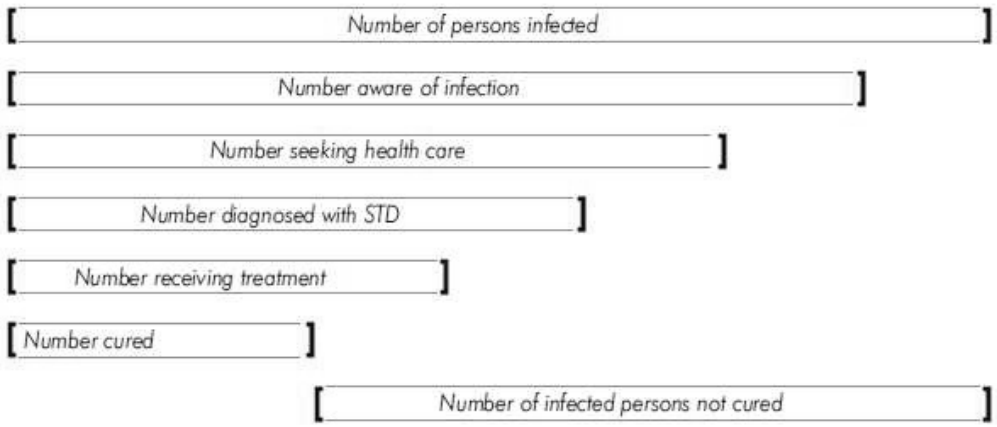
Primary prevention is aimed at preventing acquisition of infection by modifying sexual behaviour [9]. The main interventions include explicit information, education and communication on delaying onset of first sex, abstinence, promoting safer sex through mutually faithful sexual relationships, reducing number of sexual partners, and correct and consistent use of condoms [77]. Secondary prevention involves treatment and care for the infected and affected individuals. Activities thus include promoting healthcare seeking behaviours, providing effective, accessible and acceptable health care services, including screening, case finding, counselling, treatment and partner notification [79]. These strategies can reduce the incidence and prevalence of STIs by reducing the pool of infected people in a population, including asymptomatic cases [9, 79]. Screening involves testing for STIs in persons not directly seeking care, such as blood donors, while case finding involves testing for STIs in persons seeking care for different reasons other than STIs, such as pregnant women [17]. Finding infected persons can be a challenge due to asymptomatic presentation, especially in women, and lack of awareness of certain signs of the infection. Even when an STI has overt symptoms, distinguishing between normal and abnormal vaginal discharge is at times difficult, leading to patients not seeking care [29, 80]. Through screening and case finding, cases that would otherwise remain untreated can be identified and treated, and the pool of infected people is reduced. Partner notification should be part and parcel of comprehensive care and can be patient or provider-led, or conditional. Conditional referral is

where a specific period of time in which to notify the partner/s is agreed upon between the patient and the provider, and if partner notification is not done by the patient during this period, the health provider takes up the responsibility. Notification is aimed at informing partners of STI patients of their exposure to infection and treating them to limit further spread, protect the health of the partner and prevent re-infection of the index case. The World Health Organization recommends epidemiological treatment, i.e partners are treated for the same syndromes or infections as the source patient [2].

In most high income countries, treatment is based on identification of the pathogen. This is usually not feasible in developing countries where laboratory facilities are inadequate or not available or the laboratory reagents are too costly. In these settings, WHO recommends use of syndromic management of STIs based on the fact that a number of different organisms that cause STIs give rise to only a limited number of syndromes. A syndrome is a group of symptoms that a patient complains about and clinical signs the health worker observes during clinical examination. Based on the symptoms and clinical findings, a diagnosis is arrived at, i.e. vaginal or urethral discharge, genital ulcer, lower abdominal pain, scrotal swelling or inguinal bubo [81]. Treatment is based on the commonest causative agents for the syndromes [8, 81]. Further, it is recommended that treatment should be based on the local aetiologies of the syndromes and their microbial sensitivity patterns. Treatment for genital herpes should be included when the sero-prevalence is higher than 30% in a given population [5]. In practice, however, control programmes are faced with the challenge of meeting the vast need to provide services to infected persons. A large proportion of patients with asymptomatic infection who need treatment, do not get it. Others have overt disease but are missed before or after reaching the health care system, as illustrated in the figure below. A community survey in a rural district in South Africa estimated the point prevalence of STIs among women, the proportion of those who were asymptomatic, and the proportion of those who were symptomatic who did not seek care, as well as the proportion who sought care. Testing for gonorrhoeal and chlamydial infection was done using PCR, and serology was used to test for syphilis. The data showed that 50% of the women with STIs were asymptomatic and thus did not seek care. Among those that were symptomatic, the majority did not seek care [82]. As illustrated in Figure 4, there is a high number of individuals who have STIs

but do not to get treated due to varying reasons. Analysis of the impediments to care at the various levels can provide useful information and aid intervention strategies [29].

Figure 5: Loss of individuals at selected steps between infection and cure



Source: UNAIDS, WHO, 1999 [79]

The health care system and the Zambian context

Zambia has a liberal health care sector with diversity in ownership of the facilities. In total there are over one thousand eight hundred health care facilities. These include public (80%), private (14%) and mission facilities (6%). The public health care structure has three levels of care. The primary is the lowest level of care and consists of health posts, health centres and district hospitals. Provincial hospitals are the second level facilities, and the tertiary level of care consists of national referral hospitals. The informal health care sector consists of traditional and alternative health service providers and these are not regulated by the Ministry of Health [83]. Ninety-nine percent of the urban population resides within 5 kilometers of a health facility, compared to 50% in rural areas [84, 85].

In Zambia, STIs have been recognised as a public health problem [83]. There are over 200,000 cases reported annually and approximately 50% of all new infections occur in young people aged between 15 and 29 years of age [86, 87]. The quoted figures are facility-based statistics and are therefore likely to be underestimates. Some previous studies conducted in Zambia showed that individuals with STIs preferred to be seen in the private sector and confidentiality was among the

reasons cited for avoiding public facilities [87, 88]. A compilation of clinical diagnoses for genital ulceration made in the 1990s at the national referral hospital in Lusaka showed that chancroid was the commonest diagnosis in men (47%) while syphilis was the commonest in women (39%) [89]. Data from earlier published studies in the 1980s also found a high prevalence of maternal syphilis in Zambia, ranging from 8 to 18% among pregnant women based on antibody tests of blood. These probably contributed to a high number of mid-trimester abortions, still births, prematurity, morbidity and mortality of the child [90-92]. Hira et al. likened the frequency of adverse pregnancy outcomes in Zambia in the 1980s to the pre-penicillin era in developed countries [90]. Up to 40% of the stillbirths reported in Zambia have been attributed to congenital syphilis [90, 92]. A recent report showed a reduction in syphilis prevalence among pregnant women in the last one and a half decades [93]. In the general population, syphilis prevalence estimates among adults in 2001/2 and 2007 were seven and four percent, respectively [94, 95].

