The Epidemiology of HIV-1 and other STDs in Trucking Workers in Kenya: Preparations for HIV-1 vaccine trials

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- Initiating and consolidating the process of building the cohort and monitoring the project recruitment and follow-up. This included clinical diagnosis and management, and setting up the mobile primary health care and STD clinic.
- Continuous in-service training of staff, including clinical and research methodology training for the project physician from the University of Nairobi, Dr J P Rakwar.
- Data management (in addition to most of the data entry in the first year).
- Liaison with the laboratory and contribution to project laboratory direction, with Dr K Mandaliya, the Coast Provincial Pathologist. Ms Bhavna Chohan was the laboratory manager with whom the author worked on a day-to-day basis.
- Analysis of the data: exception to this was the time-trend modeling, in which the author was assisted by Dr B Richardson, biostatistician and assistant research professor from the University of Washington. Dr N Nagelkerke of the University of Manitoba (now Erasmus University, Netherlands) provided statistical advice on the urethral infection study.
- Interpretation of the results and preparation for publication and presentation. This included writing the papers, with editorial review from Professor J K Kreiss for the prospective cohort and vaccine acceptability study, and Dr S Moses for the cross-sectional urethral infection study.

Abstract

A cohort of HIV-1 seronegative male trucking company workers was established in the Kenyan coastal city of Mombasa, for the purposes of preparing them for HIV-1 preventive vaccine trials. The cohort was one of only three prospective male cohorts which have published data on heterosexual HIV-1 acquisition in sub-Saharan Africa, the continent most affected by this pandemic. HIV-1 seroincidence was measured and correlates of HIV-1 acquisition, including other STDs, were examined. Results of almost three years of follow-up, and data on anticipated acceptance of the conditions of an HIV-1 vaccine trial are presented.

- The baseline seroprevalence for antibodies to HIV-1 was 17% and the prevalence of active syphilis was 4.5%.
- HIV-1 seroincidence was 4.0% per annum in 990 person years of follow-up. Multivariate Hazard analysis revealed a strong association between HIV-1 acquisition and occupation of driver or assistant (HR 4.0, 95% CI: 2.1-7.9), any sex with a partner other than a spouse (HR 4.2, 95% CI: 1.3-13.6), and a trend towards higher incidence with uncircumcised status (HR 2.0, 95% CI: 0.9-4.6). No association between STD and HIV-1 acquisition was found with an observed incidence of symptomatic gonococcal and non-gonococcal urethral discharge of 18.2% per annum, and 4.3% per annum for genital ulcer disease.
- There were highly significant declines in extramarital sexual contacts from 50% to 40% in three month follow-up time blocks ($p<0.001$), and sex worker contacts from 12% to 6% ($p=0.001$), in a time trends analysis which included 494 person-years of follow-up. No significant change in condom use was recorded over time.

Consistent (100%) condom use remained at approximately 30% of men engaging in extramarital sex with a partner other than a spouse.

- Highly significant declines in the incidence of observed and reported sexually transmitted diseases were measured over the course of follow-up ($p<0.001$) in the first 494 person-years of follow-up. In the absence of data from the general population, it is not possible to attribute these declines to the behavioural and treatment interventions of the project, but it does document that the climate is right for behaviour change, and decrease in STD acquisition, in men in this setting.
- Prevalence of symptomatic and asymptomatic urethral infections (*N gonorrhoeae*, *C trachomatis*, or *T vaginalis*) was 11.5% in a cross-sectional study which included HIV-1 seropositive men, following the documented decline in symptomatic STDs. Over two thirds of infections were asymptomatic. The leucocyte esterase dipstick (LED) urine screening test for urethral inflammation had a sensitivity of 55% and a specificity of 82%, in asymptomatic men. The LED test was the most accurate predictor of asymptomatic urethral infection. Risk assessment on the basis of demographic and behavioural characteristics did not prove useful. *T vaginalis* was the most common urethral infection and was associated with older age.
- Eighty six per cent of 201HIV-1 seronegative men interviewed in a vaccine acceptability survey stated that they felt at personal risk of HIV infection, and 84% of men declared interest in participation in an HIV preventive vaccine trial. However, 17% of men stated that they would increase risk behaviour if they participated in an HIV vaccine trial.

Thesis Overview

The studies outlined in this thesis were initiated following a call for proposals from the United States National Institutes of Health (NIH) in 1992, in order to establish international sites for trials of preventive HIV vaccines. Cohorts at high risk of HIV infection were to be established in nine countries in Africa, Asia, and the Americas, in addition to US domestic cohorts. The research proposal sponsored by the NIH was "High Risk Cohorts in Kenya: Prostitutes and Truckdrivers", with the investigations to be carried out in a field site to be established in the coastal city of Mombasa.

The author, as field director, had overall administrative responsibility for establishment and maintenance of the field site. The studies covered in this thesis are those for which he had primary scientific responsibility for development, implementation and reporting during his tenure. The aims of these studies were:

1. To characterise a heterosexual, HIV-1 seronegative male cohort, at high risk of HIV-1 infection, for the purposes of inclusion in HIV-1 preventive vaccine trials.
2. To measure HIV-1 seroincidence and assess the following potential correlates of HIV-1 acquisition: male circumcision status, other STDs, sexual risk behaviour, and occupational travel.
3. To determine the prevalence of symptomatic and asymptomatic curable urethral infections in male trucking workers and examine strategies for control.
4. To document the potential acceptability of an HIV preventive vaccine trial in the cohort of male trucking company workers.

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Ethical Approval

The baseline and prospective studies (Studies 1-5) were approved by the Human Subjects Review Committee of the University of Washington, Seattle (appendix 1). The urethral infection study (Study 6) was approved by the Kenyatta National Hospital Ethical and Research Committee in Nairobi (appendix 2). The vaccine acceptability survey (Study 7) was not considered to require separate ethical approval by the Division of Human Subjects of the University of Washington, as no new subjects were enrolled and no new methods were introduced.

Background

The WHO Collaborative Centre for STD Research has existed at the Department of Medical Microbiology of the University of Nairobi, Kenyatta National Hospital Campus, since 1981. The first collaborators were the University of Nairobi and the University of Manitoba (Professors Frank Plummer and Allan Ronald). These institutions were joined by the Universities of Washington (Professor King Holmes), and the Institute of Tropical Medicine of Antwerp (Professor Peter Piot), in 1985. Research sites were established at Nairobi City Council special treatment (STD) clinic, and at a community site in the Pumwani slum, where many home-based female sex workers (FSWs) live.

There have been many active Kenyan research partners, the most prominent being Professor Ndinya-Achola and Dr Bwayo. It was Dr Bwayo's work with the long-distance truck drivers at the Athi River Weigh Station , on the Mombasa Road 25 km east of Nairobi, which was instrumental in the formulation of many of the research questions which we attempted to address in Mombasa [1].

Mombasa is a city of approximately half a million people and a major east African sea port. Trucking companies which service trade routes throughout east-central Africa have terminals in Mombasa where trucks are maintained and loads assigned to drivers. The drivers and their assistants stayed in Mombasa for several days during the "turn-around" period. This provided potentially regular access to the men, a problem which could not be overcome at Athi River, where the men remained for a few hours only.

A feature which potentially played a role in the importance of the transport industry in the HIV epidemic is the industrialisation process which is gaining pace in Kenya, driven in part by the economic Structural Adjustment Programmes (SAPs) of the World Bank and International Monetary Fund. These SAPs have inadvertently fuelled the HIV-1 epidemic by shifting emphasis from food to cash crops, thereby making subsistence farming less viable and accelerating rural to urban migration, improving roads and communications infrastructure, and decreasing expenditure on health and social services [2]. A cycle of uncontrolled and unregulated development has ensued, with influx of male migrant workers into the large towns, including Mombasa.

In the current pandemic, more emphasis has been placed on changing the behaviour of women for the following reasons: high-risk groups are easier to identify and access, women are more receptive to health messages, and more amenable to community activities. What is clear, however, is that the power lies with the male when it comes to sexual decision making. As long as there are poor women and gender inequality, the ability of women to effect behaviour change, such as consistent condom use, will be limited [3].

While there are now effective cures for many STDs, access to treatment in developing countries is often difficult, and conditions which prevailed in Victorian London are mimicked in modern African cities, with private wealth, public squalor, and rampant prostitution. The lifestyle of men who leave their tribal homes to come to work in the industrial centres is Spartan and often brutal. They tend to live in

bachelor groups in low-quality accommodation. There is a need for greater understanding of the determinants of male sexual behaviour in these conditions.

Ganjoni Clinic in Mombasa, the operational centre of the research in this thesis, is also of historical interest in STD control. In the 1940's and 50's, Mombasa was the sea port where the British colonial administration off-loaded their troops to police their commercial interests in east Africa. A thriving sex trade grew up around these troops, and the colonial administration found that it was losing soldiers' work time because of STDs. For this reason, a system of registration of female sex workers was set in place.

The advent of mass tourism to Mombasa in the last twenty years has been another factor which has had impact on commercial sex. While military and merchant navy ships from all over the world have traditionally docked at Mombasa, their arrival was sporadic and women would come from up-country to service the sailors. It was quite usual for buses to be hired by groups of women from Nairobi and the west of Kenya, to take them down to the coast, when the "fleet" was in port. The year-round presence of tourists with large amounts of money to spend has slowed this tidal flow of sex workers to a trickle, as many more are now resident in the town, and the differential increase in business when the ships come in has decreased.

Women who may initially have come to Mombasa to service the night-club sex trade, become bar workers as they become older and a less valuable commercial commodity, and sex with customers becomes a way of making ends meet [4]. These women are often sexual partners of trucking and other itinerant workers, and this point will be discussed in the appropriate sections.

It was planned that the prospective cohort of HIV-1 seronegative male long-distance truck drivers would form a closed cohort, which would both be followed at Ganjoni clinic. In this way, HIV-1 seroincidence would be measured and the subjects prepared for trials of a candidate HIV-1 preventive vaccine.

In a closed cohort design, a pre-determined number of subjects are recruited over a short period of time, and followed for a fixed period of time [5]. New subjects are not recruited after the recruitment period, and there is an expectation of a degree of cohort attrition due to migration, withdrawal and death. In the case of the truck drivers study, it was estimated that 750 seronegative men would be required.

This calculation was based on the assumptions that there would be an annual HIV-1 seroconversion rate of 10%, follow-up of 18 months, and a 20% loss to follow-up. This would allow 296 subjects in each arm of a randomised, double-blind, placebo-controlled vaccine trial, with 90% power to show a significant difference (alpha error 0.05) if vaccine efficacy was 50%. The first three months of the study were to be used for hiring and training of staff and establishing the field site. The following six months were to be used to screen 1,000 men from two trucking companies, in order to identify and enrol 750 seronegative men, and the next 18 months used to complete the follow-up period.

The first trucking company approached in Mombasa was the largest, Coast Hauliers. The management were orientated and gave permission for the men to come to Ganjoni clinic for follow-up. A general meeting of the men was held in the company yard and the programme was explained. The second largest company in Mombasa, Bayusuf Brothers, was contacted and the process repeated. Screening and

counselling services were planned and organised in Ganjoni clinic. After a period of two weeks, no men had been screened. One reason for this was that our arrival in Mombasa had coincided with articles in the local newspapers specifically identifying truck drivers as the cause of much of the spread of the epidemic. It was necessary to re-think the screening and follow-up strategy, and it was decided to take the clinic to the trucking company depots.

Two other issues became apparent. Firstly, there were insufficient long-distance drivers and their assistants to meet the sample size requirements, so a decision was taken to recruit all male employees. This included ancillary staff, such as mechanics, fuel men, tyre boys, storemen, and administrative staff such as clerks and personnel workers. This meant that no pre-screening process was necessary, so barriers to access were reduced. It was also made clear that if men participated in the study, there would be an open door policy on primary health care and counselling.

Secondly, it was clear that there were insufficient men in only two companies. The orientation and recruitment process was repeated in four other companies: Interfreight Panalpina, Transami, Sigiuon Freight and Anwarali Brothers. One other company, Transpares, was approached but the company management stated that they would require to have the results of HIV screening of individual men made available to them, so their participation was not pursued. The selection of companies for participation was done on the basis of size, in the absence of any prior knowledge that any company would be more or less suitable than another. All companies were located in the industrial area of the town. Each participating trucking company was visited on an arranged morning each week, and the clinic finished when everyone

who wanted to be seen for study or health care purposes had been attended to. The smallest company, Interfreight Panalpina, was visited on Wednesday afternoons, but so few men attended the clinic that it was stopped after one year. Access to health care and follow-up continued to be offered to men of all companies each weekday afternoon at Ganjoni clinic.

The mobile health team consisted of physician, nurses, health educator and counsellor, clerical assistant, and driver. Informal group discussions were held by the health educator at each company visit for men interested in joining the project or requesting information on health related issues. This was done in one of two ways. The most common was for him to place himself in a visible position in the yard, reading the newspaper. Men would then approach him and ask for verification and clarification of stories that they had heard about STDs and HIV. The second was for him to approach groups of men during their breaks and join in the conversation, which would invariably turn to requests for information on sexual safety matters.

In order to keep the clinic flowing, three separate areas were required:

- For examination and post-test counselling
- For HIV-1 screening and pre-test counselling
- For enrolment and follow-up interviews

As none of the companies would allocate more than two rooms, the project vehicle was used as an interview room for the duration of the project.

While we welcomed men who were HIV-1 seropositive at the clinic for care and follow-up, we did not actively try to trace them, as the goal was to gather a cohort of HIV-1 seronegative men. A database of the names, company, study numbers, and

date of last HIV ELISA blood test of the men were stored. Each day, after entering the results of the blood tests in the lab data file, the author or Dr Rakwar entered the date that the next follow-up was due. Only the two project physicians had access to the computer files, where the serostatus of the men was entered, in order to maintain confidentiality. Each morning, before leaving for the trucking depot, a list of men who were due for follow-up was printed out. A young man was employed to go round the depot and ask if the men on the list were in the depot on that day. If they were, he would ask them to come to the clinic, if not he would ask their friends to remind them to come next time.

As previously stated, it was necessary to adapt the recruitment design as the situation on the ground became clearer. The numbers of men recruited in the first 10 months (1993) were as follows:

March	99
April	131
May	57
June	47
July	74
August	61
September	43
October	27
November	41
December	19

This gave a total of 599 men after 10 months, over 150 less than the 6 month target. Measurement of the total number of men who may have been eligible for recruitment and follow-up was not attempted for the reasons outlined below. It was important that the project was seen by the men to be independent of the company management, in order to maintain the trust of the men that confidentiality was

observed. There was a perception among men that, if the company knew that a man was HIV seropositive, he would be dismissed. This was not stated company policy, but it is now becoming increasingly common practice in Kenya for companies to send prospective employees for a medical examination, including a blood test. The HIV test result is conveyed directly to the employer. In the event of a positive HIV blood test the man is simply informed that he has not been successful and will not be employed. Formal contact with the management was kept to a minimum and employee information was not sought from the companies, in order to avoid the expectation that exchange of information would be reciprocated.

The companies all had up-country depots in Nairobi, or the west of Kenya. Men were moved between depots depending on the balance of the company work-load. The types of contracts secured by companies could also cause fluctuations in the employment and deployment of men. An example of this was a large contract won by Bayusuf Brothers in 1993, to supply relief food to southern Somalia. Often at that time the depot would be almost empty, with the trucks all out and the mechanics doing running repairs between Mombasa and the Somali border, or full with the trucks being loaded, depending on the arrival of ships in the port.