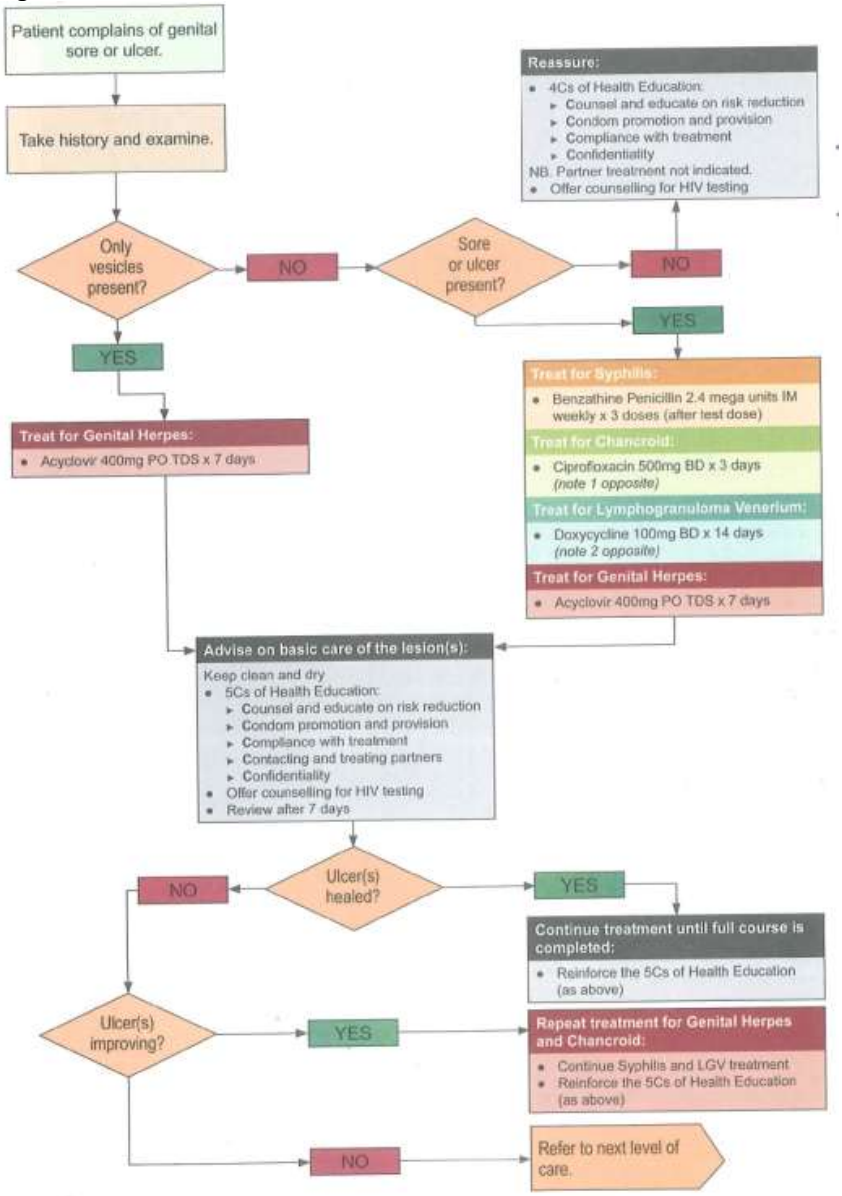
STI prevention interventions in Zambia

A national STI control programme has been in existence since 1980, and was run with a vertical approach. As part of the national response to STIs, clinical officers, who are the frontline health workers, were trained to manage STI patients, and job aids and information, education and communication materials were printed and distributed in more than 50 clinics around the country [96]. Diagnosis for syphilis was based on dark field microscopy and serology. For chancroid, diagnosis was based on clinical features. In 1990, Zambia adopted the WHO syndromic guidelines for management of STIs and these are still in use in primary health care facilities [89]. Figure 6 shows the flow chart for GUD treatment in Zambia, based on WHO adapted guidelines. According to the guidelines, a patient with GUD should be treated for syphilis, chancroid, Lymphogranuloma venereum and HSV-2 with benzathine penicillin, ciprofloxacin, doxycycline and acyclovir, respectively.

In 2002 the National HIV/AIDS/STI/TB Council was formed by an Act of Parliament as a response to the AIDS epidemic in Zambia. This council coordinates the multi-sectoral national response for prevention and control of all STIs, including HIV/AIDS. The HIV/AIDS component of the programme, however, received more financial support, thus limiting specific STI activities. Strong advocacy by national programme managers has led to improved funding since 2004 and

revamping of activities such as staff trainings, production of job aids and improved supply of drugs for STI management. This also included improvement of diagnostic facilities at higher level health facilities (secondary and tertiary) and improvement of referral systems by sensitizing and training traditional healers.

Figure 6: Flow chart for GUD treatment in Zambia



Source: National STI guidelines for Zambia, 2008 [97]

Rationale

Only a few studies on STIs in Zambia have been conducted in the last three decades, and thus there is limited information available about trends in the prevalence of different pathogens. A study that examined STI trends during the early HIV era from 1987-1989 showed an increase in STIs. The analysis was based on the absolute number of STI patients (including all syndromes and pathogens) attending the STI clinic at the University Teaching Hospital in Lusaka [98, 99]. The clinic has a limited geographical coverage. Being a specialist clinic, it caters for patients referred from lower level facilities within the city. Reports from the antenatal sentinel surveillance rounds between 1994 and 2008 showed declines in maternal syphilis prevalence in Zambia during this period [93, 100], and reports based on ZDHS data also indicated a decline in syphilis prevalence among men and women in the general population in Zambia [94, 95]. Powell et al used ZDHS data from 2001/2002 and found several risk factors for syphilis among men and women in the general population such as province of residence, age, education, and age at first marriage [101]. A further search of literature showed that there were no studies that had analysed recent trends/changes in syphilis prevalence in different geographic areas and socioeconomic groups in Zambia. Some research had been conducted on providers of healthcare for STIs, sexual behaviour among STI patients, healthcare seeking and partner notification in the late 1990s and between 2000 and 2001 in Zambia [87, 96, 102-105]. There were, however, no recent studies on sexual behaviour and healthcare seeking for STIs. Further, aetiologic studies on genital ulcers based on laboratory tests were lacking, but high prevalence estimates of Herpes Simplex Virus type 2 in men (36%) and women (55%) based on serological testing had been demonstrated in Ndola in 1997/1998 [106]. The study conducted at the University Teaching Hospital in Lusaka by Hanson et al in 1995 did not include laboratory tests to confirm the clinical diagnoses made on patients with genital ulceration [89]. Based on the fact that STIs are dynamic it is important to monitor their local epidemiological profile as this can provide relevant information that can guide the treatment policy for GUD. To add to the body of knowledge about STIs in Zambia, we sought to examine what pathogens cause genital ulcers among patients seeking care in Lusaka, predictors of genital ulceration in the general population in Zambia, care seeking practices among individuals with GUD and sexual behaviours after onset of symptoms. We also examined

trends/changes in syphilis prevalence in different geographic areas and socioeconomic groups in Zambia based on available data.

Aims and study objectives

Overall aim

The overall aim was to examine syphilis trends in Zambia and the predictors for genital ulcers, care seeking patterns and the aetiological pattern of genital ulceration in a high HIV prevalence setting.

Specific objectives

The specific objectives were to:

1. To investigate syphilis trends among pregnant women over a fourteen year period (ANC data) – Paper I
2. To compare trends among pregnant women against changes in syphilis prevalence in the general population (ANC and ZDHS data) – Paper I
3. To explore risk factors for self-reported symptoms of genital ulcers (ZDHS 2007) – Paper II
4. To determine predictors for healthcare seeking among people with self-reported symptoms of genital ulcers (ZDHS 2007) – Paper II
5. To examine sexual behaviour and the potential for transmission of infection among GUD patients (GUD study) – Paper II
6. To establish the microbiological causes of genital ulcers among patients with GUD attending government health centres in Lusaka (GUD study) – Paper III

Methods

Study area and population

Figure 7: Map of Zambia in Africa

Source:http://maps.google.com maps/teleatlasmaps+zambia

Zambia is a landlocked country and is situated in the southern part of Africa and lies between 8 and 18^0 south latitude and 20 and 35^0 east longitude. The country was colonised by Britain and gained her independence in 1964, and has enjoyed political stability since then. It shares borders with eight countries: Tanzania and the Democratic Republic of Congo to the north, Namibia and Angola to the west, Botswana, Mozambique and Zimbabwe in the south and Malawi on the

eastern side. Zambia has been a haven for most of its war-torn neighbours. The country has a population of slightly above 13 million residents [107]. The surface area is 752,612 square kilometres and the climate is tropical. Administratively, the country is divided into 10 provinces and 92 districts. At the time of the study, there were nine provinces and 72 districts. Two provinces are predominantly urban (Lusaka and Copperbelt) while the remaining 8 are predominantly rural. Sixty-one percent of the total population is rural and 39% is urban. There are 73 tribes and languages in Zambia and the official language is English. The adult literacy rate in 2005 was 64% and 82 % in women and men respectively [95]. Box 2 shows some of the key health indicators in Zambia.

Box 2: Health indicators

Health indicators	**Estimate**
Total population (million)	13, 092, 666
Annual growth rate (%)	2.8
Total fertility rate (children per woman)	6.0
Maternal mortality rate (per 100, 000 live births)	591
Life expectancy at birth (years)	50
Infant mortality rate (per 1000 live births)	70
Under-five mortality rate (per 1000)	119
HIV/AIDS prevalence (%)	14.3

Source: CSO, 2009 [95]

Data sources

The papers were derived from different data sources (See Table 3).

Table 3: Data sources

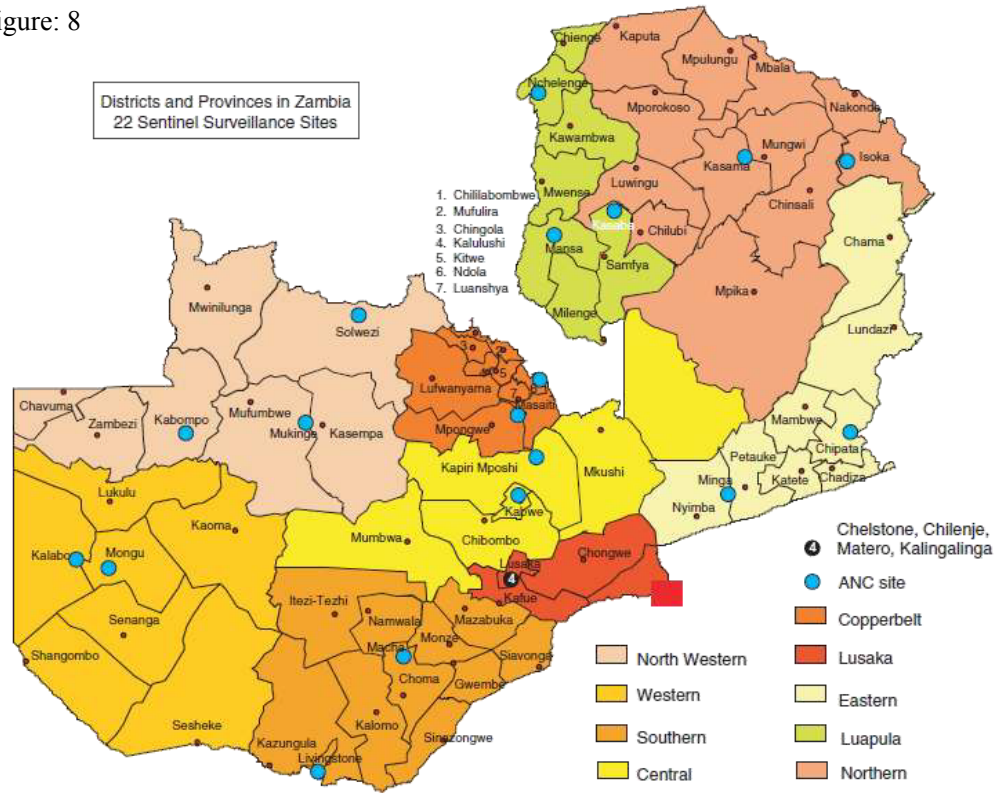
Paper	Data source/s	Period	Main variables	Main focus
I	a) ANC	1994, 1998, 2002, 2004, 2006, 2008	Syphilis test result & Socio- demographics	Syphilis trends
	b) ZDHS	2001/2, 2007	Syphilis test result & Socio- demographics	Syphilis changes
II	a) ZDHS	2007	Symptoms of GUD, Care seeking, Socio-demographics	Predictors of GU, Healthcare seeking,
	b) GUD study	2010	Sexual behaviour	Condom use after onset of symptoms
III	a) GUD study	2010	HSV 1&2 *Treponema Pallidum* *Chlamydia trachomatis* *Haemophilus ducreyi*	Prevalence estimates

Ante Natal Clinic (ANC) sentinel surveillance system for HIV and syphilis

Study site, study population and sampling

Zambia has an ANC sentinel surveillance system that has monitored HIV and syphilis prevalence since 1993. The sentinel sites were conveniently sampled to include all 9 provincial capitals and at least one rural site in the 9 provinces of Zambia at the time [108]. The 22 original sites of 1994 have been maintained in all the subsequent surveys, with a few additional sites over the years. Data from all the 22 sites that were consistently included in all the 6 surveys years between 1994 and 2008 were analysed.

Figure: 8



Source: Adapted from MoH, 2008 [93]

Pregnant women coming for the first time in that pregnancy were recruited consecutively from every site during the surveillance rounds. The target sample size per site was 500 women. Four sites in Lusaka and the Ndola site each had target samples of 800 women. The maximum period of data collection was 4 months. Demographic information and the obstetric history were obtained and blood was collected in two tubes; one with personal identifiers for routine syphilis screening, the second for unlinked anonymous HIV and syphilis testing for surveillance purposes. The Rapid Plasma Reagin (RPR) card test (Omega Diagnostics Ltd) was used for syphilis screening. Confirmatory testing of all specimens that tested positive with RPR was done using IMUTREP Treponema Pallidum Haemagglutination (TPHA) Test (Omega Diagnostics, UK) [109].

Demographic and Health Surveys

Study site, study population and sampling

The Zambia Demographic Health Surveys (ZDHS) of 2001/2002 and 2007 were nationally representative population-based surveys which captured data on a wide range of topics. Of particular interest to our study was the information on self-reported symptoms of STIs and HIV and syphilis test results. The ZDHS surveys were based on 2-stage random cluster sampling. The sampling frame from the 2000 census of population and housing of Zambia was used, which consists of more than 16,000 Standard Enumeration Areas (SEAs). A SEA is a small geographic unit consisting of 130 households on average. An urban-rural stratification was done for each of the 9 provinces of Zambia, making a total of 18 strata. In the first stage, 320 SEAs were sampled from these strata, based on probability proportional to size. In the second stage, 25 households were randomly picked from each selected SEA using equal systematic probability sampling. Men aged 15-59 and women aged 15-49 years who were permanent residents or visiting the night before were eligible for interview and HIV testing. The overall response rates were 89% and 91% in 2001/2002 and 2007 respectively. One third of the respondents were asked to consent to syphilis testing using venous blood. Rapid Plasma Reagin (RPR) was used for syphilis screening, and Treponema Pallidum Haemagluttination Assay was used as a confirmatory test [94, 95]. Coverage rates for syphilis testing were 76% in 2001/2002 and 52% in 2007, while the refusal rates were 15% and 24% for the respective years. In 2007, 17% of the specimens went missing in the laboratory and 4% had no sample identifiers, while absence during the time of blood collection was recorded for 6% in 2001/2 and 3% in 2007.