The conditions of employment of truck drivers specifically dictate that they are highly mobile. Work assignments may take them from Mombasa to Sudan, Ethiopia, Rwanda or the east of Democratic Republic of Congo (Zaire) for well over a month at a time. This interfered with the regularity of availability for follow-up, and the accuracy of calculations of STD incidence. For example, if a man developed symptomatic urethritis while he was in Uganda or Rwanda, he would attend a

practitioner for treatment at that location. If cure was achieved, he would not have had any investigations done at the research clinic and have may chosen not to report the episode at the research clinic.

Because of these factors, and the fact that there was no candidate vaccine to test in early 1994, the study moved into an open cohort design by necessity rather than by any process of active planning. Heyward and associates, in a paper examining the methods for the determination of HIV-1 incidence for the purposes of phase III vaccine efficacy trials, determined that the open cohort may be more suitable for the development of a well characterised cohort available to participate in HIV-1 preventive vaccine efficacy trials [5]. For this reason, men were not designated as "lost to follow-up" from the cohort studies, and any time between follow-up visits included for the purposes of calculation of HIV-1 seroincidence. The primary outcome, HIV-1 acquisition, could still be measured with infrequent or intermittent follow-up, but collection of data on time varying correlates of HIV-1 infection, such as symptomatic STDs, would be less complete. The collection of data on symptomatic STDs was also incomplete due to the nature of the cohort, with men who were travelling seeking treatment at other clinics between follow-up visits.

Section 2: Literature Review

The HIV Pandemic

Since the first cases of a new clinical syndrome, which was named the acquired immunodeficiency syndrome (AIDS), were identified in the United States 1981, the HIV epidemic has spread throughout the world, often at an alarming pace [6]. The epidemic was initially called the "gay plague" in the media, because the clinical syndrome was first recognised in homosexual men. The laboratories of Gallo in the USA, and Montagnier in France reported the isolation of a human T-cell leukemia virus in subjects with AIDS, and at high-risk of AIDS respectively, in the same edition of the journal Science in 1983 [7,8]. This became known as HTLV-III before the current nomenclature of human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) came into common use. HIV-1 has been the strain responsible for the global pandemic, and is the strain which has been discussed exclusively in this thesis. HIV-2 is primarily found in west Africa, although some HIV-2 infections have been found in Angola and Mozambique in the southern hemisphere [9,10].

There are three main modes of HIV transmission. Sexual transmission is the most important, with over 80% of infections worldwide [11], and it is this mode of transmission which has been addressed in this thesis. Homosexual transmission was the predominant mode of transmission in the industrialised countries of Europe, North America and Australasia in the early phases of the epidemic, but there is evidence of rising trends in heterosexual transmission in industrialised countries [12]. Heterosexual transmission has been responsible for the rapid spread in developing countries in Africa and Asia, and the percentage of infected adults in the world who are women rose from 25% in 1990 to 45% by 1995 [13]. A mixed picture of the

importance of homosexual, heterosexual and injecting drug use in the evolution of the epidemic has been seen in South America and the Caribbean, with heterosexual spread now becoming more important [14,15].

Perinatal transmission, from mother to child in utero, intra-partum and through breast-feeding, is the next most important mode of spread globally [16]. Parenteral transmission is the third most important, either through transfusion of blood or blood products, or injecting drug use [17,18]. A striking example of different modes of transmission leading to identifiably separate epidemics has been reported from Thailand [19,20]. However, although two different sub-types of HIV-1 were identified in adults presumed infected through heterosexual intercourse (sub-type E) and through injecting drug use (sub-type B) early in the epidemic, later studies have shown a considerable degree of cross-over of subtypes [21].

While the HIV-1 risk for recipients of blood products in many countries has been eliminated, blood transfusions still play a role in HIV-1 transmission in Kenya, especially to the groups most likely to receive transfusions in malarious regions, women of reproductive age and children [22].

Quinn outlined four main phases of evolution of the HIV pandemic [23]:

- emergence
- dissemination
- escalation
- stabilisation.

He also asserts that the long incubation period, of up to and beyond ten years between HIV infection and HIV disease, has helped the epidemic to spread throughout the world unchecked. A study from Zaire in west-central Africa by Nzilambi and colleagues [24], is of special importance in this regard. In 1985, investigators tested 659 human serum samples collected in the remote Equateur province of Zaire in 1976, for antibody to HIV. Five (0.8 %) were found to be positive. Follow-up investigations in 1985 revealed that three of the five seropositive persons had died of illnesses suggestive of AIDS, and two remained healthy but seropositive. They conducted a repeat serosurvey in the same region in 1986, and showed a seroprevalence of 0.8% in 389 randomly selected residents. The investigators concluded that the long-term stability of HIV infection in residents of rural Zaire suggested that social change may have promoted the spread of AIDS in Africa, allowing the infection to emerge from remote rural areas, to be spread in urban centres.

The infection was disseminated to various parts of the world by travel, both internationally and through internal migration. Escalation occurred when the epidemic was amplified through high-risk populations, such as female sex workers, the male clients of sex workers, intravenous drug users and promiscuous homosexual men. The fourth stage, stabilisation, has occurred in Europe, North America, and Australia, and Quinn suggests that this may represent a degree of success in HIV prevention programmes, or equally that the epidemic is moving to an endemic phase. There is also evidence that the epidemic is stabilising, albeit at a much higher level, in parts of Africa, and this will be discussed in the appropriate section.

The UNAIDS/WHO Working Group on Global HIV/AIDS and STD estimated that 30.6 million people were living with HIV/AIDS by November 1997, in their update in the Weekly Epidemiological Record, published by the World Health Organisation [11] Almost six million of these were estimated to have been newly infected in 1997. The breakdown by geographic region is given in Table 1 below.

Table 1: Estimated number of adults and children living with HIV/AIDS as of November 1997, by region. [11]

Adults and children estimated to be living with HIV/AIDS as of November 1997	
Industrialised Regions	
North America	860,000
Western Europe	530,000
Australia & New Zealand	12,000
Regions with Mixed (Rich and Poor) Economy Countries	
Eastern Europe & Central Asia	150,000
North Africa & Middle East	210,000
East Asia & Pacific	440,000
Caribbean	310,000
Developing Regions	
Latin America	1.3 million
South & South-East Asia	6.0 million
Sub-Saharan Africa	20.8 million
Total	**30.6 million**

In addition, it was estimated that 11.7 million people had died of AIDS by the end of 1997, 2.3 million of these deaths occurring in that year. Forty six percent of those who have died of AIDS were women, and 460,000 were children. A total of

1,736,958 AIDS cases had been reported by all countries up to November 20, 1997. There is great variation by country in the proportion of AIDS cases which are reported, with the lowest proportion reported from the developing countries which are worst affected.

Over 90% of HIV infections have occurred in developing countries, with two thirds of the total in sub-Saharan Africa, which has an estimated overall HIV seroprevalence of 7.4% in adults aged from 15 to 49 years.

The seroprevalence rates in the general population in Asia are lower but, because of the large population size, there is a danger of explosive epidemics. Warning of this was given in the documented HIV-1 seroprevalence of 47% and seroincidence of 16% per annum in sex workers in Bombay in the early 1990's [25]. In addition, epidemics may vary widely in speed and intensity of progress, even within countries, as evidenced by the fact that the HIV-1 seroprevalence in sex workers in New Delhi remained stable at less than 1%, during the period of rapid rise in their peers in Bombay [26].

Although there have been some successes in the developed world, for example the decreased HIV-1 seroincidence among homosexual men as a result of safer sex practices [27], reduced acquisition of infection among injecting drug users because of altered needle sharing or cleaning practices [28], the failure of current approaches to control the epidemic in many high risk communities is obvious. Research demonstration projects have documented that HIV-1 acquisition can be slowed among female sex workers (FSW), an important core group in many developing countries [29,30]. However these programs have not been replicated on a large scale in most

countries, and the number of infected individuals globally continues to climb rapidly. The explosive spread of HIV-1 in Thailand, despite the accepted intervention tools of behaviour modification, condom promotion, and sexually transmitted disease control, was a reminder of the limitations of existing control strategies in the initial phases of the epidemic [31]. It is true, however, that serial surveys of twenty one year old male army conscripts in Thailand, up to 1995, produced convincing evidence of decreased risk behaviour, leading to a decrease in HIV-1 infection in young men [32].

Because of the global nature of the epidemic, outlined above, the United States National Institutes of Health sponsored nine international sites to prepare for vaccine and non-vaccine HIV-1 preventive interventions, four of which were in countries in sub-Saharan Africa: Malawi, Uganda, Rwanda (subsequently moved to Zambia), and Kenya.

HIV-1 Epidemiology in Sub-Saharan Africa

As stated above, it has been estimated that by the end of 1997, 68% (20.8million) of people living with HIV/AIDS were in sub-Saharan Africa, with the vast majority of adult infections transmitted through heterosexual intercourse. The National AIDS and STD Control Programme (NASCOP) of the Ministry of Health of the Government of Kenya, estimate that 75% of all HIV-1 infections in Kenya are attributable to heterosexual vaginal transmission, 20% are attributable to vertical transmission from mother to child, and 5% are due to blood transfusion [22]. Injecting drug use and anal intercourse are uncommon and not thought to contribute significantly to the magnitude of the HIV-1 epidemic in sub-Saharan Africa. UNAIDS has estimated that 90% of the three million infants born with HIV-1 infection were in sub-Saharan Africa [33].

Within each country, and regions of each country, the epidemic has progressed with varying speed in different population groups. The reasons for the full extent of the diversity of transmission rates have not been fully elucidated. Work done in Malawi, and published in the Journal of Infectious Diseases in June 1998, compared semen HIV-1 RNA concentrations of HIV-1 infected Malawian men with HIV-1 infected men who had never had anti-retroviral therapy, from Switzerland and the USA [34]. HIV-1 RNA concentrations in semen were over three times higher in Malawian men, in the absence of any urethritis and, while the reasons for these differences are not yet known, the investigators suggest that they may help explain the high rates of HIV-1 transmission in sub-Saharan Africa.

While information on male groups is sparse, studies have recorded HIV-1 seroprevalence among women attending antenatal clinics in eastern and southern Africa for more than ten years, with levels increasing in recent years to 30% or more in many urban centres [35-39]. Pregnant women are valuable as a sentinel group because they are sexually active, attend health facilities for reasons other than illness, and come from all social groups within society. They also give an indication of differences in background seroprevalence between different locations. However, a recent study from the Rakai area in Uganda has suggested that HIV-1 seroprevalence among pregnant women may underestimate true seroprevalence in similar birth and gender cohorts, due to an adverse effect of HIV-1 infection on fertility [40]. Another community study from Uganda has shown that prevalence has started to stabilise, and that an endemic phase of the HIV-1 epidemic may have begun in some areas [41]. Community HIV-1 seroprevalence in this advanced phase will be sustained by young people commencing sexual activity entering the susceptible population pool, and it is anticipated that peak HIV-1 incidence will be concentrated in the younger age groups [42]. In Malawi, pregnant women under 20 years of age were shown to have the highest annual HIV-1 seroincidence, at almost 6% per annum [43].

Antenatal surveillance data from municipal clinics in Nairobi, with lower socio-economic group catchment areas, are presented below. These data have contributed to the National Sentinel Surveillance Survey data, and have also been published, in part, in the AIDS in Kenya 1998 edition [22]. Table 2 shows province of origin of the 6,828 women who were screened, by HIV-1 serostatus.

Table 2: Province of origin and HIV-1 serostatus of 6,828 pregnant women screened in Nairobi, 1991-1997.

	Total [n=6828] (Percentage of total)	HIV-1 Positive [n=1004] (Percentage from each Province)
Province of Origin		
Coast	61 (0.9%)	5 (8.2%)
North eastern	137 (2.0%)	10 (7.3%)
Eastern	748 (11.0%)	68 (9.1%)
Central	1810 (26.5%)	162 (9.0%)
Nairobi	94 (1.4%)	9 (9.6%)
Rift valley	219 (3.2%)	34 (15.5%)
Western	1485 (21.7%)	207 (13.9%)
Nyanza	2274 (33.3%)	509 (22.4%)

One-third of women reported that their province of origin was Nyanza, in the extreme west of the country, and one quarter came from Western or Rift Valley provinces, two neighbouring provinces in western and central-west Kenya (Figure 1). The location of Mombasa, the study site, is indicated on that map. HIV-1 seroprevalence was significantly higher in Nyanza than in the other provinces (22.4%, 95% CI 20.9-23.9), and women from Western and Rift Valley provinces were more likely to be HIV-1 infected (14.1%, 95% CI 12.7-15.5) than women from Nairobi, Central, Eastern, North Eastern and Coast provinces (8.9%, 95% CI 8.3-9.5), $P < 0.001$. Some issues related to these differences are discussed below.

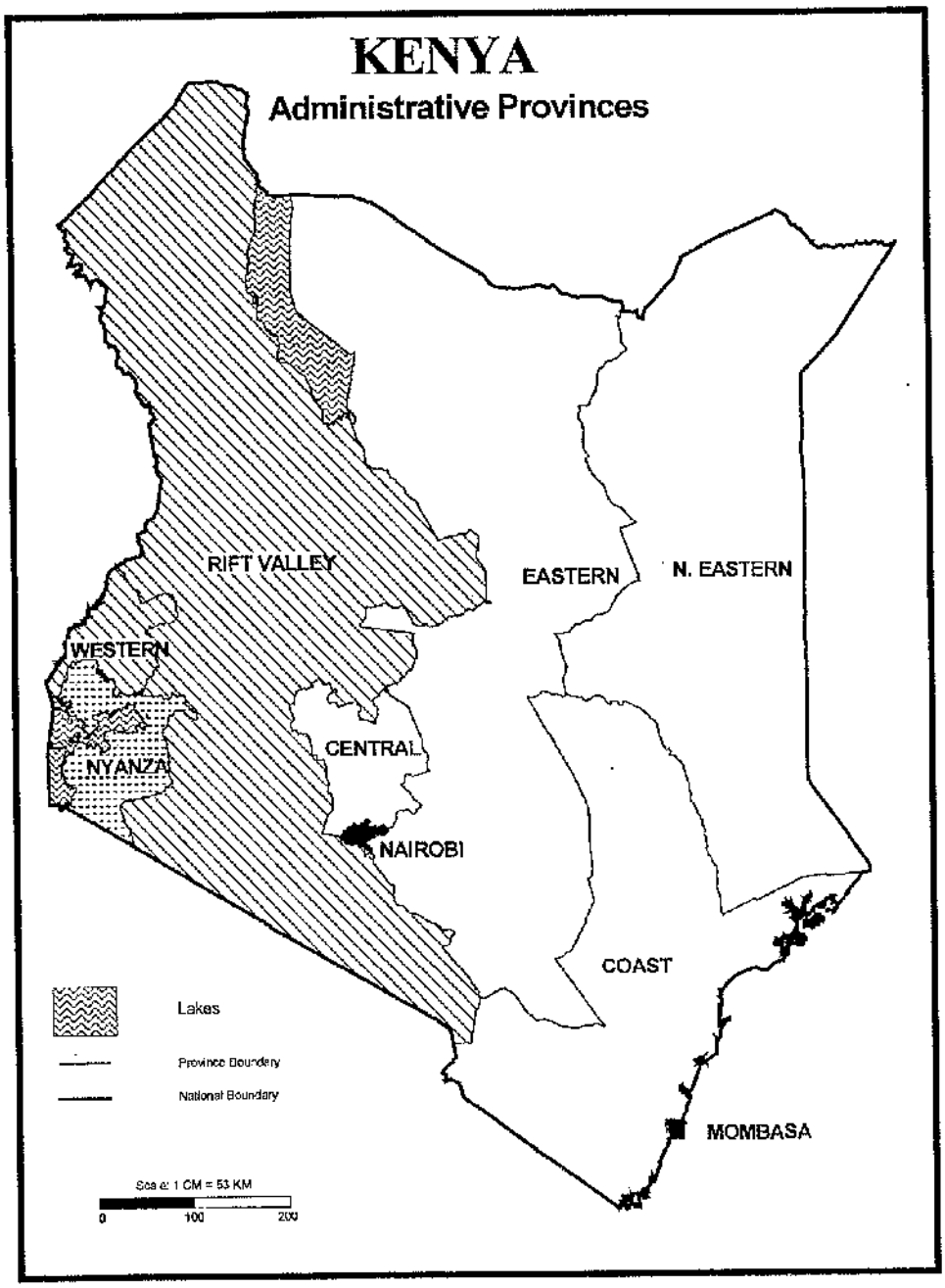
It is noteworthy that less than 2% of women gave their province of origin as Nairobi. One reason for this is that while people may have lived in Nairobi for many years, they still perceive themselves as part of a family group which lives in the ethnic home province. Another observation, made by Ferguson in a fertility and family planning study in two rural areas in Western and Eastern Provinces [44], is that there

are regional differences in male migration, with 59% of men from the rural Western Province study area living away from home compared with 21% from Eastern Province. The temporal pattern of migration also differed, with men from Western Province returning home only at Christmas and Easter, whereas men from Eastern Province often returned home on a monthly basis, because of easier access. The potential importance of this difference will be discussed in the body of the thesis.