Genital Ulcer Disease Study

Study site, study population and sampling

Lusaka district is the capital city of Zambia with an estimated population of 1.7 million residents [107]. The Lusaka District Health Management Team (DHMT) is responsible for provision of primary health care services and operates twenty six government-owned health centres dotted around the city. The facilities vary in size (small, medium and large) and type of services provided (i.e. out-patient department only, out-patient department and birthing services and in- and out-patients and birthing services, respectively). Each health centre has a designated

catchment population. The ten health centres in Lusaka district with the highest incidence of STIs (i.e. cases per 1000 inhabitants in catchment area during the year preceding the study, according to the District Health Management Information System) were purposively selected for this study. Consenting patients aged 16 and above presenting with GUs were recruited consecutively between April and May 2010. Interviews and PCR results were obtained for 100 men and 100 women. The questionnaire included questions on demographic data, sexual behaviour and knowledge of cause of the ulcer. A physical examination of the genital area was done and a swab collected from the genital ulcer. In addition, women had a vaginal speculum examination performed on them. Consenting patients were given an Oral Mucosal Transudate HIV test, using the Oraquick® rapid HIV 1/2 antibody test.

Details of specimen collection, storage and sample processing are described in paper 3. Specimens were transported to the laboratory within 3 hours of collection. PCR testing was done at a molecular laboratory at the national referral hospital in Lusaka, operated under the auspices of University of Nebraska, Centre for virology. The laboratory has both internal and external quality assurance procedures for PCR methods, and the latter is done with the assistance of CDC Atlanta. All the laboratory work for this study was done by 2 experienced laboratory scientists and 1 laboratory assistant. The assistant was responsible for receiving specimens. Five real-time qualitative PCRs were done on each extracted DNA specimen; each using a set of two primers and a probe to target one of the five organisms *T. pallidum*, *H. ducreyi*, *C. trachomatis*, HSV-1 and HSV-2. Specimens were stored at the recommended temperature, -80^{o}C, before extraction. To avoid DNA degradation due to thawing and refreezing, 6 aliquots were made after extraction, and frozen at -20^{o}C until amplification. To increase the accuracy of interpretation of the results, positive and negative controls were included in each run. An internal extraction control was also included in each well, to rule out PCR inhibition. Ideally inhibitory substances, if any, should be removed during the DNA extraction process. If this fails, they may inhibit PCR amplification, thereby giving false negative results. For each extract, a "housekeeping" gene, β-actin, was checked for to determine the quality of the extracted DNA. ("Housekeeping" genes occur in all human nucleated cells and are commonly used as controls for presence of human DNA in genetic studies [110]). Only specimens where β-actin gene was detected were considered appropriate for further determination of the results. Commercially available kits, Qiagen QIAmp mini extraction

kit and PrimerDesign detection kit were used. The PrimerDesign detection Kits and the master mix were received on dry ice and immediately stored at -20ºC. Before use, the primers/probes, internal extraction control and positive control were reconstituted as per reconstitution protocol in the kit insert. Reconstituted materials were stored at -20ºC until used. Before storage, reconstituted primers/probes were also aliquoted and the master mix, which was received ready to use, was aliquoted too and stored at -20ºC.

Ethical considerations

The ANC surveillance system is an on-going process to monitor HIV trends in the country. The protocol was approved by the National AIDS Research Committee in 1990. The ZDHS are population-based serial surveys undertaken by the Central Statistical Office in Zambia. Approval by government through Ministry of Finance was given in 1992. For the GUD study ethical clearance was obtained from the Biomedical Research and Ethics Committees of the University of Zambia and Western Norway, and approval to conduct the study in Lusaka was granted by The Ministry of Health and LDHMT. In addition, written informed consent was obtained from participants in the ZDHS and GUD-studies, prior to participation. The patients were also asked to give separate consent for the syphilis testing in the ZDHS, and the HIV testing in both ZDHS and GUD.

Data analysis

The statistical software for social sciences PASW for Windows version 18.0 (SPSS) was used for data analysis for all the papers. The cluster design and weighting were considered during analysis of the ZDHS data. For the GUD study, data were cleaned, double entered into Epi-data, validated, and then exported to SPSS. Associations were assessed through Chi square tests and logistic regression. Trends were tested for using the chi-square test for trends in paper 1. A p-value of less than 0.05 was considered significant. Multivariate analysis was done to assess predictors for genital ulcer symptoms in paper II. Specific details of the analyses are provided in the individual papers.

Summary of results

Paper I

A significant reduction in syphilis prevalence between 1994 and 2008 was noted from 9.2% to3.2% and from 7.8% to 3.2% in both urban and rural pregnant women aged 15-49 years old (p-value < 0.001). Provincial analysis showed fluctuations in syphilis estimates especially in the earlier surveys. However, there was an overall tendency towards a decline. At site level 14 out of 22 sites showed declines in the prevalence. Women with syphilis infection were 1.9 times more likely to be HIV infected than those who did not have syphilis, while HIV positive women appeared with a similar difference in risk of syphilis infection compared to HIV negative (AOR 1.9; CI = 1.8, 2.1).

A sharp decline in syphilis prevalence was noted in all women irrespective of the educational status and age. However, it was noted that women with 10 or more years in school had a lower prevalence throughout the period under study and had a sharper decline compared to those with 7 or less years of schooling. Data from the ZDHS surveys also showed an overall relative reduction in syphilis prevalence of 50% and 60% in rural and urban women aged 15-49 years respectively. A discrepancy in the direction of change between the ANC and ZDHS estimates was noted in some provinces, i.e. declines in the ANC data versus increases in the ZDHS data, for urban participants in Luapula, Southern and Western provinces and rural participants in Central, Copperbelt and Lusaka provinces (but none of the increases were significant). A tendency toward reduction in the prevalence across all ages was observed.

Paper II

The prevalence of self-reported GU in 2007 in the general population of Zambia was 3.6%. Important predictors for genital ulcers were age (25-29 years), being widowed/separated/divorced and having a high number of lifetime sexual partners. No differences in care-seeking were observed by age and gender, and 60% of the respondents sought care from public health facilities. Among patients with GUs in Lusaka, 14% sought care >2 weeks after symptom onset. Fifty-seven percent reported sex after onset of symptoms. About 60% of the GUD patients in Lusaka reported that their partners knew about their genital ulcers. Those who had been sexually active after the onset of symptoms were more likely to have partners who were aware of the ulcer than those who

had not been sexually active. Consistent condom use was reported by only 15% of the respondents who engaged in sex after onset of symptoms. Among those whose partners were aware of the ulcer, 16% reported consistent condom use versus 12% of those whose partners were unaware (p=0.837)

Paper III

The prevalence of the detected pathogens was as follows; Herpes Simplex Virus type 2: 28%; *Treponema Pallidum*: 11%; *Chlamydia trachomatis*: 3%; Herpes Simplex Virus type 1: 0.5%, *Haemophilus ducreyi* 0%. Co-infection with HSV-2 and *Treponema pallidum* was found among 1.5%, and co-infection of HSV-*2* and *Chlamydia trachomatis* among 1%. Fifty five percent of the patients had no pathogens detected from their ulcers.

Discussion

Methodological considerations

Study design

This thesis is based on studies that were cross-sectional in nature. Cross-sectional studies measure both exposure and outcome simultaneously and can measure multiple exposures, but temporal associations and causal relationship cannot be established [111]. Repeated cross-sectional studies of independent samples using standardised eligibility criteria and the same methodology may be useful in providing indications of prevalence trends [112, 113].

Analyses were done using existing ANC-based and ZDHS data, while primary data were collected and analysed for the GUD study. The advantages of using the secondary ANC and ZDHS data are that they offer the opportunity to investigate trends of change over time based on comprehensive data sets on important information. A drawback, however, to using secondary data is that the researcher is restricted to the available information and as a result may not be able to address some important questions pertaining to the subject under study. For example in the analyses of predictors of reported genital ulceration among ZDHS respondents, in paper II, some of the potentially important behavioural factors such as concurrency, frequency of sex or consistent use of condoms were not controlled for as this information was not available. Furthermore, information on many of the factors that are known to predict healthcare seeking was not available and thus healthcare seeking models could not be employed in the analysis of care seeking for GUD.

As the main aim of paper I was to assess trends in syphilis, this was possible as data were available from serial surveys of pregnant women. The ZDHS are population–based surveys and are nationally representative, thus allowing for generalisable inferences. However, since the sub-sample (in the ZDHS) of persons with reported symptoms of GU was small, this probably limited the ability of the survey to establish significant associations in the analyses of predictors of GU and predictors of healthcare seeking [114].

Internal validity

Internal validity is the ability of a study to measure what it sets out to measure [115]. Bias is a threat to internal validity and has three components: selection and information bias as well as confounding. Selection and information bias are due to systematic error in the design, conduct or analysis of a study. A study with little systematic error is likely to have valid results which can be extrapolated to the source population. Internal validity thus refers to the ability to make inferences from the findings of a study to members of the source population [114].

Selection bias

Selection bias refers to a systematic difference in characteristics between study participants and those not participating in the study, and can lead to an apparent association between an exposure and outcome when in reality there is no association, or can lead to a null association when in fact an association exists [113, 116].

The ANC-based data have a good geographical coverage countrywide, with representation of both rural and urban areas. Only the sites that were consistently included in all the surveys were included in the analyses. Double recruitment of the same woman is avoided in these surveys by including only women coming for their first antenatal visit during that pregnancy within the period of data collection. Selection bias due to non-participation in the ANC data is unlikely to be an important factor since all the women attending their first ANC visit during the time of data collection were included in the sentinel surveillance rounds. Since surveillance rounds are not announced, but are conducted as part of the routine care, and the women are not asked to consent to the HIV testing of their blood, ANC attendance is not affected by the surveillance [93] and thus there are no refusals. Wealthier urban pregnant women are more likely to attend private clinics and some of them may have been missed. However, data from the public facilities are likely to be relatively representative of pregnant women in the country since more than 95% of pregnant women attend public facilities for antenatal care in Zambia [117].

Selection bias due to non-response could have affected the results of the ZDHS if respondents were more likely to be infected with syphilis than the non-respondents, or vice versa. Overall

response rates of about 90% were achieved for participation in both surveys (2001/2 and 2007), whereas the coverage for syphilis testing was 76% and 52% and for HIV testing it was 76% and 75%, respectively [94, 95]. The difference in the collection methods of HIV and syphilis specimens (finger prick versus venopuncture) could have contributed to the higher refusal of syphilis testing in the 2007 survey. The proportion of respondents that were absent during the surveys was small and so was the number of missing samples in 2001. However, even though about 17% of the samples went missing in the 2007 survey, it is unlikely that this caused selection bias since they were reported to have been lost at random. A comparison of age, sex and education level, showed no difference between participants who tested and those who did not test for syphilis. Thus, although it is clear that the coverage of syphilis testing in the 2007 survey was low, it is likely that selection bias only had minor effects on the syphilis estimates. Nevertheless, future surveys should adopt less-invasive techniques for obtaining blood specimens in order to improve coverage rates for syphilis testing.

In the GUD study, there is an obvious selection bias in that not all patients with genital ulceration seek care, e.g. those with mild disease or groups with limited resources. The wealthiest may seek treatment at private clinics, and some patients may prefer traditional healers or self-medication. These would not be captured since data were collected from public clinics only. Patient recruitment was done from 8am to 4pm to allow for samples to be transported to the laboratory in time for processing and storage on the same day. Patients coming outside these times were likely to have been missed, and this is another possible source of bias since such patients may be different from those attending during the day, e.g. in terms of employment status. Clinic records, however, showed that on average only six percent of the patients attended to in the out-patients department were seen outside the data collection times and of these, less than one percent presented with STIs (unpublished data). The study did not seem to be affected by non-response bias as the research assistants did not record any refusals for participation. Although one cannot exclude the fact that some of the participants could have perceived a pressure to participate, it is not very likely since data collectors were different from the health personnel treating the patients, and the patients were thoroughly informed that they would get the same treatment independent of participation.

Information bias

Information bias occurs when the means of gathering information about participants are inadequate or not uniform [115, 116], and it has the potential to lead to misclassification of exposure or outcomes. Misclassification can be differential or non-differential [112], and occurs because the sensitivity and specificity of a procedure or test is not perfect. Thus the procedure or test may fail to accurately determine who is diseased and who is not. The sensitivity of a test can be defined as the ability to identify correctly those who have the disease, while specificity is the ability to identify correctly those who do not have the disease [116].

In all the three surveys, measures were taken to minimise misclassification. The data collectors were trained prior to the surveys on how to ask questions in a standardised way. The data collection tools were translated into the local languages commonly spoken in each area. In the GUD study, back translation of the questionnaire was done prior to data collection to ensure that the meaning of the questions remained the same. To increase clarity and minimise ambiguity, the tools were pre-tested in role plays. Notwithstanding, the flow of some questions may have been confusing and some level of ambiguity exists in the GUD questionnaire, but it is likely that the research assistants were able to compensate for this after the thorough training they received.

Further effort was made in the GUD study to avoid measurement errors by ensuring the quality of specimen storage and testing. Real-time PCR was used to detect the pathogens and though expensive, molecular methods are the gold standard as they detect the actual pathogen causing infection and have high sensitivity and specificity [118, 119]. The extraction process for real-time PCR is automated and involves less manipulation of the specimens, thereby minimising contamination, compared to standard PCR. (Cross-contamination of other specimens or contamination of the working space may result in reproducing the result of the "pollutant"). Microbiological cultures are less expensive and can also be used as the gold standard as they isolate the actual agent. However, they have lower sensitivity compared to PCR [120]. Moreover, some organisms, such as *T pallidum,* are difficult to culture, while *H. ducreyi* does not survive long outside the human body and thus diagnostic methods using culture are difficult [121]. Ascertaining the cause, only based on clinical diagnosis, is unreliable because of the changing genital ulcer morphology and atypical presentations, especially when there is HIV co-infection

[119, 122]. The possibility of missing mixed infections is also high. Thus, by using PCR, the probability of measurement error was reduced. Nevertheless, the proportion of specimens in which no pathogens were detected was high compared to previous studies of GUD using PCR, but similar to the findings in a study by Scott et al involving patients with symptoms of genital ulceration presenting to a sexual and reproductive healthcare centre in Glascow, UK. They also used real-time PCR to detect HSV1/2 and syphilis, and had negative PCR results of up to 57% [123]. It was not clear why there was a high proportion of specimens without any pathogens detected in the study. Transportation issues for molecular detection are not critical since even non-viable organisms can be detected. However, false negative results due to insufficient genomic material can be ruled out as the housekeeping β-actin gene was added to the reactions and only the results of specimens that had the β-actin gene detected in them were reported. Although real-time PCR has high recovery of nucleic acids and achieves consistent and reproducible results [110, 119], it is possible that resolving ulcers may have had inadequate number of organisms for the pathogen to be detected from the swabs.