It has been noted that fertility levels vary widely in different localities in Nairobi, localities with the highest fertility being those occupied largely by ethnic groups from western Kenya [45]. This was interpreted as suggesting that the cultural practices of the ethnic groups who migrate to Nairobi remain the same, perpetuating customs which led to the differential fertility rates. It may be that this also applies to sexual network patterns, contributing to differences in HIV-1 seroprevalence.

Hunter, Kapiga and colleagues have reported that lack of circumcision in the husband is associated with increased HIV-1 seroprevalence in pregnant women in Nairobi [3] and with increased seroincidence in pregnant women in Dar es Salaam [46]. The association in general between lack of male circumcision and risk for HIV-1 infection has also been reviewed in detail [47], and will be addressed in this thesis. The main ethnic group which does not practice male circumcision in Kenya comes from Nyanza Province [3].

Figure 1: Map of Kenya



Gregson and colleagues have suggested methods of using age-specific HIV-1 prevalence data to measure HIV-1 incidence in stable endemic conditions [42]. They note that very young women who attend for antenatal care may be at higher risk of infection, as they are sexually active at a young age. On the other hand, caution should be exercised in interpreting falls in HIV-1 seroprevalence as falls in incidence, even in young people, as apparent falls in age-specific prevalence may at least in part be accounted for by mortality, migration, aging and higher absenteeism among HIV-1 positive individuals [48].

HIV-1 seroprevalence was almost 15% in the "low-risk" group of pregnant women, included in the Nairobi surveillance data. Differences by province of origin were consistent with those found in unlinked, anonymous antenatal testing performed by the Kenyan National Sentinel Surveillance Survey up to 1997, extracts of which which are reported in table 3 below [22]:

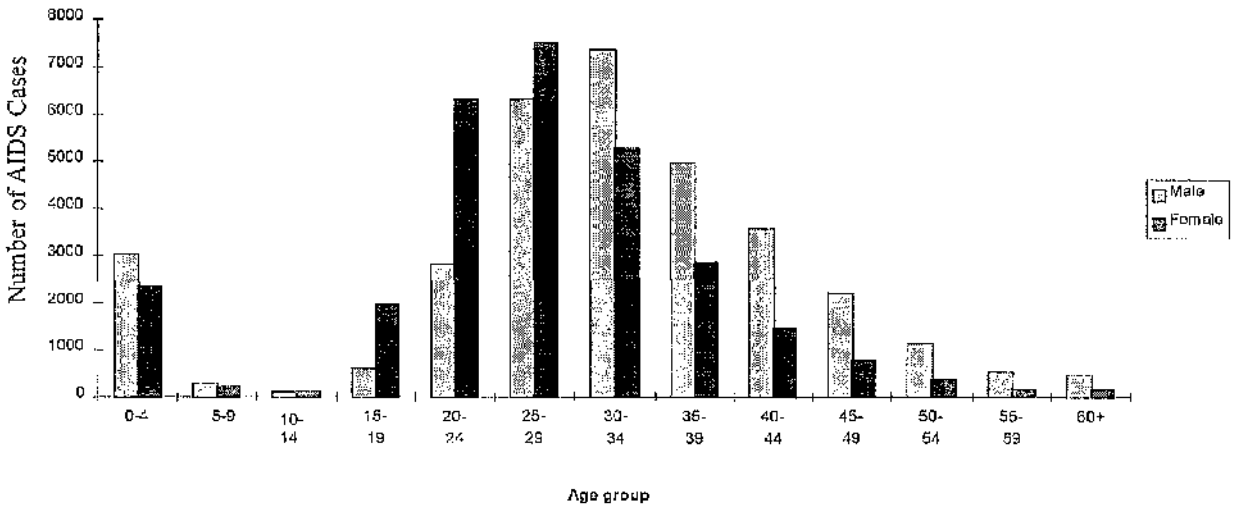
Table 3: Percentage of pregnant women testing positive for HIV-1 in unlinked anonymous testing in Kenya. (AIDS in Kenya [22]: reproduced by permission of the author, Elizabeth Pisani)

Sentinel Site	1990	1993	1995	1997
Garissa (NE)	4.9	3.8	5.8	8.1
Mombasa (Cst)	10.2	16.5	15.8	17.4
Meru (E)	2.7	2.3	8.7	13.8
Nyeri (Cnt)	2.9	5.4	9.6	10.1
Nakuru (RV)	10.0	22.5	27.2	24.6
Busia (W)	17.1	22.2	22.0	28.1
Kisumu (Nyz)	19.2	19.6	25.3	34.9
North Eastern = NE, Coast = Cst, Eastern = E, Central = Cnt, Rift valley = RV, Western = W, Nyanza = Nyz				

Past experience has shown that the peak age of HIV-1 prevalence (and incidence) in males is approximately 5 years older than in females in East Africa

[49,42]. This is reflected in the age and gender pattern of AIDS cases reported to the kenyan National AIDS and STD Control Programme between 1986 and 1997, represented in figure 2, and consistent with mortality data from Uganda [49]:

Figure 2: Age and sex distribution of reported AIDS cases in Kenya, 1986-1997



(AIDS in Kenya [22]: reproduced by permission of the author, Elizabeth Pisani)

The Kenya Demographic and Health Survey (KHDS) of 1993 reported that men were older at first marriage with a median age difference between husband and wife of seven years [50]. The median age at first marriage of urban Kenyan women was 20.6 years. Under 5% of women of any age reported more than one sex partner in the previous six months. Forty five per-cent of men aged between 20 and 24 reported more than one sex partner in the previous six months, and 24% of men in that age group reported 3 or more sex partners in the previous six months. It follows that, if a twenty year old woman now marries a twenty seven year old man, he has been through his period of highest risk sexual activity in an extremely high risk environment, and she may be in grave danger because of his past activity. A reflection of this is that, of 1059 pregnant women in Nairobi, screened between 1991

and 1997, who reported one lifetime sexual partner and who had no perceived risk of AIDS, 80 (7.6%) were infected with HIV-1 (unpublished data).

AIDS is already the leading cause of adult death in some urban and rural areas of sub-Saharan Africa [49, 51], and models of the course of the epidemic predict that this will soon be the case for many other parts of this region [52]. The importance of co-factors, such as STDs and uncircumcised status in men, in the rapid spread of the HIV-1 epidemic among heterosexual STD high-frequency transmitter core groups (see below) in east and central Africa is well documented [47,54]. Interventions to decrease the prevalence and incidence of STDs in the general community have been targeted at core groups, such as female sex workers (FSWs) and their clients [54]. Truck drivers have been identified as a male occupational group at high risk of STD and HIV-1 acquisition, who may play an important role in dissemination of these infections because of their geographic mobility [1].

The need for more effective interventions to control the current HIV-1 epidemic is clear, and a vaccine would hold the most promise for prevention of new infections. The ideal vaccine would be cheap, safe, highly efficacious when given as a single dose, and associated with lifelong sterilising immunity to all strain types of HIV. While the quest for a vaccine with these properties continues in many centres, preparation for safety and efficacy trials in human populations has been conducted simultaneously in many countries worldwide.

In the absence of a vaccine of high preventive efficacy, there is a clear need to identify other practical interventions which will reduce HIV-1 transmission. Behaviour change programmes, incorporating health education, risk reduction

counselling, STD care and condom promotion, have been successful in decreasing HIV-1 incidence in female sex workers in Africa and homosexual men in North America [27,30]. However, there are few examples of prospective data on the ability of educational interventions to effect changes in sexual behaviour and incidence of HIV-1 or STDs in heterosexual male cohorts.

This cohort is one of only four non-clinic based studies in Africa (3 prospective) which have published data examining male HIV-1 and STD acquisition, and that of male circumcision as an HIV-1 public health intervention strategy [55-57].

STD Epidemiology

STDs are ranked among the top five categories for which adults attend health care clinics in developing countries [58]. They are an important cause of morbidity, including neonatal infections and stillbirths, and infertility in sub-Saharan Africa [59], and they contribute to the scale of the current HIV-1 epidemic in this region [54].

Apart from HIV-1, the STDs which were the focus of this thesis are the five most common curable STDs: gonorrhoea (*N gonorrhoeae*), chlamydia (*C trachomatis*), trichomonas (*T vaginalis*), chancroid (*H ducreyi*) and syphilis (*T pallidum*). The world Health Organisation estimated in 1995 that there were 333 million new cases of curable STDs worldwide; 12 million syphilis, 62 million gonorrhoea, 89 million chlamydia, and 170 million trichomoniasis [59]. They also estimate that sub-Saharan Africa has the highest incidence, with 11-35% of 15 to 49 year-olds becoming infected with a curable STD over the course of a year.

Transmission co-efficients for sexually transmitted diseases can be defined as the fraction or percentage of sexual contacts which result in transmission, when one partner is infected. An equation which has been used to describe the basic reproductive rate (R_0) of an STD is [60]:

$$R_0 = \beta c D$$

where β is the transmission co-efficient, c is the effective rate of partner change and D is the duration of infectivity.

The transmission co-efficients of the STDs between partners, as given in the appropriate chapters of the standard text, Sexually Transmitted Diseases, edited by Professor King Holmes and others, are outlined below [61]:

T pallidum	30% from patients in primary or secondary phase
H ducreyi	59% from men with ulcers to female sex partners
T vaginalis	33% female to male, 85% male to female
C trachomatis	45% male to female
N gonorrhoeae	20% per sexual contact female to male
	50%-90% per sexual contact male to female.

Clearly such estimates are approximate, as tracing all contacts and persuading them to attend for investigation is at the very least difficult, and there is a potential for bias in that people with low-grade symptoms of infection may be more likely to attend as a result of tracing.

Thomas and Tucker reviewed the development and use of the concept of a sexually transmitted disease core group, which is central to the calculation of c, the effective rate of partner change [62]. They noted that the term has been used to refer to people who are infected a large proportion of the time, infect more than one person, are repeatedly infected, are sex workers and their clients, or to geographic areas with large numbers of cases. They call for more research interventions targeting strategic groups to evaluate their effectiveness. A percentage of trucking workers fit into more than one category outlined, and they have been the subject of sexual behaviour and STD research and intervention on three continents [1,63,64]. They can also be viewed as a "bridging population" for STD spread between members of high-prevalence and low-prevalence sub-populations. Wasserheit and Aral believe that these bridging populations may be the most important agents of STD spread throughout society, as they have concurrent sexual relationships with high and low risk groups [65]. FSWs

living in Kenya, who are often the "high-risk" partners of these men, have seroprevalences of HIV-1 infection as high as 80% in Nairobi and 55% in Mombasa [66,67]. The seroprevalences of the "low-risk" partners was addressed in the preceding section of the introduction. Stigum and colleagues have pointed out the importance of people who move between "core" and "non-core" behaviour in HIV-1 transmission [68]. While their work refers to the Norwegian situation, the same principle applies to men in Kenya who act as part of a core group when they live away from home, and as non-core when they live at home.

The durations of infectivity (D), quoted in the respective Natural History sections of the King Holmes standard text, are given below:

T pallidum	during primary or secondary phases - can be years.
H ducreyi	until ulcers or discharging buboes heal. One or two weeks with antibiotics, usually approximately 10 weeks without antibiotics
T vaginalis	Can be several years in women.
C trachomatis	Unknown. Thought to be many years
N gonorrhoeae	Resolution occurs within six months in over 95% of untreated men

Ulcerative STDs, such as chancroid, have been implicated most often as facilitating HIV-1 transmission [69], but prospective studies from Kenya [70] and Zaire [71] have found that the presence of non-ulcerative sexually transmitted diseases also increase the risk of acquiring HIV-1 infection. Retrospective studies from Kenya [1], Côte d'Ivoire [72] and India [73] have also reported associations between history of urethral discharge and HIV-1 infection. However, little is known about the

epidemiology of urethral infection among men in Africa, and about asymptomatic infection in particular.

Asymptomatic urethral infections may be more common than is generally supposed. Cross-sectional studies in the United States have revealed prevalences of up to 5% for *Neisseria gonorrhoeae* and 11% for *Chlamydia trachomatis* infections among asymptomatic young men who were at high-risk for STDs [74]. A study involving a general population of 6,000 men in rural Tanzania reported a combined prevalence of gonococcal and chlamydial infection of almost 3%, 85% of whom were asymptomatic [75]. Studies in the United States have shown that *Trichomonas vaginalis* can cause symptoms of urethritis [76], and that it can be carried asymptomatically in the male urethra for at least four months [77]. It is unclear whether pathogens causing asymptomatic urethral infection are as efficiently transmitted as those associated with symptomatic infection, but it is likely that they do play a role in transmission, and must be identified and treated if the chain of transmission is to be successfully interrupted. Cohen and colleagues, working in Malawi, have shown that appropriate treatment of urethral infections decreased HIV-1 viral shedding in semen, potentially decreasing the transmission co-efficient for HIV-1 [78].

Active case finding is required to identify asymptomatic infection. In most African countries, however, because of cost and lack of infrastructure, it is usually not possible to perform laboratory diagnostic tests on even symptomatic individuals. Risk assessment strategies and the use of simple, inexpensive screening tests have therefore been advocated to identify sexually transmitted infections in such populations [79]. In

this regard, several North American investigators have evaluated the urine leucocyte esterase dipstick (LED) test, which identifies pyuria, and it has shown promise as a screening tool to identify *N. gonorrhoeae* and *C. trachomatis* infections among asymptomatic men [80-82]. It has also been favourably evaluated in two studies from Africa in screening for urethral infection among primarily symptomatic men attending health facilities [883,84].

The prevalence of the three most important urethral pathogens in a workplace population of both symptomatic and asymptomatic East African truck drivers and ancillary workers has been measured in this thesis. Various potential strategies for the screening and management of urethral infection, including use of the urine LED test, have been evaluated and we have attempted to identify demographic and behavioural risk factors which could provide guidance in the development of risk scores for screening purposes.

With the recent finding in a randomised, controlled community trial in Tanzania that HIV-1 transmission can be significantly reduced by an effective STD treatment programme [85], identifying and treating urethral infections in Africa becomes an even higher public health priority.

HIV-1 and STD epidemiology

That transmission of HIV-1 is enhanced in the presence of many classical STDs (epidemiological synergy) is now well accepted [53,86-92]. As male acquisition of HIV-1 and STDs is the main focus of this thesis, emphasis will be placed on aspects of that interaction.

Mathematical modelling work done in Thailand on the probability of female-to-male transmission of HIV-1 infection per sexual contact [86], found that overall probability of of HIV-1 male acquisition was up to 5.6% per sex worker contact in the presence of STDs, and 1.2% with no STDs. A history of STDs increased this probability by a factor of between 3.3 and 5.1, and the degree of effect varied with different STDs. These estimates of probability of transmission of HIV-1 per sex act (β) are higher than those seen in Western countries [93], but consistent with high rates of HIV-1 acquisition in men and women with genital ulcer disease in Nairobi [87]. The investigators concluded that the high prevalence of STDs in the sex worker population and the frequency of of sex worker contact by the military recruits may have contributed to to the rapid spread of HIV-1 infection in this population. This is given credance by the fact that studies in discordant couples in Thailand with a low prevalence of STDs found transmission coefficients similar to those in the West [94].

Historical data was used by Job Bwayo and colleagues on long-distance truck drivers, screened and surveyed at the Athi River weigh bridge station, 25 km east of Nairobi [1]. They found that a history of genital ulcer or history of urethritis in the past 5 years were statistically significantly associated with HIV-1 infection on multivariate analysis.

Increased numbers of CD4 lymphocytes, which are target cells of the HIV-1 virus, are present in increased numbers in the endocervical canal of women with nonulcerative STDs [95]. Work done by Moss in Nairobi showed that HIV-1 DNA detection on urethral swabs could be reduced by appropriate treatment of gonorrhoea and resolution of urethritis [92], and research by Cohen in Malawi showed that treatment of gonorrhoea also decreased quantitative shedding of HIV-1 in semen [78]. Lillienfield outlined the discriminators used in making a causal inference between risk factors and disease or infection [96]:

- Strength of association

As we have seen, odds ratios of over 5 have been observed for history of STDs and identification of HIV-1 infection [86,91].

- Consistency of the observed association

The relationship between STDs and HIV-1 has been reported from a wide range of populations in different continents[10,27,32,37].

- Specificity of the association

Specificity has become less important as a criterion for causality, as the recognition of multifactorial aetiologies leading to disease has gained ground. An example of this is ischaemic heart disease, in which smoking and high cholesterol levels are exposures which are both accepted as causal. In this thesis, evidence will be examined as to why STDs, lack of male circumcision and occupational travel should all be viewed as having a causal association for HIV-1 infection.

- Temporal sequence of events

This was well demonstrated by Cameron and colleagues in the Special Treatment Clinic in Nairobi, when men presented with genital ulcer disease and urethritis [91]. The initial HIV-1 seroprevalence was 12%, and 8.2% of seronegative men who returned for follow-up seroconverted to HIV-1 in the following 12 weeks.

- Dose-response relationship

It is often difficult to identify a gradient of exposure and incidence of infection or disease and sometimes proxy indicators of exposure must be used. In the study by Cameron, referenced above, it was noted that newly acquired infection was independently associated with frequent sex worker contact (risk ratio 3.2), which may be considered as a proxy indicator of increased exposure to STDs, given the high prevalence of STDs in sex workers in Kenya [54,66].

- Biological plausibility of the observed association

Association of an infection which is transmitted by sexual contact (HIV-1) with diseases which either disrupt the integrity of the mucosal barrier or increase the shedding of the infectious particles at the site of infection is entirely plausible.

- Experimental evidence

Double-blind, randomised, controlled clinical trials are the gold standards of intervention research. However, there are very clear ethical problems in the design of any experimental study which involves exposure to an infection with such devastating effects as HIV-1. A community randomised and controlled research study, with improved STD management as the intervention, was carried out in Mwanza,

Tanzania [85]. That study was carefully constructed to conform to ethical criteria, and reported a 42% decrease in new HIV-1 acquisition in the intervention communities.

As stated above, WHO estimate that sub-Saharan Africa is the region most seriously affected by the epidemics of HIV-1, and other STDs. The literature on the relationship between HIV-1 and STD transmission, and the other potential correlates of sexually transmitted infections, male circumcision status and migration, are discussed below.

HIV-1 and Circumcision Status

There has been considerable debate over the role of uncircumcised status in the male in HIV-1 transmission over the past ten years. A Medline database search was conducted for entries from October 1993 to February 1998, to identify studies investigating the relationship between HIV-1 transmission and lack of male circumcision. The preceding period was covered by a review of this subject by Stephen Moses, referenced above. That review identified 30 epidemiological (26 cross-sectional, two prospective and 2 ecological) studies, 15 of which were published in peer-review journals and 15 as conference abstracts. Eighteen of the 26 cross-sectional studies from 6 countries (Cote d'Ivoire, Kenya, Rwanda, Uganda, United States and Zambia) found a significant association between HIV-1 infection and lack of circumcision. Trends towards an association were found in four other countries (The Gambia, Mexico, Tanzania and the United States). The two prospective studies (from Kenya) and the two ecological studies from Africa all showed positive associations between the presence of a foreskin and HIV-1 acquisition. Measures of increased risk (odds ratios or relative risks) of between 1.5 and 8.4 were recorded in studies which showed a significant positive association. Many of these studies are referenced elsewhere in the thesis, but they are not referenced individually in this section. There is, however, one study which merits special mention, as it was carried out in the Dept of Medical Microbiology of the University of Nairobi and is still widely quoted, almost 10 years after it was first published. Cameron and colleagues followed 293 male STD patients prospectively, and found that lack of circumcision was associated with a rate ratio of 8.1 (95% CI, 3.4-19.7) for HIV-1 acquisition [91].

Since that review, which was published in 1994, both Nasio and Tyndall have confirmed associations in Nairobi [97,98]. Nasio found an odds ratio of 4.67 for HIV-1 infection and uncircumcised status at enrollment of patients who presented with an STD at the Special Treatment Clinic (STC), and Tyndall found an odds ratio of 4.8 (95% CI, 3.3-7.2) in men who presented with genital ulcer disease, after adjusting for sexual exposure variables in a multivariate logistic regression model. Another cross-sectional study was carried out in Kigali, among married men who volunteered for counselling and HIV testing [99]. Uncircumcised men in that study had a relatively low-risk profile in that they reported fewer lifetime sex partners and sex worker contacts than circumcised men, and were more likely to live in rural areas where HIV-1 seroprevalence is lower. In spite of this, uncircumcised men were more likely to be infected with HIV-1 (29% vs. 21%, p=0.02). After adjusting for sexual behaviour variables, lack of circumcision remained significant (OR 1.69, 95% confidence interval 1.16-2.47). Urassa and colleagues published results of five population-based studies in north-western Tanzania [56]. On univariate analysis, circumcision status was unrelated to HIV-1 infection in most of those five studies. However, after adjusting for confounding variables, HIV-1 seroprevalence was found to be less in circumcised men (OR 0.62; 95% CI 0.48-0.81). The effect was strongest in urban areas (OR 0.46; 95% CI, 0.32-0.68), and in roadside settlements (OR 0.65; 95% CI, 0.42-1.01). The introduction of male circumcision as a mass HIV-1 prevention strategy has been under serious discussion since 1993 [100].

Causal inferences on lack of circumcision status and HIV-1 acquisition are discussed below:

- Strength of association

The prospective study of Cameron and colleagues revealed a rate ratio over 8 [91], and another study by Tyndall, on 413 genital ulcer patients [98], published in 1996, found an odds ratio of 4.8.

- Consistency of the observed association

Twenty two of the 30 studies reviewed by Moses [47], and all five of the referenced studies since, have shown a significant association between presence of the foreskin and HIV-1 infection.

- Specificity of the association

As uncircumcised men are also more susceptible to other STDs, especially genital ulcer disease, the specificity of the association is not certain [91]. This will be discussed further in the body of the thesis.

- Temporal sequence of events

In the published prospective study, the exposure clearly preceded infection. However, as circumcision is generally carried out either in infancy or at puberty, it could usually be shown to fulfill the temporal criterion.

- Dose-response relationship

Usually circumcision either has, or has not, been carried out and so the concept of a biological gradient does not apply. Bongaarts and colleagues did, however, apply this criterion to percentage of the population circumcised in their ecological study [101], and they found a strong inverse correlation ($r=0.9$, $p<0.001$) with seroprevalence of HIV-1.

- Biological plausibility of the observed association

That an increased surface area of non-keratinised mucosa exposed to trauma during sexual intercourse may lead to an increase in the incidence of sexually transmitted diseases is clearly plausible. It is also possible that sub-clinical balanitis is a frequent occurrence in uncircumcised men, especially if levels of hygiene are not optimal [47]. These areas of minor inflammation could act like small ulcers, by disrupting the integrity of the mucosa. In addition, there may be recruitment of target lymphocytes to the inflamed area. It has been demonstrated that Langerhans cells, which are plentiful in the foreskin of male macaque monkeys, are highly susceptible to Simian Immunodeficiency Virus [102]. If this is also true for humans and HIV-1 infection, it may be that the foreskin is more vulnerable than the other tissues in the male genital tract.

- Experimental evidence

Experimental evidence, i.e. deliberately attempting to infect circumcised or uncircumcised humans, is not available for clear ethical reasons. However, the research in macaques referenced above is interesting in this regard.

In the absence of an authoritative randomised controlled trial, there continues to be debate on the potential benefits of the introduction of male circumcision as a population HIV-1 prevention strategy. Professor King Holmes suggested that we use this cohort to perform a randomised controlled trial to examine the relationship between HIV-1 and male circumcision status, but the available sample size was inadequate for this purpose.

HIV-1 and Migration

There is now a large body of literature on the relationship between migration and HIV-1 infection, and throughout the ages any mass movement of men without their wives or girlfriends, whether for reason of war or economic necessity, has led to increased transmission of sexually transmitted diseases [103-114]. It is sub-Saharan Africa's great misfortune that this turbulent period of development, urbanisation and wars has co-incided with this epidemic of a new sexually transmitted disease, which is enhanced by other STDs.

Causal inference criteria:

- Strength of association

A prospective cohort study in a rural Ugandan area found that seroincidence of HIV-1 in people who had moved into the area in the past five years was more than double that of people who had been living in the area for more than ten years [103]. The investigators concluded that this was likely to be a result or more risky behaviour, and less stable sexual relationships, among those who were more mobile.

- Consistency of the observed association

Travel outside of the region or country of origin was one of the original recognised risk factors, and the reason why Dr Bwayo started the long-distance truck drivers study in Athi River in June 1989. Examples of HIV-1 transmission studies in five continents are included in the references [1,63,64,110,111].

- Specificity of the association

As in all three of the factors (STDs, circumcision and migration) subjected to causal inference examination here, specificity is the most difficult of the criteria to speak clearly on, as there are so many societal, cultural, economic, physical and psychological factors which make people vulnerable to HIV-1 infection.

- Temporal sequence of events

The prospective study by Nunn and colleagues from Uganda referenced above clearly showed that the relationship satisfied this criteria, in that the study subjects were not infected before they moved from home.

- Dose-response relationship

It is still difficult for us to quantify the migration and HIV-1 association in a dose-response way, but Nunn and colleagues did find a gradient in HIV-1 seroprevelance with increased distance of migration in Uganda [103]. Anderson, in a paper on aspects of sexual behaviour and the potential demographic impact of AIDS in developing countries, called for more quantitative analysis of patterns of sexual mixing in communities [114]. The experience from Nakuru, in the Rift Valley (Figure 1), is possibly instructive in this respect. Sentinel surveillance in pregnant women in 1990 revealed an HIV-1 seroprevalence of 10.0%, which had increased to 27.2% in 1995 (Table 3). Compare this with Mombasa, a port city also on the trans-Africa highway, for which the seroprevalences in 1990 and 1995 were 10.2% and 15.8%, respectively. Nakuru is the capital of Rift Valley Province, which has been the site of internationally publicised "tribal clashes" throughout in early 1990's. These violent

clashes were about land ownership and resulted in displacement of many people from their rural homes, forcing them to re-locate in urban Nakuru.

- Biological plausibility of the observed association

It is entirely plausible that disruption of family life, with separation of husband and wife, and removal of cultural safeguards leads to increased sexual risk behaviour and HIV-1 transmission.

- Experimental evidence

Ethical considerations preclude experimentation in this subject.

The findings of Nzilambi and colleagues in Zaire are of great interest in the relationship between HIV-1 transmission and migration [24]. They found that HIV-1 remained endemic at a low level (<1%) over a ten year period, between 1976 and 1986, in a stable rural population and concluded that social disruption and mobility led to the explosive epidemics seen in urban centres.

The existing literature gives sufficient *a priori* evidence that different types of sexual risk behaviours, other STDs, circumcision status and migration patterns would potentially act as confounders in any vaccine or non-vaccine HIV-1 preventive intervention. The quantification of these variables, and their interaction with HIV-1 acquisition, was deemed by the US National Institutes of Health to be important in the preparations for such research interventions.

Section 3: Aims, Objectives and Project Timetable

Aims

1. To characterise a heterosexual HIV-1 seronegative male cohort, at high risk of HIV-1 infection, for the purposes of inclusion in HIV-1 preventive vaccine trials.
2. To measure HIV-1 incidence and assess the following potential correlates of HIV-1 acquisition: other STDs, sexual risk behaviour, male circumcision status and occupational travel.
3. To determine the prevalence of symptomatic and asymptomatic curable urethral infections in male trucking workers and examine strategies for control.
4. To document the potential acceptability of an HIV preventive vaccine trial in the cohort of male trucking company workers.

Objectives

1. Determine the HIV-1 seroprevalence in men screened for antibodies to HIV in trucking companies in Mombasa (Study 1).

2. Estimate the prevalence of symptomatic STDs at first presentation in the HIV-1 seronegative male cohort (Study 2).

3. Calculate HIV-1 seroincidence and examine the effects of potential correlates of HIV acquisition in the male trucking company worker cohort (Study 3).

4. Monitor sexual risk behaviour over time in the male cohort of trucking company workers (Study 4).

5. Monitor STD incidence over time in the male cohort of trucking company workers (Study 5).

6. Measure the prevalence of symptomatic and asymptomatic urethral infection in the male cohort of trucking company workers (Study 6).