In order to achieve valid syphilis results in the ANC and ZDHS surveys, testing was done using both RPR and TPHA. The RPR is a non-treponemal test which detects a non-specific treponemal antibody, cardiolipin [49]. It is used as a screening test and is able to detect primary, secondary and latent syphilis [124]. Non-treponemal tests become positive four to eight weeks after syphilis infection is acquired [125]. However, false positive test results of non-treponemal tests occur since non-specific treponemal antibodies (cardiolipin or lipoidal antibody) are also produced in other non-treponemal diseases, infections, in pregnancy autoimmune diseases, and after immunisations [49, 124, 126]. False positive results only occur in one to two percent of the cases and estimates of false positive results in pregnancy are not higher than those in the general population [127]. False negative results also occur in about one to two percent of the cases, mostly pregnant women or people with early primary infection and HIV co-infection [125, 127]. This happens as a result of the “prozone phenomenon”, where excess treponemal antibody titres interfere with the proper formation of the antigen-antibody matrix complex, which is necessary to visualise a positive flocculation test [49, 128]. The sensitivity for non-treponemal tests ranges from 60-90% for primary and latent syphilis and up to 100% for secondary syphilis [125]. In contrast to RPR, the TPHA is a treponemal test. Its sensitivity and specificity are 95% and

99%[129]. Since treponemal tests remain reactive for years after infection, they are mainly used as confirmatory tests to validate a positive result of a non-treponemal test. In low prevalence settings, they are also utilised as screening tests followed by presumptive treatment [49, 127]. Alternatively, follow-up testing using non-treponemal tests can be done to determine active infection before treatment is given [127]. Treponemal tests detect a specific antibody that reacts with *Treponemal* antigenic compounds [124, 127]. They are also useful for diagnosing late syphilis, where a non-treponemal test is negative, but suggestive signs and symptoms are present [127]. A reactive non-treponemal test is suggestive of active infection and it does not detect adequately treated disease [49]. On the other hand, treponemal tests persist for years after infection despite successful treatment. Thus a reactive test can denote present or past infection [125]. Ideally, a confirmatory test should have at least the equivalent sensitivity with a screening test and a higher specificity [49]. In both the ANC and the ZDHS, the tests were performed at two designated laboratories, at the University Teaching Hospital in Lusaka and at the Tropical Diseases Research laboratory in Ndola. By using trained staff and performing the tests in the laboratory with controlled temperature and minimal dust particles in the environment, error was minimised.

Recall bias results from imperfect recollection of events related to the exposure or outcome and can lead to distortion of the estimates in either direction if one group in the study is more likely to recall events of interest more accurately. Factors that reduce the accuracy of recall include a long interval since the occurrence of an event, low frequency and low severity [130-132]. The ZDHS measured self-reported symptoms of genital ulceration in the last 12 months prior to the survey, and these measurements may be subject to substantial recall bias because of the long recall period [131]. It is also possible that respondents that suffered severe or recurrent episodes of genital ulceration, and those who sought care, were more likely to recall symptoms compared to those with mild infection. Equally, patients in the GUD study who had suffered less severe symptoms may have been less likely to recall past episodes, or if the time lapse from the time they used a condom was long, they may have reported a less accurate frequency of condom use.

As sexual behaviour is a private activity and observation is not possible, its research mainly relies on self-reports. Self-reporting of events, however, is sometimes distorted in a socially desirable

direction, manifested through under-reporting, over-reporting or omitted information. For instance, behaviours that are associated with stigma tend to be under-reported [130, 131, 133]. Gender discrepancies also exist when reporting sexual behaviours [134, 135]. Men tend to over-report behaviours that are associated with male prestige or prowess and are likely to exaggerate their total number of lifetime sexual partners. Women on the other hand are more secretive and tend to minimise their number of sex partners, due to fear of stigma and cultural pressures that expect women to have few sexual partners and to be less sexually experienced compared to men [136]. Zaba et al., in a population survey conducted in 2000 in rural Tanzania, found that women were selective in reporting their relationships and were more likely to report partners with a higher socioeconomic status. Younger women tended to under-report relationships with older men [137]. Similarly, our data showed that men in the ZDHS reported more lifetime sexual partners than the female respondents. In the GUD study some questions such as "when last did you have sex?", "did you use a condom?" and "with whom did you have sex?" may have been perceived to be intrusive, and thus could have been associated with under-reporting, especially among women. To minimise social desirability bias, sensitive questions were posed towards the end of the interview, after rapport had been built with the interviewee. Self-administered questionnaires are sometimes used as a way of improving the accuracy of the reports. They have an advantage of maintaining a respondent`s privacy and reduce the need to disclose sensitive information to the interviewer [133]. However, they are more suitable for populations with a higher literacy level than Zambia.

Confounding and interactions

Confounding is referred to as mixing or blurring of effects between exposure, outcome and a third factor. An extraneous factor is a confounder if it is a risk factor for the outcome and is associated with the exposure, but is not in the causal pathway between the exposure and the outcome. Unlike bias, confounding is not due to error, but occurs when the extraneous factor is unevenly distributed across groups and can lead to overestimation or underestimation of effect sizes. The most common confounding variables are age, sex, and social class [112, 113]. In randomised controlled trials, randomisation and stratification are ways of reducing possible confounding since important baseline differences between study arms with respect to characteristics such as age, sex, race, severity of disease etc. are avoided. Another method to avoid confounding is restriction, i.e.

when study participation is limited to individuals with particular characteristics. Thus, those with a known confounder to the variable of interest may be excluded. In case-control studies, matching of study participants may be used to ensure that possible confounders are evenly distributed [112]. At the analysis stage, stratification and multi-variate analysis can be done to control for confounding.

Since the three papers in this thesis were based on cross-sectional data, confounding was controlled for at the analysis stage through stratification by age, education, site and province in paper I. Stratification by sex was done in paper III. Despite the steps taken, residual confounding may be present since adjusting for all confounders is not possible as some may be unknown.

Interaction is present when a difference in the effect of one risk factor is observed between the strata of another risk factor [116]. When assessing syphilis trends in paper I, interaction was found between residence and province, residence and parity, and residence and education, while in paper II, interaction was found between self-reported genital ulceration and sex. The analyses were thus stratified by residence and sex, respectively.