7. Survey male trucking workers for potential acceptance of the probable conditions of an HIV-1 preventive vaccine trial (Study 7)

Table 4: Project Timetable and Thesis Study Guide (Also on inside cover)

October, 1992 United States National Institutes of Health approve research project "High-risk cohorts in Kenya: Prostitutes and Truckdrivers" as part of the Preparation for the AIDS Vaccine Evaluation (PAVE) initiative
December 9, 1992 Author recruited to be Project Field Director by University of Washington. Discussions in Seattle regarding project planning
January 25, 1993 Investigators gathered in Nairobi to begin preparations for implementation
February 7, 1993 Investigators travel to Mombasa to begin Laboratory and clinic and trucking company preparations
February 15, 1993 Screening centre opened at Ganjoni Clinic
March 1-4, 1993 Mobile clinic started at Coast Hauliers (First men screened), Bayusuf Brothers, Interfreight, and Transami. (Siginon Freight added 31/3/93 and Anwarali Brothers added 14/7/93)
Studies 1 and 2: March 8, 1993 – January 31, 1996 **Prevalence** ***Enrollment and HIV-1 prevalence data on 1367 men screened. STD prevalence data on 955 seronegative men.*** 1136 HIV-1 seronegative, 955 (84%) enrolled 231 HIV-1 seropositive, 118 (51%) enrolled
Study 3: March 15, 1993 – January 31 1996 **Incidence** ***Routine follow-up and interim STD evaluation data collected for HIV-1 seroincidence and correlates of HIV-1 acquisition study*** 955 HIV-1 seronegative men enrolled. 716 HIV-1 seronegative men (75%) contributed incidence data. **990 person years of follow-up**
Studies 4 and 5: March 15, 1993 – October 14, 1994 **Incidence** ***Routine follow-up and interim STD evaluation data collected for time-trends analysis of sexual behaviour and symptomatic STD incidence*** 1195 men screened, 206 (17%) seropositive. 836 (85%) of 989 seronegative men enrolled. 556 men HIV-1 seronegative men contributed incidence data, 72 % of 771 men eligible for routine follow-up (enrolled for 3 months or more by October 14, 1994) **494 person years of follow-up**
Study 6: October 17, 1994 – July 28, 1995 **Cross-sectional** ***Prevalence of symptomatic and asymptomatic urethral infection*** 504 men: 429 HIV-1 seronegative, 75 (15%) HIV-1 seropositive. 4 (1%) men refused urethral swabs
Study 7: September 8 1993 – May 20, 1994 **Cross-sectional** ***Vaccine acceptability survey*** 201 HIV-1 seronegative men surveyed

Section 4: Methods

Methods

Enrolment and Routine Follow-up (Studies 1-5)

All of the health educators, nurses, and physicians received training in an HIV/STD counselling course by specialists from the University of Nairobi Department of Community Health, encompassing pre- and post-test HIV counselling, condom negotiation, demonstration, and promotion, and HIV/STD risk reduction. The course was spread over a period of three months, with course work interspersed with in-service training provided by a professional counsellor employed by the project. Risk reduction health education materials, produced by the Kenyan Ministry of Health and local non-governmental organisations, were distributed. These materials contained information on HIV-1 and STD transmission, actively promoted 100% condom use and sought to educate the men on the dangers of unprotected sex (without a condom) with a partner other than a spouse (extramarital sex). A supply of free condoms was offered to each man, after demonstrations of condom use, using penile models. A large box of condoms was placed in the waiting area at each company visit. Men were encouraged to come and take as many as they wanted, whether they wished to attend the clinic that day or not. Condom use with the spouse was not actively advocated for HIV-1 seronegative men, as it was considered practically unattainable and a distraction from the concept that they were in control of their own risk. Also, it was considered that the wife was the lowest risk sex partner, albeit not zero risk, and results are presented which are supportive of this assumption. The materials also advocated a reduction in the number of sexual partners, and sought to heighten the perception of personal risk.

Verbal informed consent was obtained after individual pre-test counselling. Pre-test counselling contained all of the elements outlined in the consent form approved by the University of Washington Human Subjects Review Committee in August 1992 (Appendix 3). This document was not read verbatim, but rather explained section by section, in Kiswahili. Dr Rakwar did this, or the project counsellor, if the author was doing the clinic. If the project counsellor was doing the pre-test or post-test counselling, the author sat next to him, in order to answer any technical questions during the process of counselling and obtaining consent. After verbal consent was obtained, a 10 ml blood sample was then drawn for HIV and syphilis serology. Each individual was given a return appointment for the next week, at which time the physician provided results and post-test counselling. Post-test counselling after a negative HIV screening result had been conveyed consisted of the following elements:

- Advice on risk reduction, including the decreasing the number of sexual partners, condom promotion, and avoidance of other STDs.
- A discussion on the individual HIV/STD risk profile of the man and how he thought he might reduce that risk.

Post-test counselling after a confirmed positive HIV test included:

- The meaning of the positive test, and the state of current knowledge about what that meant for the way of life of the individual and his family.
- Information on the risk to others which his positive HIV serostatus carried, and detailed information on the activities which both could transmit the virus and those which would be unlikely to.

- 100% Condom use was emphasised, especially with spouse.
- Men were informed that, while no cure for HIV disease currently existed, it was important to live positively, avoiding STDs by reducing the number of sexual partners and 100% condom use. In this way, if a cure became available, they may still be strong enough to benefit from it.

An enrolment questionnaire regarding demographic and occupational variables, medical history, and sexual behaviour was completed and a physical examination, including genital examination, was performed. The enrolment and examination questionnaires are included as appendices 4 and 5, respectively. The coding sheets are included in the appendices.

Subjects were informed that they would have open access to free primary health care, including sexually transmitted disease diagnosis and management, at each mobile team visit, regardless of serostatus. All men were appropriately treated and counselled according to Kenya national STD/HIV management guidelines (Figure 3). The guidelines are in the form of a flowchart and employ clinical algorithms, so that men could be treated immediately, without waiting for laboratory results. This was important in this highly mobile population, for individual compliance and for secondary prevention of STD transmission. The algorithmic approach is now favoured for STD control in developing countries and has been shown to assist in decreasing STD prevalence [115]. These procedures were carried out in all 1367 study subjects who have data included in study 1.

Men returned for follow-up at three monthly intervals, or as close as their work schedule allowed. At each follow-up visit, a questionnaire on interim sexual

behaviour and medical history was completed, physical examination performed, and a 10 ml blood sample drawn for HIV serology. Further risk-reduction counselling and condom promotion was carried out at these follow-up visits, and one week later when they returned for results. The follow-up questionnaires (appendix 6), physical examination and counselling procedures were carried out on all subjects who contributed to the incidence data included in studies 3, 5 and 6.

Interim Follow-up and STD evaluation (Studies 3 - 5)

Men with symptoms or signs of STDs, at either scheduled or interim visits, underwent additional evaluation. A follow-up questionnaire was completed (appendix 6) and physical examination performed, as for routine follow-up. Urethral discharges were investigated by gram stain and culture for *Neisseria gonorrhoeae*. Urethritis was defined as positive *N gonorrhoeae* culture or urethral inflammation on gram stain (see below) and confirmed urethral discharge. Genital ulcers were evaluated for the presence of *Haemophilus ducreyi* by culture. Syphilis serologic testing was repeated on all subjects after 12 months of follow-up.

This full STD evaluation was carried out in all HIV-1 seronegative male study subjects, either at screening, for the prevalence studies, and follow-up for the incidence studies. The formal enrolment of seropositive men was not anticipated, and only started two months after the project had started. The HIV-1 seropositive men received full, free and equal treatment for STDs (Figure 3) and were encouraged to attend the clinic with symptomatic STDs and other illnesses. They were not, however, traced for follow-up in the same way as seronegative men or had STD specimens fully evaluated. For this reason, only the STD data for the seronegative men are included in Studies 2, 3, and 5.

Urethral infection Study Enrolment (Study 6)

All men, HIV-1 seropositive and HIV-1 seronegative, were invited to participate in the cross-sectional urethral infection study (Study 6), when they attended the clinic for follow-up or illness, between October 1994 and August 1995. A medical and sexual history (Appendix 7) was taken and a physical examination was performed (Appendix 5) on all men. As can be seen from the questionnaire, the study was initially planned as a prospective study, but it became obvious that, while asymptomatic men would submit to one urethral swab, they would refuse more. Plans to repeat evaluation after treatment of any infection found were also abandoned for that reason. Only 1% of the men who attended the clinics declined participation in the study, by refusing to have a urethral swab taken. A total of 504 men were recruited consecutively in this manner. All men were appropriately treated and counselled on the basis of laboratory findings, using the antibiotics outlined in the Kenya national STD/HIV management guidelines (figure 3), with the addition of metronidazole 2gm as a single oral dose for T *vaginalis* infection.

MANAGEMENT OF SEXUALLY TRANSMITTED INFECTIONS (STI)

THE CHART PRESENTS THE MOST COMMON STI SYNDROMES AND TREATMENTS — FOR MORE INFORMATION CONSULT THE NATIONAL GUIDELINES FOR THE CONTROL OF STI

Urethral Discharge

Urethritis, usually caused by gonorrhoea and chlamydia

EXAMINE FOR DISCHARGE

- DISCHARGE PRESENT → URETHRITIS ℞ and 4C's → IF DISCHARGE PERSISTS AFTER 7 DAYS → Alternative URETHRITIS ℞ and 4C's → IF DISCHARGE PERSISTS AFTER 7 DAYS → REFER FOR INVESTIGATIONS
- DISCHARGE ABSENT → SYMPTOMATIC ℞

URETHRITIS ℞

Norfloxacin 800mg stat
AND
Doxycycline 100mg BD x 7 days

Alternative ℞

Ampicillin 3.5g AND Probenicid 1g
AND Augmentin® 1 tab
AND
Doxycycline 100mg BD x 7 days

Vaginal Discharge or Pruritus

Vaginitis, usually caused by candida and trichomonas
Cervicitis, usually caused by gonorrhoea and chlamydia

ENQUIRE ABOUT LOWER ABDOMINAL PAIN AND EXAMINE FOR LOWER ABDOMINAL TENDERNESS

- NO LOWER ABDOMINAL PAIN OR TENDERNESS → VAGINITIS ℞ and 4C's → IF NO IMPROVEMENT AFTER 7 DAYS → CERVICITIS ℞ and 4C's → IF DISCHARGE PERSISTS AFTER 7 DAYS → REFER FOR INVESTIGATIONS
- LOWER ABDOMINAL PAIN OR TENDERNESS → FOLLOW THE FLOWCHART FOR LOWER ABDOMINAL PAIN

VAGINITIS ℞

Nystatin 1 pessary
BD x 5 days
AND
Metronidazole 2g stat

If pregnant

Clotrimazole
1 tab intra-vaginally
daily x 6 days

CERVICITIS ℞

Norfloxacin 800mg stat
AND
Doxycycline 100mg
BD x 7 days

If pregnant

Ampicillin 3.5g AND
Probenicid 1g
AND Augmentin® 1 tab
AND
Erythromycin 500mg
QID x 7 days

Lower Abdominal Pain in Women

PID, caused by gonorrhoea, chlamydia and anaerobes
Surgical and obstetrical conditions

DO ABDOMINAL & BIMANUAL EXAMINATIONS

- ABDOMINAL TENDERNESS DUE TO SURGICAL OR GYNAECOLOGICAL CAUSES* → REFER FOR SURGICAL OR GYNAECOLOGICAL ASSESSMENT
- ABDOMINAL TENDERNESS OR TENDERNESS ON MOVING THE CERVIX → PID ℞ and 4C's → IF NO IMPROVEMENT AFTER 7 DAYS → REFER FOR INVESTIGATIONS
- NO TENDERNESS ON ABDOMINAL EXAMINATION → SYMPTOMATIC ℞ OR VAGINITIS ℞ IF THERE IS VAGINAL DISCHARGE → START FLOWCHART AGAIN AFTER REPEATING ABDOMINAL EXAMINATION

*Surgical or gynaecological causes are determined by rebound tenderness and/or guarding; last menstrual period overdue; recent abortion or delivery; menorrhagia or metrorrhagia.

PELVIC INFLAMMATORY DISEASE (PID) ℞

Norfloxacin 800mg stat
AND
Doxycycline 100mg BD x 7 days
AND
Metronidazole 400mg BD x 10 days

If pregnant

Refer for obstetric evaluation if PID is suspected

Genital Ulcer Disease (GUD)

GUD, usually caused by chancroid, syphilis and herpes genitalis

EXAMINE FOR ULCER

- MULTIPLE VESICLES GROUPED TOGETHER WITH A HISTORY OF RECURRENCE (HERPES GENITALIS) → SYMPTOMATIC ℞ and 4C's → IF NO IMPROVEMENT AFTER 7 DAYS* → FOLLOW OTHER GUD COLUMN
- OTHER GUD → GUD ℞ and 4C's → IF NO IMPROVEMENT AFTER 7 DAYS* → ALTERNATIVE GUD ℞ and 4C's → IF NO IMPROVEMENT AFTER 7 DAYS → REFER FOR INVESTIGATIONS

*GUD heals slowly. Improvement is defined as signs of healing and reduction of pain. People with HIV infection will be slower in responding to GUD treatment.

GENITAL ULCER DISEASE (GUD) ℞

Erythromycin 500mg TID x 7days
AND
Benzathine Penicillin 2.4 MU IM stat

If Penicillin allergy, use
Erythromycin 500mg qid x 14 days

Alternative ℞

Ciprofloxacin 500mg stat

FIGHT AIDS! REMEMBER THE *4C's* OF GOOD STI MANAGEMENT

COUNSELLING

- Empathise with your patient (put yourself in your patient's place)
- Dialogue with your patient
- Discuss the other 3C's

COMPLIANCE

Your patient should

- avoid self-medication
- take the full course of medication and not share or keep it
- follow your other instructions

CONDOMS

- proper condom use is the only alternative to abstinence
- give condoms to your patient
- explain and demonstrate the proper use of condoms

CONTACT TREATMENT

Your patient should

- tell all his/her sexual partners to seek medical attention

REPUBLIC OF KENYA, NATIONAL AIDS/STD CONTROL PROGRAMME (NASCOP), MINISTRY OF HEALTH, P.O. BOX 20781, NAIROBI - 1996

PRODUCED IN CONJUNCTION WITH THE BELGIAN ADMINISTRATION FOR DEVELOPMENT CO-OPERATION BADC

Figure 3: Kenyan Ministry of Health STD Guidelines

Vaccine Acceptability Survey Enrolment (Study 7)

The vaccine acceptability questionnaire (Appendix 8) was administered to a convenience sample of 201 seronegative men during a follow-up visit for the cohort study between September 1993 and May 1994.

A convenience sample selection process was chosen for logistic reasons. In effect, 5 or 6 men attending the clinic on any given day were asked to participate, as they left the clinic after a follow-up visit. The vaccine acceptability questionnaire took longer to complete than the follow-up visits. The vaccine acceptability interviewer waited until a subject left the examination area and requested their participation. This was done to prevent subjects waiting, which would have caused a problem with the company management. Selection of a random sample of all subjects enrolled was not attempted, because of the uncertain date of attendance at follow-up visits and the time constraint. No individual refused participation. The questions were developed in English, with a Flesch-Kincaid grade level of 7.0 and a Flesch Reading Ease score of 68 [116]. These "readability" scores were obtained using Microsoft Word version 6, and are a measure of the educational level required to understand the language in a document. In this case, a grade level of 7.0 indicates that a person with seven years of formal education should understand the level of language in the document. It must be stated, however, that we have no such measure of the document in the language in which it was administered, Kiswahili. It was translated into Kiswahili by the Kenyan physicians and health educators, piloted in 30 subjects and finalised after holding focus group discussions with subjects at the clinics. The questionnaire was administered as a standardised interview, comprising four sections:

- General knowledge of prophylactic vaccines currently in use.
- The magnitude of the HIV-1 epidemic in Kenya, and perception of individual risk.
- Anticipated willingness to participate in an HIV-1 vaccine trial.
- Anticipated behaviour change resulting from such participation.

Standardised information regarding the nature and limitations of prophylactic vaccines was given after questions in the vaccine knowledge section, in order to resolve basic misconceptions.

Laboratory Methods: Studies 1 to 5

All specimens were transported to the laboratory at Coast Provincial General Hospital for processing. These laboratory methods were uniform in studies 1 to 5.

Sera were tested by synthetic peptide enzyme-linked immunosorbent assay (ELISA) for HIV-1 and HIV-2 antibodies (Behring Werke, Marburg, Germany). Positive samples were confirmed for HIV-1 using a recombinant ELISA system (Recombigen, Cambridge Biotech Corporation, Worcester, Massachusetts). Western blot assays (Cambridge Biotech Corporation, Worcester, Massachusetts) were performed if the two ELISAs produced discordant results, and to confirm all HIV-1 seroconversions.

Serological testing for syphilis with rapid plasma reagin card test (RPR, Becton-Dickinson, Cockeysville, Maryland) and *Treponema pallidum* haemagglutination assay (TPHA, Becton-Dickinson, Cockeysville, Maryland) was carried out on all subjects at enrolment. Active syphilis infection was defined by a positive RPR and TPHA. A negative RPR and positive TPHA provided evidence of past syphilis infection. RPR testing was performed at the 12 month follow-up visit, with TPHA confirmation if positive.

Gonorrhoea was diagnosed by positive culture of *N gonorrhoeae* from urethral swab on modified Thayer-Martin media.

Non-gonococcal urethritis was defined by the presence of 5 or more polymorphonuclear cells per high power field (urethral inflammation) on a gram stained slide of urethral secretions, in the absence of *N. gonorrhoeae* infection.

Genital ulcer disease was a clinical diagnosis, with no attempt made to differentiate ulcers caused by different aetiological agents on clinical grounds. *H. ducreyi* was cultured on activated charcoal media.

Laboratory Methods: Study 6

A "first catch" specimen of 20 ml. of urine, collected at least two hours after men had last passed urine, was obtained and tested in the clinics by leucocyte esterase dipstick (LED, Boehringer-Mannheim). The dipsticks were read at 120 seconds as "negative", "trace", "1+" or "2+", according to the manufacturer's colour chart, and a positive LED test was defined as a reading greater than "trace". Urethral swabs were taken from all men, plated on modified Thayer-Martin media for *N. gonorrhoeae* culture, and placed in modified Diamond's media for *T. vaginalis* culture. The urine sample was transported in a cool box to the laboratory of the Coast Provincial General Hospital in Mombasa and centrifuged for 10 minutes at 3,000 rpm. The urine pellet was then re-suspended in one millilitre of supernatant, assayed for *C. trachomatis* by enzyme immunoassay (EIA, Syva MicroTrak Chlamydia EIA) and cultured for *T. vaginalis* by inoculation into modified Diamond's medium.

Statistics

For cross-sectional comparisons, the Wilcoxon rank sum test was used to test differences for continuous variables and Pearson's chi-square test and Fisher's exact test for categorical variables.

Stata, version 3.0, was used for multivariate logistic regression analysis of determinants of urethral infection in Study 7. Multivariate Cox Proportional Hazards regression analysis to estimate hazard ratios for HIV-1 infection in Study 3, assuming uniform levels of exposure for risk variables for each individual throughout the period under study. Exceptions to this were when STDs were examined as time-dependent co-variates. An entry and removal (at p-value for adjusted hazard ratio greater than 0.1) method was used in the construction of the models.

SPSS for Windows, version 6.0, was used to perform descriptive and comparative analyses, and S-Plus used for time-trend modelling techniques (Studies 4 and 5). Generalised estimating equations with a binomial link and exchangeable correlation structure were employed for examination of changes in the sexual behaviour variables over time, and generalised estimating equations with a poisson link and exchangeable correlation structure were used to model changes in STD incidence [117]. Use of the exchangeable correlation structure was based on the assumption that there should be no difference over time in the correlation between observations made on the same man.

Section 5: Results

Some discussion on the use of some of the study variables is included in this section, to aid understanding as the reader progresses through this section.

Results

Study 1: HIV-1 prevalence

A total of 1367 men were screened for antibodies to HIV-1 between March 1, 1993 and January 31 1996, of whom 231 (17%) were seropositive. Nine hundred and fifty five HIV-1 seronegative men were enrolled in the prospective cohort study. Of the 181 (16%) seronegative men who were screened but not enrolled, 174 did not return for results. The remaining 7 men declined participation.

Seventeen percent (231/1367) of those screened were HIV-1 seropositive, but only 51% (118/231) of HIV-1 seropositive men were enrolled. As no data were collected on those not enrolled, it is not possible to confirm that this is a representative sample of the HIV-1 positive men.

HIV-1 seropositive men were older (33 vs 29), and consequently more were married (85% vs 63%). Although religion was not significantly associated with HIV-1 serostatus if examined as a 2 X 4 table, if moslem religion is examined against all others in a 2 X 2 table, the odds ratio for being HIV-1 seropositive and moslem is 0.52 (95% CI 0.28-0.93, p=0.02). The most mobile occupations (driver and assistant) were significantly more likely to be HIV-1 seropositive, but no such association was seen when the data was stratified by days of occupational travel. A lifetime history of sex worker contact or ever having used a condom were strongly associated with positive HIV-1 serostatus, as was increasing numbers of sex partners in the previous year.

The high percentage of uncircumcised men who were HIV-1 positive (26% vs 13%) was highly significant (OR 2.43 [95%CI 1.51-3.91, p<0.001]).

Table 5: Demographic, occupational, behavioural and medical characteristics at enrolment of all men, by baseline HIV-1 serostatus

	HIV-1 Negative n=955	HIV-1 Positive n=118	p value
Demographic			
Age, median (range)	29 (16-62)	33 (21-57)	<0.001
Marital status			
Married	599 (63%)	100 (85%)	<0.001
Never married	345 (36%)	17 (14%)	
Widowed / divorced	11 (1%)	1 (1%)	
Years of education, mean (range)	9.1 (0-16)	9.0 (0-16)	0.1
Religion			
Protestant	379 (40%)	51 (43%)	0.2
Catholic	303 (32%)	42 (36%)	
Moslem	210 (22%)	15 (13%)	
Other	63 (6%)	10 (9%)	
Occupation			
Driver	130 (14%)	32 (27%)	0.001
Assistant driver	210 (22%)	29 (25%)	
Mechanic	237 (25%)	28 (24%)	
Ancillary worker	376 (39%)	29 (25%)	
Travel			
No travel	531 (56%)	59 (50%)	0.3
<15 days/month	203 (21%)	24 (20%)	
≥15 days/month	221 (24%)	35 (30%)	
Sexual behaviour			
Lifetime			
Age at first sex, median	15	16	0.5
History of sex with FSW	532 (56%)	95 (81%)	<0.001
History of condom use	503 (53%)	79 (67%)	0.007
Past 1 year			
Sex Partners			
0	34 (4%)	0	<0.001
1	368 (39%)	21 (18%)	
2-5	455 (48%)	75 (64%)	
>5	98 (10%)	22 (19%)	
Sex with FSW	297 (31%)	64 (54%)	<0.001
Unprotected FSW sex	188 (20%)	48 (41%)	<0.001
Sex with girlfriend or casual partner	522 (55%)	58 (49%)	0.3
Any unprotected extramarital sex	537 (56%)	76 (64%)	0.1
Used alcohol In past year	429 (45%)	61 (51%)	0.1
Uncircumcised	122 (13%)	31 (26%)	<0.001

Study 2: STD prevalence in Seronegative Men

The prevalence of active syphilis was 4.5% (43/955), and a further 2.6% of men (25/955) had evidence of past infection. The prevalence of symptomatic STDs at first presentation and association with occupational travel (>14 days a month), circumcision status, and an indicator of sexual risk behaviour, sex worker contact, are given below. Table 6 shows the number, percentage of the total, and age of men who presented with symptomatic STDs or serological evidence of active syphilis. Men with urethritis included men with a positive gonorrhoea culture or who had five or more polymorphonuclear cells per high power field on gram stain. Men with either diagnosis were significantly younger, while those diagnosed with syphilis were older. Overall, men with any STD were older, largely due to the number of men with syphilis.

Table 6: Mean age of HIV-1 seronegative men with and without STDs at first presentation

STD	Number (% of all men)	Mean Age in years		
		Positive	Negative	p value
Urethritis	29 (3.0%)	27.5	30.4	0.05
Gonorrhoea	20 (2.1%)	26.8	30.4	0.04
Genital ulcer	9 (0.9%)	32.0	30.3	NS
H. ducreyi	4 (0.4%)	27.5	30.3	NS
Active Syphilis	43 (4.5%)	36.0	30.0	<0.001
Any STD	80 (8.4%)	32.0	30.1	.03

Circumcision status was not significantly associated with any category of STD, as shown in Table 7.

Table 7: STDs in HIV-1 seronegative men at first presentation, by circumcision status

STD	Number (% of all men)	Circumcision Status		
		Circumcised (n=833)	Uncircumcised (n=122)	p value
Urethritis	29 (3.0%)	25 (3.0%)	4 (3.3%)	NS
Gonorrhoea	20 (2.1%)	16 (1.9%)	4 (3.4%)	NS
Genital ulcer	9 (0.9%)	7 (0.8%)	2 (1.6%)	NS
H. ducreyi	4 (0.4%)	3 (0.4%)	1 (0.8%)	NS
Active Syphilis	43 (4.5%)	36 (4.3%)	7 (5.7%)	NS
Any STD	80 (8.4%)	67 (8.4%)	13 (10.7%)	NS

Occupational travel more than 14 days each month was used as a cut off between long-distance workers and short distance and sedentery workers. Two hundred and twenty one (23.1%) fell into the long-distance category. Table 8 details the associations between STDs and travel status. The urethral infections were both highly significant, as was syphilis, but genital ulcer disease was not, perhaps due to the small numbers.

Table 8: STDs in HIV-1 seronegative men at first presentation, by occupational travel

STD	Number (% of all men)	Occupational Travel		
		Long distance (n=221)	No Long distance (n=728)	p value
Urethritis	29 (3.0%)	14 (6.3%)	15 (2.1%)	<0.001
Gonorrhoea	20 (2.1%)	7 (3.2%)	13 (1.8%)	<0.001
Genital ulcer	9 (0.9%)	5 (2.3%)	4 (0.5%)	NS
H. ducreyi	4 (0.4%)	2 (0.9%)	2 (0.2%)	NS
Active Syphilis	43 (4.5%)	24 (10.9%)	19 (2.6%)	0.002
Any STD	80 (8.4%)	43 (19.4%)	37 (5.1%)	<0.001

At least one visit to a female sex worker was reported by 297 (31.1%) of seronegative men in the year prior to enrollment. This variable was significantly associated with every STD. All of the men with proven chancroid (*H ducreyi*) infection reported FSW contact. Although unprotected sex worker contact was the behaviour variable which was most sensitive in the HIV-1 prevalence analysis, any reported FSW contact proved to be more significant for the STDs noted. This is represented in Table 9.

Table 9: STDs in HIV-1 seronegative men at first presentation, by reported sex worker contact

STD	Number (% of all men)	Sex worker Contact in Previous Year		
		No (n=658)	Yes (n=297)	p value
Urethritis	29 (3.0%)	14 (2.1%)	15 (5.1%)	0.02
Gonorrhoea	20 (2.1%)	9 (1.4%)	11 (3.7%)	0.02
Genital ulcer	9 (0.9%)	2 (0.3%)	7 (2.4%)	0.005
H. ducreyi	4 (0.4%)	0 (0%)	4 (1.3%)	0.01
Active Syphilis	43 (4.5%)	23 (3.5%)	20 (6.7%)	0.03
Any STD	80 (8.4%)	39 (5.9%)	41 (13.8%)	<0.001

Study 3: HIV-1 incidence

There were a total of 40 seroconversions to HIV-1, in the course of 990 person years of follow-up (py) to January 1996, giving an overall HIV-1 seroincidence of 4.0% per annum. Seventy five percent (716) of the 955 HIV-1 seronegative men enrolled contributed to follow-up. The full follow-up statistics are given in table 10. Men enrolled in the first 4 months contributed over half (496 person years) of total follow-up time, with a median follow-up time of 549 days. The subsequent enrolment quarter (3 months) yielded 174 person years or, 17.6% of total follow-up, decreasing thereafter.

The number of seroconversions and the person years of follow-up in each risk group are given in Table 11. The "moslem" variable, was included as potentially protective. Five (12.5%) seroconversions occurred in 299 person years in men who reported no partner other than a spouse, giving an incidence of 1.7 per 100 person years of follow-up. This was the group with the lowest incidence. Thirty five (88%) of seroconversions occurred in men who reported sex with a partner other than a spouse during follow-up. This group accounted for 70% of total follow-up time. Fourteen (35%) of seroconversions occurred in 21% of total follow-up time in men who reported unprotected sex with a sex worker.

The HIV-1 incidence rates, univariate and multivariate analysis of risk for the different variables are given in Table 12. The only variables which remained significant in the multivariate models were being a driver or driver's assistant, reporting any extramarital sex during follow-up. For uncircumcised status, there was an adjusted hazard ratio of 2.0 ($p = 0.1$).

Table 10: Cohort follow-up statistics by enrolment Quarter.

		Follow-up Visits				Follow-up Time in Days	
Enrolment Quarter	Number of men	mean	median	Percentage with no follow-up	maximum	mean	median
Mar 1993- June 1993	334	4.5	4	14.8%	12	542.1	549.0
July 1993- Sept 1993	178	2.8	2	34.4%	10	357.6	269.0
Oct 1993- Dec 1993	87	2.2	1	38.4%	9	308.7	247.0
Jan 1994- Mar 1994	92	2.3	2	31.2%	9	303.3	235.0
Apr 1994- June 1994	94	1.8	1	32.9%	7	248.7	182.0
July 1994- Sept 1994	71	1.3	1	44.9%	6	173.0	105.0
Oct 1994- Dec 1994	45	1.5	1	37.8%	4	189.6	126.0
Jan 1995- Mar 1995	19	1.1	1	36.8%	5	162.3	147.0
Apr 1995- June 1995	17	0.8	1	35.3%	2	98.3	98.0
July 1995- Sept 1995	7	0.7	1	42.9%	2	71.0	84.0
Oct 1995- Jan 1996.	11	0.4	0	54.5%	1	20.3	0

The sexual behaviour variable with the highest HIV-1 incidence was reported unprotected sex with a sex worker (6.7 per 100 person years), but the most significant variable in the multivariate model was any reported extramarital sex (5.1 per 100 person years). However, men who reported any extramarital sex accounted for almost 70% of the total follow-up time, compared to just over 20% for the men who reported unprotected sex worker contact. The same applies to the occupational travel variables. The driver or assistant (8.1 per 100 person years and 30% of total follow-up) and travel over 14 days a month (8.9 per 100 person years and 21% of follow-up) probably measure the same risk. Driver or assistant includes more men who go on shorter trips, can potentially return for better follow-up, and have an increased risk due to the exposure to risk behaviour which travelling away from home entails.