External validity

External validity refers to the ability to infer the results to other populations or groups outside the study population [113]. In relation to the syphilis and HIV results from ANC data, there are two populations to which it would be desirable to make inferences to: 1) pregnant women in the whole country and 2) the general adult population of women and/or men. Regarding the first, there are some potential problems due to the fact that the selection of the ANC sentinel sites was done using convenience sampling, and thus they are not necessarily representative of all ANC clinics. At least one urban and one rural site are included in each province, however some rural sites are actually peri-urban. This may introduce under-representation of rural sites and rural women when the analyses are pooled, and the "rural" pregnant women attending a health facility may not be "the typical" pregnant women living in the most remote areas.
Pregnant women have on average been more sexually active than women in the general population, particularly in younger age groups, and thus their risk of sexually transmitted

infections may be different from men and women in the general population. Thus there are, in addition to the limitations mentioned above, some reasons to raise two important questions on the representativeness of pregnant women before inferences are made to the general population. The first question concerns the representativeness of ANC-based estimates at specific points in time. Two previous validation studies have been conducted in Zambia on the representativeness of the ANC-based HIV estimates. The first compared HIV prevalence estimates from one urban and one rural antenatal clinic in 1994 with population-based data collected from the catchment areas of the same ANC clinics in 1995. The findings showed closely similar overall HIV prevalence estimates for men and women together, but with differences in the age-specific estimates [138]. In the second validation, ANC-based estimates from 2002 were compared with nationally representative population-based data from 2001/2002 (ZDHS) [139]. This validation showed that the two data sources provided similar national HIV estimates for urban and rural Zambia. In paper I, the same was the case for the syphilis point estimates when the 2008 ANC data was validated against ZDHS 2007 data [140].

The second question is on the generalizability with regard to estimating trends over time versus the respective trends in the general population. This has previously been validated for HIV using trend data from one sentinel site (ANC-based versus population-based). The findings showed that ANC-based trends somewhat underestimated HIV prevalence declines [141]. Since there is only data on syphilis prevalence in the general population from two points in time, we are less confident whether the same tendency may be seen for syphilis. The comparison of the changes in the ANC-based estimates from 2002 to 2008 and the ZDHS estimates from 2001/02 and 2007 in paper I indicated that declines of similar magnitude occurred among pregnant women and men and women in the general population in urban areas, whereas the declines were slightly sharper in the ANC-based estimates in rural areas.

In the ZDHS, exclusion of non-household populations such as people residing in prisons, barracks, hostels or the homeless, could have led to bias if their risk of syphilis infection was different from those living in households. For example, the “Corridors of Hope”, a behavioural and biologic survey conducted between 2000 and 2003 involving over one thousand female sex workers from selected border towns in Zambia showed a syphilis estimate of 25% [142].

Similarly, Simooya et al in a study conducted between 1997 and 1998 which involved prison inmates in two towns (Kabwe and Ndola) in Central and Copperbelt provinces in Zambia, showed syphilis prevalence of 18% among the inmates [143]. The prevalence in the general population estimated by the 2001/2002 ZDHS was 2.7% for Central and 9.7% for Copperbelt province. Since the ZDHS do not capture data from non-household populations, they are likely to somewhat underestimate the actual prevalence in the whole population. However, since these non-household populations are relatively small, their exclusion is not likely to substantially affect the overall estimate [144].

Since the GUD study was the only aetiological study conducted on STIs in Zambia in the last 15 years, it is difficult to judge the external validity of its findings. Further studies in other urban and rural populations should thus be conducted.

Discussion of findings

Trends in syphilis prevalence

Data from the ANC sentinel surveillance surveys from 1994 to 2008 showed an overall significant decline in syphilis prevalence among rural and urban pregnant women. Similar declines in syphilis among pregnant women have also been reported in other developing countries such as Botswana, Ethiopia and India [19, 145, 146]. The high antenatal attendance, screening and treatment for syphilis in pregnancy could have contributed to the drop in syphilis prevalence, but it could also be a reflection of the overall drop in other bacterial STIs as reported in different countries in Africa and Asia. The latter has been attributed to increased availability and use of antibiotics [19, 31, 32, 147]. The drop in syphilis could also be an indication of changes in behaviour, as changes in syphilis estimates is a sensitive indicator for recent sexual behaviours [146]. Behaviour change has been documented in Zambia, such as increase in condom use, reduction in number of sexual partners and delayed age at first sex and first birth [95, 148-150]. Positive changes in behaviour could be a result of HIV prevention campaigns which have been on-going since the late 1980s in Zambia. Among them are the widespread use of electronic and print media, radio and television spots, billboards and awareness campaigns at health facilities and in the community.

Provincially, striking variations were observed in the prevalence estimates, though in the later surveys there was a tendency towards a reduction in all provinces. The differences at provincial level could suggest distinct risk factors for syphilis in the various areas as seen for instance in areas with migrant populations and fishing communities where prevalence estimates of STI and HIV infection have been shown to be high [151, 152].

The pooled population-based ZDHS data showed a non-significant reduction in syphilis prevalence estimates among urban and rural men and women between 2001/2 and 2007, and this was in agreement with ANC data. Monitoring of future surveys will provide a better picture of the trends in the general population since data from at least three points in time are required to determine trends.

Predictors of genital ulcers

Risky sexual behaviours predispose individuals not only to genital ulcers, but also to other STIs, including HIV. Age 25-29 years was a predictor for genital ulceration. Based on the proximate-determinants framework, the finding that widowhood and separation/divorce were predictors of genital ulceration could be interpreted as follows; widows/widowers, and divorced people may, because of their lower socio-economic position, be more likely to engage in transactional sex (proximate variable), predisposing them to biological factors that can result in infection. When the total number of lifetime sex partners was adjusted for, the association between widowhood and GUD weakened. This probably indicates that the increased risk of widows/widowers is partly mediated by differences in sexual behaviour. Having had multiple partners implies increased probability of exposure with an infected person from whom the infection can be contracted, especially if condom use is inconsistent or non-existent. However, there are a number of other sexual behaviour indicators that were not adjusted for, but could be equally important. An alternate interpretation could also be that, in the cases where their spouses died due to AIDS, it is likely that they too may be sero-positive for HIV and also HSV-2. The latter is the commonest cause of GUD, and as shown in this study, the likelihood of having HSV-2 is higher in HIV positive individuals than the HIV negative. Dual infection with HIV and HSV-2 causes frequent HSV-2 recurrences and more severe and prolonged episodes, which could explain the association between being widowed and having genital ulceration.

The push for strengthening prevention and control of STIs has been more on the biological and proximate factors. A review of the enabling factors and barriers for operationalising control programmes is key when designing control strategies for STIs. It is also important to understand the broader context, the role of the socioeconomic or cultural determinants, and these can form part of the long term strategy for STI control. However, these are beyond the scope of the STI control programme, and thus require intersectoral involvement and political will.

Healthcare seeking

The success of STI control programmes lies not only in providing appropriate treatment, but also in patients seeking care. Although the majority of the ZDHS respondents with GU sought treatment, a considerable proportion (20%) did not. This is comparable with findings from other countries in Africa [153-155] and in Asia [156]. A clinical cohort study in the early 1990s in rural Uganda showed that patients did not seek treatment despite the presence of a nearby clinic which was well stocked with drugs, had trained staff and offered free services [155]. Stigma, lack of confidentiality and inefficient or poor quality of services, are among the common reasons for not seeking care [105, 156]. A study on determinants for healthcare seeking among STI patients in Uganda, conducted between 1998 and 1999, found that socio-demographic characteristics did not influence the choice of public versus private health care. Attitudinal, normative and self-efficacy variables were, however, important predictors for choice of service provider. These were encouraging findings since all the factors are modifiable [157]. As also shown in other studies [153, 158], we found no significant difference in care seeking between men and women, nor across different age groups. In contrast, Meyer Weitz et al in a study of patients seeking care from selected STI clinics in South Africa, showed that men sought treatment earlier than women [159]. Men often suffer more severe and overt symptoms compared to the asymptomatic infections that are commonly manifested in women [29, 160]. Respondents with higher education tended to be more likely to seek care than those with less education (although the association was not significant), perhaps suggesting a higher awareness of what symptoms imply the presence of GUD, the consequences of GUD and where treatment may be sought. This is in line with a South African study which also showed that less educated people were less likely to seek care for STIs [159]. The finding in the ZDHS data that both educated and wealthy people tended to have a higher likelihood of care seeking could also suggest that these groups feel more confident about treatment options and less prone to financial barriers. Consultation fees have up to very recently (early 2012) been compulsory at government health centres in Lusaka, and this could be an impediment to accessing care by less wealthy people as found by Ndulo et al. in a rural and urban setting in Zambia [105]. The fact that only a minority sought care more than 2 weeks after symptom onset is encouraging and could suggest that patients are informed of the consequences of delaying care.