Table 11: HIV-1 seroconversions in risk groups, and follow-up time for each category.

Variable	Exposed		Unexposed	
	Seroconversions	Person-years	Seroconversions	Person-years
Age <25 at enrolment	15	239.7	25	750.3
Driver or assistant	24	296.1	16	693.9
Occupational travel >14 days per month	19	213.2	21	776.8
Moslem	4	213.8	36	776.2
Uncircumcised	8	132.5	32	857.5
Any reported extramarital sex during follow-up	35	691.0	5	299
Any reported unprotected extramarital sex during follow-up	30	598.5	10	391.5
Any reported sex worker contact during follow-up	15	280.0	25	710.0
Any unprotected sex worker contact during follow-up	14	208.7	26	781.3
Genital ulcer during follow-up	8	72.5	32	917.5
Urethral discharge during follow-up	13	184.4	27	805.6

Table 11: HIV-1 seroconversions in risk groups, and follow-up time for each category

Table 12. Analysis of risk factors for HIV-1 seroconversion

Variable	Incidence (per 100 person years of follow-up)	Univariate			Multivariate		
		Hazard Ratio	95% Confidence Interval	p-value	Hazard Ratio	95% Confidence Interval	Adjusted p-value
Age <25 at enrollment	6.3	2.0	1.0-3.8	0.04	1.6	1.0-3.2	0.2
Driver or assistant	8.1	3.7	2.0-7.0	<0.001	4.0	2.1-7.9	<0.001
Occupational travel >14 days per month	8.9	3.3	1.8-6.2	<0.001	1.6	0.7-3.5	0.3
Moslem	1.9	0.4	0.1-1.1	0.08	1.9	0.6-5.4	0.3
Uncircumcised	6.0	1.5	0.7-3.3	0.3	2.0	0.9-4.6	0.1
Any extramarital sex during follow-up	5.1	4.5	1.4-14.6	0.01	4.2	1.3-13.6	0.02
Any unprotected extramarital sex during follow-up	5.0	2.2	1.0-4.9	0.05	0.9	0.3-2.2	0.7
Any sex with sex worker during follow-up	5.4	1.5	0.8-2.8	0.2	0.4	0.1-3.1	0.4
Any unprotected sex with sex worker during follow-up	6.7	2.0	1.0-3.8	0.05	3.0	0.4-23.2	0.3
Genital ulcer during follow-up	11.0	2.9	1.3-4.7	0.005	2.0	0.8-4.7	0.1
Urethral discharge during follow-up	7.0	2.0	1.1-4.0	0.03	1.2	0.6-2.6	0.6

The STD incidence in seronegative men to January 1996, which were included in the models, are detailed in Table 13, below.

Table 13: STD incidence in seronegative men.

STD	Number of Cases	Incidence
any genital ulcer	43	4.3 per 100 py
H ducreyi	12	1.2 per 100 py
Treponema pallidum	4	0.4 per 100 py
gonorrhoea	131	13.2 per 100 py
non-gonococcal urethritis	50	5.0 per 100 py

The acquisition of genital ulcer disease is represented by "any genital ulcer". Ulcers which were *H ducreyi* culture positive are included in this classification, but also presented separately.

It is recognised that the risk for men with genital ulcer and urethral discharge was not uniformly raised throughout the study period, and that some men suffered from STDs while they were travelling, which we did not see at the clinic. However, the men most at risk of STDs, and not being seen at the clinic, are the long-distance drivers and their assistants. This gave difficulty when urethral discharge and genital ulcers were examined as time-dependant covariates, because even approximate dates were difficult to obtain and there were often periods of six months or more between visits for such men. For this reason, STDs which were not observed were not included in the analysis.

When observed urethral discharges and genital ulcers were examined as time-dependent covariates with a six month inclusion period, no increase in the hazard ratios, or the precision of the measure of effect was noted. Univariate indices for genital ulcer moved to 2.0 [95% CI: 1.0-4.2, p=0.05] and multivariate hazard ratio 1.3 [95% CI: 0.5-5.1, p=0.5]. The same pattern was seen for urethral discharge, with a univariate hazard ratio of 1.7 [95% CI 0.9-3.2, p=0.1] and multivariate hazard ratio of 1.1 [95% CI: 0.5-2.9, p=0.5].

Study 4: Behavioural risk reduction time trends

A total of 1195 men were screened for antibodies to HIV-1 between March 1, 1993 and October 14, 1994, of whom 206 (17%) were seropositive. Eight hundred and fifty six HIV-1 seronegative men were enrolled in the prospective cohort study. Of the 133 seronegative men who were screened but not enrolled, 126 did not return for results. The remaining 7 men declined participation. No demographic or risk profile characteristics are available from these 133 men.

Of those enrolled, 556 men returned for at least one follow-up visit. This represented 72% of 771 who were scheduled for follow-up on or before October 14, 1994. Four hundred and ninety four person years (py) of follow-up were accrued, and 1620 follow-up visits recorded. Mean follow-up was 326 days (range 21 to 588). The mean number of follow-up visits was 2.9 (range 1-8), and the mean interval between follow-up visits was 101 days (range 3-431).

There were no significant differences between men who did or did not return for follow-up with regard to median age (29 vs 29), unmarried status (35% vs 37%), median age at first sex (15 vs 15), lifetime history of sex with an FSW (57% vs 57%), median number of sex partners in the previous year (2 vs 2), or history of condom use (52% vs 55%). There was a significant difference in occupational travel, with 37% of those men who travelled more than two weeks each month failing to return for follow-up, compared with 25% of men who travelled two weeks or less each month ($p=0.001$).

The enrolment demographic, occupational, behavioural, and medical characteristics of the 556 participating men are detailed in Table 14. The median age

was 29 years and 63% were married. The majority of men had completed primary education. Almost half of the cohort travelled in the course of their work, often for extended periods of time. Over 50% reported a history of sex with an FSW and only 51% had ever used condoms. Fifty nine per cent reported more than one sex partner in the previous year, and 20% reported unprotected sex with an FSW.

Table 14: Demographic, occupational, behavioural and medical characteristics at enrolment of seronegative men included in the time-trends study

Characteristics	No. (%) Total = 556
Demographic	
Age, median (range)	29 (16-62)
Marital status	
Married	348 (63%)
Never married	199 (36%)
Widowed / divorced	9 (2%)
Years of education, mean (range)	9.1 (0-16)
Religion	
Protestant	223 (40%)
Catholic	190 (34%)
Moslem	121 (22%)
Other	22 (4%)
Occupation	
Driver	62 (11%)
Assistant driver	102 (18%)
Mechanic	172 (31%)
Ancillary worker	220 (40%)
Travel	
No travel	299 (54%)
<15 days/month	142 (26%)
≥15 days/month	115 (21%)
Sexual behaviour	
Lifetime	
Age at first sex, median	16
History of sex with FSW	314 (57%)
History of condom use	286 (51%)
Past 1 year	
Sex Partners	
0	19 (3%)
1	213 (38%)
2-5	270 (49%)
>5	54 (10%)
Sex with FSW	168 (30%)
Unprotected FSW sex	109 (20%)
Sex with girlfriend or casual partner	311 (56%)
Any unprotected extramarital sex	309 (56%)
Used alcohol In past year	262 (47%)
Uncircumcised	72 (13%)

During follow-up, 53% (184) of married men reported extramarital sex, 42% (145) unprotected (without condom) extramarital sex , 20% (71) FSW sex, and 16% (54) unprotected FSW sex. One hundred and fifty two (44%) married men reported at least one three month period when they did not have sex with their wife, and they were significantly more likely to report sex with another partner during these periods (OR 2.5: 95% CI 1.8-3.4, $p<0.001$) or acquire an STD (OR 4.9: 95% CI 2.7-8.7, $p<0.001$). Eighty six percent (178) of unmarried men reported sex with any partner, 73% (152) unprotected sex, 27% (55) sex with an FSW, and 15% (32) unprotected sex with an FSW. No sex with a male partner was reported.

Of the 126 men who reported sex with an FSW, 46 (36%) never used condoms, 40 (32%) sometimes used condoms, and 40 (32%) consistently used condoms. One hundred and fifty nine (49%) of 323 men who reported sex with a girlfriend never used condoms, 116 (36%) sometimes used condoms, and 57 (18%) consistently used condoms. Significantly more men reported consistent condom use with FSWs than with girlfriends ($p=0.002$). Overall, condoms were used for 27% of sex acts with girlfriends and 50% of sex acts with FSWs.

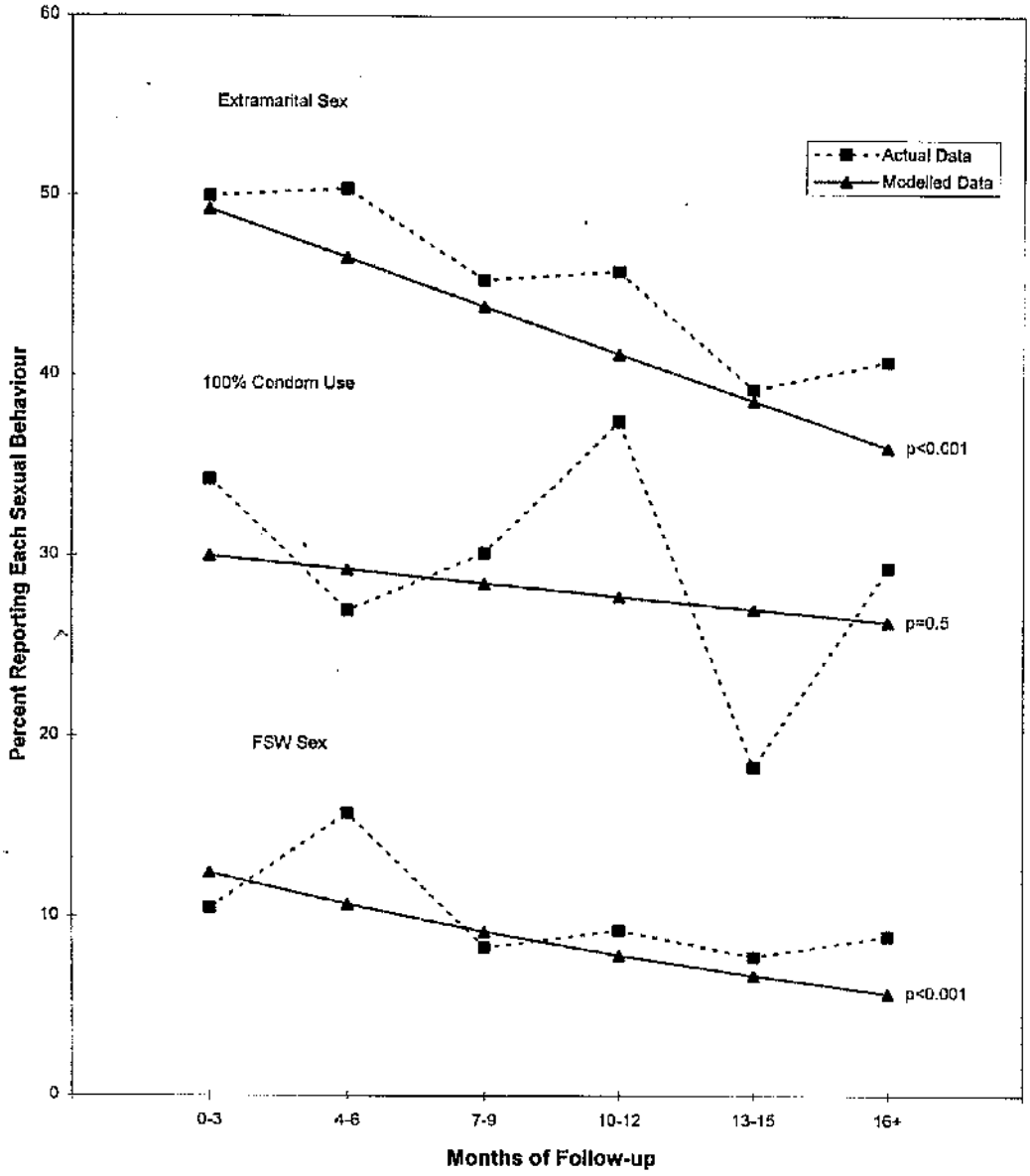
There were significant declines in the percentage of men reporting extramarital sex or sex with an FSW during the prior 3 month period, as shown in Table 15 and Figure 4. Statistical modelling indicates that the percentage reporting extramarital sex decreased from 49% during the first quarter to 36% during the last quarter of follow-up ($p<0.001$). The analogous decline for sex with an FSW was from 12% to 6% ($p=0.001$). To determine if loss to follow-up was causing biased results (e.g. differential loss to follow-up of men with higher levels of risk behaviour would produce an artifactual decline), we modelled separately the trends in sexual behaviour of men who attended <75% and >75% of their scheduled follow-up visits. In both subgroups, there were significant reductions in the percentage of men reporting extramarital sex ($p=0.02$ and $p=0.006$, respectively) and FSW sex ($p=0.02$ for both).

These declines in high risk sexual exposure were not accompanied by changes in condom use. As shown in Figure 4, the percentage of men who reported consistent (100%) condom use during extramarital sex remained fairly constant at approximately 30% ($p=0.5$). Men with <75% and >75% follow-up compliance had similar rates of consistent extramarital condom use, and no significant changes were observed over time ($p=0.2$ and $p=0.9$ respectively). Average frequency of condom use and the percentage of men who reported ever using condoms with any partner type also remained stable over time.

Table 15. Proportion of men in time-trends study with selected sexual behavioural variables, by quarter of follow-up

Table 15. Proportion (perceniage) of men in time-trends study with selected sexual behavioural variables by quarter of follow-up

Sexual Behaviour	0-3 months	4-6 months	7-9 months	10-12 months	13-15 months	16+ months	p-value of change
Any FSW Sex	22/210 (10%)	59/375 (16%)	23/278 8%)	21/227 (9%)	14/181 (8%)	12/145 (8%)	0.001
Any Extramarital Sex	105/210 (50%)	189/375 (50%)	126/278 (45%)	104/227 (46%)	71/181 (39%)	58/145 (40%)	<0.001
Consistent Condom Use During Extramarital Sex	36/105 (34%)	51/189 (27%)	38/126 (30%)	39/104 (38%)	13/71 (18%)	17/58 (29%)	0.5


Extramarital Sex
Actual Data
Modelled Data
100% Condom Use
p<0.001
p=0.5
FSW Sex
p<0.001
Percent Reporting Each Sexual Behaviour
Months of Follow-up
0
10
20
30
40
50
60
0-3
4-6
7-9
10-12
13-15
16+

Study 5: STD incidence time-trends

In the course of follow-up for the time-trends studies, there were 20 seroconversions to HIV-1 (4.0/100 py), 62 cases of gonorrhoea (12.6/100 py), 37 cases of non-gonococcal urethritis (7.5/100 py), and 35 cases of genital ulcer disease (7.1/100 py), including 7 which were H. ducreyi culture positive. One incident syphilis infection was seen (0.3/100 py). Histories of an additional 117 episodes of urethral discharge and an additional 38 cases of genital ulcer disease which occurred between clinic visits were recorded. Men who travelled more than two weeks each month had significantly more STDs than those with less occupational travel (risk ratio 1.9: 95% CI 1.5-2.6, $p<0.001$).