Sexual behaviours

Data on sexual behaviour is essential for predicting future STI epidemics and for prevention policies. Patients with active ulcers in the GUD study continued to have unprotected sex after the onset of symptoms although many of them were HIV infected. This is in line with findings by other researchers in Africa of continued sexual activity among patients despite having active genital ulcers [155, 159, 161]. Even more disturbingly, this was despite their partners knowing about the condition. Such behaviour poses a threat to STI and HIV control, and requires more education for patients and their partners on how and why to prevent transmission of the infection. Effective control entails reversing the epidemic by having a reproductive number (R_o), of less than 1 through a reduced rate of partner change, efficiency of transmission and duration of infection [51]. Combined efforts operating through different mechanisms may work in synergy to reverse an STI epidemic, as illustrated in the 100% condom use programme in Thailand and Cambodia, which promoted and enforced 100% condom use among female sex workers, and offered targeted screening and treatment for STIs. The programme led to a drop in the partner change rate among the clients of the sex workers, and resulted in a reduction of both STI and HIV prevalence [51, 162].

Microbiological causes of GUD

Based on PCR detection, HSV-2 was the commonest pathogen causing genital ulceration in the GUD study. This is in line with findings from studies from other parts of Africa where HSV-2 has been shown to be the commonest cause of GUD [22, 24, 163]. *H.ducreyi* was not detected in any of the patients included in our study. Declines in chancroid prevalence have been reported in different regions in the world, including Africa [51]. However, in South Africa, Lai et al. in a study conducted between 1993 and 1994 to determine the causes of GUD among men in a mining community, using PCR, found *H. ducreyi* as being the commonest cause. They also found an association between having chancroid and HIV sero-positivity [118]. Chancroid has a short incubation period and requires a high partner change rate and hence the prevalence in a tight sexual network can be high. Sexual behaviour information was not collected in the South African study. However, it is likely that high risk sexual behaviour, defined by low condom use and engaging in sex with sex workers who are a “core group”, could explain the high prevalence of chancroid among the miners in South Africa. The fact that *H. ducreyi* was not detected in this

study could suggest a reduction or elimination of chancroid, i.e. a shift from what was previously reported in the 1990s by Hanson [89]. However, Hanson's estimates were based on clinical features and thus may have overestimated or underestimated the prevalence. As seen in other countries, it is plausible that antibiotic therapy may have played a role in the reduction of chancroid and other bacterial STIs [19, 51, 55]. Elimination of chancroid in this setting would entail revising the GUD treatment guidelines by omitting the treatment for chancroid in patients with genital ulceration, and treating for syphilis, LGV and HSV-2 only. It is also important to ensure that patients are treated for genital herpes considering the high HSV-2 estimates. Although the guidelines show that HSV-2 ought to be treated for [164], in practice, patients are usually treated with antibiotics only. This in part could arise from the fact that Acyclovir is not on the essential drug list at primary health care level facilities, and therefore it is rarely available in such facilities [165]. Where it is available, it is mostly through donor funded projects and is usually confined to departments such as the anti-retroviral unit, and not at the out- patients' pharmacy.

Conclusions

The first paper in this thesis showed a substantial decline in syphilis among pregnant women between 1994 and 2008. The findings of the ZDHS (two time points) also showed an indication of a decline in syphilis estimates in the general population. Analyses of subsequent ZDHS surveys, however, will be useful in providing population-based trend data on syphilis. Among patients that presented with GUD at public primary health care facilities in Lusaka in 2010, *T. pallidum* was found to be the second most common pathogen according to molecular testing techniques. Herpes simplex virus type 2 was the most common pathogen, while *H. ducreyi* was not detected in any of the patients. The absence of *H.ducreyi* could suggest a decline or possibly elimination of chancroid in Zambia. If confirmed, this would entail changing the treatment guidelines for GUD by removing treatment of chancroid, but continuing treatment for syphilis, LGV and HSV-2. At national level, there were no significant differences in the reporting of GU by gender, education or urban/rural residence in the general population (according to the ZDHS), but the prevalence differed by age and marital status. Care seeking was common in almost all subgroups, and the respondents who reported symptoms of genital ulceration mainly sought care from public health facilities. However, a considerable proportion reported seeking care outside the public health sector, indicating a mix of providers for STI care in this setting. Condom use was found to be low among patients with GUD in Lusaka, despite the high HIV sero-prevalence. Failure to abstain from sex or to use condoms while having symptoms of genital ulceration poses a challenge to the efforts to limit transmission of STIs.

Recommendations

Implications for policy

It is encouraging to note the significant decline in syphilis estimates among pregnant women in Zambia. However, there is an urgent need to strengthen preventive programmes at provincial and district level, especially in areas that are still facing high prevalence levels. Effective strategies should also be identified in an effort to further reduce the prevalence to negligible levels in Zambia. Public awareness needs to be raised on the importance of early treatment seeking, long-term consequences of STIs and sexual risk reduction as strategies for reducing transmission of STIs. Intensified health campaigns on abstinence and promoting condom use when one has ulceration are key in order to limit the spread of STIs. Interventions ought to pay attention to counselling and educating patients on the importance of having their partners treated to prevent re-infection. Taking into consideration that there are various health care providers for STI care, programme managers need to ensure that involvement of all stakeholders is on-going in order to promote better partnership and referral of patients.

There is need to validate the findings for *H. ducreyi,* but if elimination of chancroid is confirmed, it would require a change in treatment policy by omitting treatment for this pathogen from the GUD algorithm, and instead treating only for syphilis, LGV and HSV-2.

Through multisectoral involvement and with the political will, underlying factors such as improving the socio-demographic conditions, for instance by empowering widows or women economically, are long term strategies for STI control. These ought to be implemented in tandem with sexual risk reduction interventions. Programmes should tap into the evidence from current research in order to improve services and control STIs.

Future research

Although the data from pregnant women have shown a significant reduction in syphilis prevalence since the mid-1990s in Zambia, there is need for continued efforts to further reduce it. New strategies through operational research should be embraced to ensure a sustained reduction. Continued monitoring of syphilis in the general population will provide insight into the actual

trends in the population. This would also allow proper validation of the findings from the ANC data. Less invasive techniques for collecting blood for syphilis testing to reduce refusals should be explored.

The provincial differences noted in syphilis trends require further research with a focus on the contextual and proximate determinants of syphilis in the respective settings. A mix of both qualitative and quantitative work will provide an in-depth understanding of the problem, and can aid the design of specific interventions relevant to the different geographical areas.

Aetiological surveillance systems for genital ulcer diseases ought to be considered to monitor disease trends and resistance patterns. Corroboration of the findings from the GUD study can be done through repeated cross-sectional surveys to ascertain the causes of genital ulcers in Zambia. This information is vital for guiding treatment policies.

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