Change in incidence of STDs during follow-up quarter are shown in Figure 5 and Table 16. There was a decrease in the modelled incidence of observed STDs from 34 cases per 100 py during the first quarter to 10 cases per 100 py during the last quarter of follow-up ($p=0.001$). Similar declines were seen when each of the STDs of interest (gonorrhoea, non-gonococcal urethritis, and genital ulcer disease) were modelled separately or when self-reported STD symptoms (urethral discharge or genital ulcers) occurring between clinic visits were added to the model ($p<0.001$). The incidence of observed STDs underwent significant reductions in men with <75% follow-up compliance (from 20% to 4%, $p=0.05$) and >75% follow-up compliance (from 47% to 11%, $p=0.001$). No significant time trends were discernible with respect to HIV-1 seroincidence, but the statistical power of this study was insufficient to definitively address this issue.

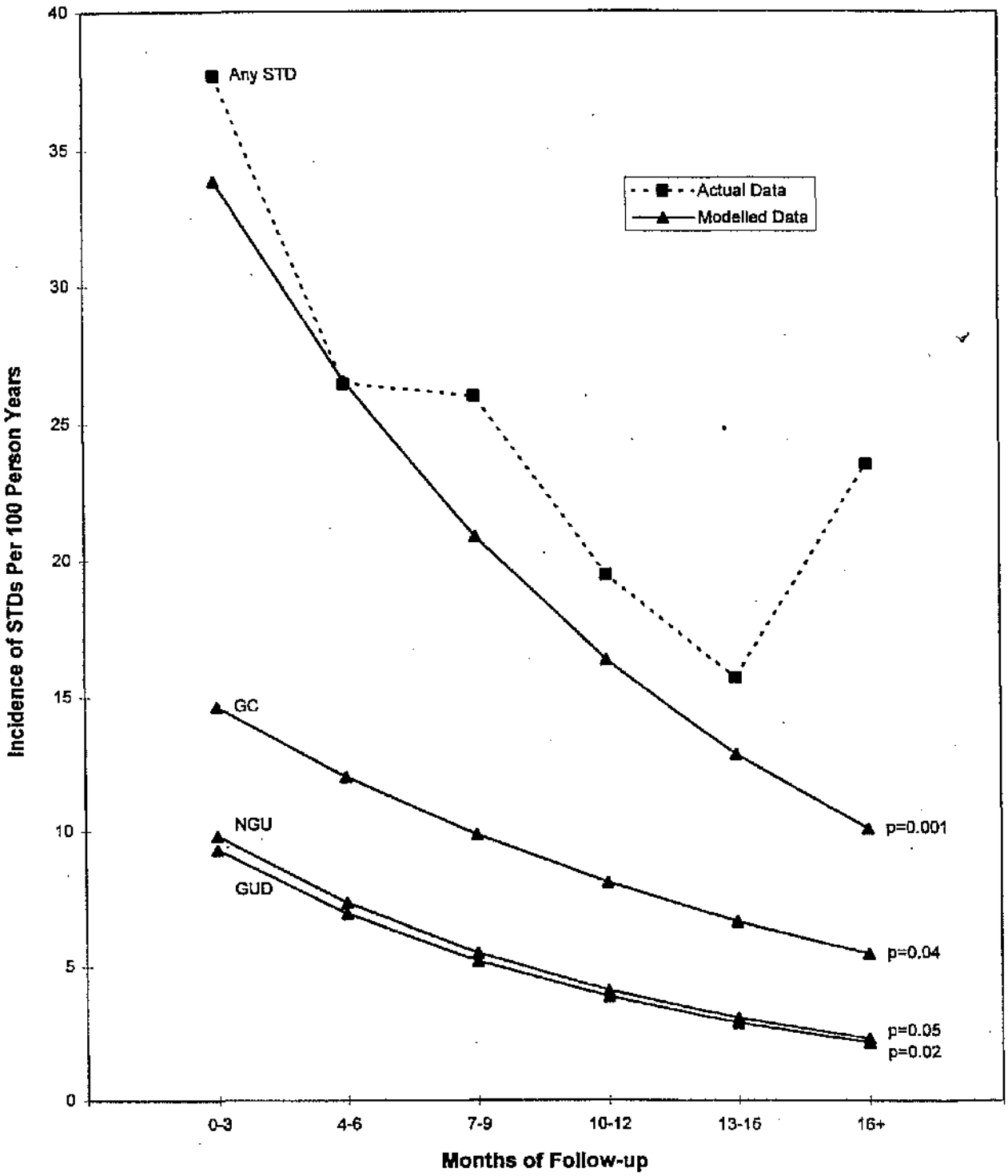
Table 16. Incidence of STDs in time-trends study, by quarter of follow-up*

STD	0-3 months py=135.3	4-6 months py=113.4	7-9 months py=92.3	10-12 months py=72.0	13-15 months py=51.0	16+ months py=29.8	p-value of change
GC	24 (17.7)	10 (8.8)	13 (14.1)	7 (9.7)	3 (5.9)	5 (16.8)	0.04
NGU	14 (10.3)	10 (8.8)	6 (6.5)	3 (4.2)	3 (5.9)	1 (3.4)	0.05
GUD	13 (9.6)	10 (8.8)	5 (5.4)	4 (5.6)	2 (3.9)	1 (3.4)	0.02
Any Observed STD	51 (37.7)	30 (26.5)	24 (26.0)	14 (19.4)	8 (15.7)	7 (23.5)	0.001
Any Reported STD	64 (47.3)	54 (47.6)	26 (28.2)	9 (12.5)	2 (3.9)	0 (0.0)	<.001
Any Observed or Reported STD	115 (85.0)	84 (74.1)	50 (54.2)	23 (31.9)	10 (19.6)	7 (23.5)	<.001

*py = person years of follow-up in a quarter. The number of episodes of STDs is presented, followed by the incidence rate per 100 person years in parentheses.

Table 16. Incidence of STDs in time-trends study, by quarter of follow-up

Figure 5: STD incidence during follow-up in time-trends study



Study 6: Urethral infection study

Demographic, occupational, behavioural and circumcision characteristics of the 504 men did not differ significantly from those in table 5. HIV-1 positive men were included in this analysis. The mean age was 30 years and they had a mean of 9 years of education. Almost three-quarters of the men were married, 73% were Christian and 19% were Muslim. Thirty-three per cent were drivers or drivers' assistants, and 22% travelled for more than two weeks each month in the course of their work. Over half of the men reported having ever paid for sex, having ever used condoms, and having had an STD at least once in the past. In the previous three months, 37% of married men reported sex with a partner other than their wife, and 20% reported more than one sex partner. Sixty per cent of married men had spent less than half of the previous month living with their wife. Fifteen per cent of men were uncircumcised, 8% had serological evidence of past or current syphilis infection (positive TPHA), and 15% tested positive for antibodies to HIV-1.

Table 17 indicates the pathogens which were identified, stratified by presenting symptoms. Over 11% of men had at least one urethral pathogen detected. Four per cent of men had a symptom of urethral discharge and 5.4% had a symptom of dysuria alone. The remainder (90.7%) were asymptomatic. Seventy per cent of men with urethral discharge, 22.2% of men with dysuria and 8.3% of asymptomatic men had at least one urethral pathogen identified. However, because most men were asymptomatic, the majority of infections (about two-thirds) occurred in asymptomatic men. *T. vaginalis* infection was found in 15% of symptomatic men (urethral discharge and/or dysuria), 5% of asymptomatic men, and 6% of men overall. Urethral infection rates did not differ significantly between drivers and non-driving ancillary workers. Mean age was significantly lower in men with gonorrhoea compared to those without

gonorrhoea (25.0 vs 30.0 years, P = 0.006), whereas there was a strong trend towards older age in men infected with *T. vaginalis* (32.2 vs 29.9 years, P = 0.06).

Table 17. Prevalence of urethral infections, stratified by symptom (N=504).

Pathogen	Symptom of urethral discharge N=20 (4.0%)	Symptom of dysuria alone (no discharge) N=27 (5.4%)	Asymptomatic N=457 (90.7%)	Prevalence of urethral infection N=504 (100%)
Neisseria gonorrhoeae (Ng), by urethral swab culture	8 (40%)	2 (7.4%)	5 (1.1%)	15 (3.0%)
Chlamydia trachomatis (Ct), by EIA on urine	2 (10%)	1 (3.7%)	10 (2.2%)	13 (2.6%)
Trichomonas vaginalis (Tv), by urethral swab culture	2 (10%)	2 (7.4%)	19 (4.2%)	23 (4.6%)
Ng & Tv (mixed)	1 (5%)	1 (3.7%)	0	2 (0.4%)
Ct & Tv (mixed)	1 (5%)	0	4 (0.9%)	5 (1.0%)
Total	14 (70%)	6 (22.2%)	38 (8.3%)	58 (11.5%)

There was considerable discordance between those men with a symptom of urethral discharge and those who were found to have discharge on physical examination (Table 18). In the overall population, of the 20 men with the symptom of

urethral discharge, only 10 had discharge on physical examination, and of 22 men among whom discharge was elicited on physical examination, only 10 had given a complaint of discharge. Among the 58 men with confirmed urethral infection, there were 14 who had a symptom of urethral discharge. Of these, only 9 (64.3%), had evidence of a discharge on physical examination.

Table 18. Agreement between the complaint of urethral discharge and the finding of urethral discharge on physical examination.

			Complaint of discharge		
			Yes	No	Total
	Yes		10	12	22
Discharge on physical examination	No		10	472	482
		Total	20	484	504

B. Among men with confirmed urethral infection

			Complaint of discharge		
			Yes	No	Total
	Yes		9	3	12
Discharge on physical examination	No		5	41	46
		Total	14	44	58

Table 19 shows the performance parameters of the LED test in symptomatic and asymptomatic men. For symptomatic men, treatment based on a positive LED test would pick up 95% of infections. With a positive predictive value of 63%, 37% of LED positive men would be treated unnecessarily. By contrast, if management were based on treating all symptomatic men, 57% would be treated unnecessarily, although

of course 100% of infections in this group would be picked up. Among asymptomatic men being screened, treatment based on a positive LED test would pick up 55% of infections. Of those treated, 77% would be treated unnecessarily. The three men with confirmed urethral infection who did not complain of discharge, but had discharge on physical examination (Table 19), all had positive LED tests.

Table 19: Performance parameters of the leucocyte esterase dipstick (LED) test by symptom group.

Any urethral Infection (Ng, Ct or Tv)	Symptomatic men (urethral discharge and/or dysuria)	Asymptomatic men	All men
LED positive (> trace)	63.8% (30/47)	20.4% (93/457)	24.4% (123/504)
Sensitivity	95.0% (19/20)	55.3% (21/38)	69.0% (40/58)
Specificity	59.3% (16/27)	82.8% (347/419)	81.4% (363/446)
Positive predictive value	63.3% (19/30)	22.6% (21/93)	32.5% (40/123)
Negative predictive value	94.1% (16/17)	95.3% (347/364)	95.3% (363/381)

If asymptomatic men with an LED equal to trace were included as positive, 83% (379/457) would be considered positive.

viii) Strategies for Control of Urethral Infection

Table 20 compares how three potential detection and treatment strategies for urethral infection would have performed in this population. Strategy 1, treatment on the basis of presenting symptoms, is the strategy used in most treatment settings in Africa. Strategy 2, treating only men who present with discharge and whose discharge is confirmed on physical examination, is a strategy that is advocated by many medical practitioners, and is the one recommended in World Health

Organisation (WHO) treatment guidelines [58]. Strategy 3 is a combination of Strategy 1 for symptomatic men, with use of the LED test for asymptomatic men. Note that Strategy 1 (treatment based on symptoms alone) was more than twice as sensitive as treatment requiring both symptoms and signs of discharge to be present (Strategy 2). Strategy 2 was highly specific, but at the price of low sensitivity. The highest sensitivity was obtained using Strategy 3, although this strategy did not perform significantly better than using the LED test alone.

Table 20: Performance parameters of potential strategies for detecting and treating urethral infection.

Treatment strategy	Strategy 1: Treat only men with symptoms (urethral discharge and/or dysuria)	Strategy 2: Treat only men with urethral discharge that is confirmed on physical examination	Strategy 3: Treat all men with symptoms (urethral discharge and/or dysuria), and asymptomatic men with a positive LED test
Sensitivity	34.5% (20/58)	15.5% (9/58)	70.7% (41/58)
Specificity	93.9% (419/446))	99.8% (445/446)	77.8% (347/446)
Positive predictive value	42.6% (20/47)	90.0% (9/10)	29.2% (41/140)
Negative predictive value	91.7% (419/457)	90.1% (445/494)	95.3% (347/364)

Potential behavioural risk factors for predicting urethral infection were explored by means of multiple logistic regression, as we wished to examine whether a risk score could be constructed that would improve diagnostic accuracy. The regression model used proven infection as the dependent variable, and signs, symptoms, LED test, age, and behavioural variables as independent variables. LED positivity was the predictor with the highest adjusted odds ratio (OR = 6.8, 95% CI 3.3 -14.0), followed by the

presence of symptoms of urethral discharge and/or dysuria (OR = 3.2, 95% CI 1.4 - 7.3), and urethral discharge on examination (OR = 3.0, 95% CI 1.0 - 9.0). Extramarital sex, commercial sex, unprotected sexual contacts, occupational travel and age were not associated with urethral infection. Non-cohabitation with a spouse for more than half the previous month was also not significantly associated with urethral infection. However, when infections were analysed separately, recent non-cohabitation with a spouse was associated with *C. trachomatis* and/or *N. gonorrhoeae* infection (adjusted OR = 5.4, 95% CI 1.2 -24.4), but not with *T. vaginalis* infection.

Differences between the results of HIV-1 positive nad negative men were also sought. A significantly higher number of HIV-1 positive men (21/75, 28%) had 5 or more urethral polymorphs per high power field on gram stain than HIV-1 negative men (79/429, 18%), OR 1.7 (1.0-3.0, p=0.05). HIV-1 positive men had a higher mean number of sex partners in the three months prior to examination (1.28 vs 1.02, p=0.02). STD prevalences and odds ratios are outlined in Table 21.

Table 21: Urethral infection by HIV-1 serostatus.

STD	HIV-1 positive	HIV-1 negative	OR (95% CI)	p value
any STD	11/75 (15%)	47/429 (11%)	1.4 (0.7-2.8)	0.4
Ng	4/75 (5%)	13/429 (3%)	1.8 (0.6-5.7)	0.2 FET*
Ct	5/75 (7%)	13/429 (3%)	2.2 (0.8-6.6)	0.1 FET
Tv	5/75 (7%)	25/429 (6%)	1.2 (0.4-3.1)	0.8 FET

*FET, fishers exact test

No significant differences in STD prevalence was detected between HIV-1 positive and negative men.

Age, presence of urethritis on urethral swab gram stain and urine leucocyte esterase urine dipstick (LED) results of men with unmixed infections with Tv, *Neisseria gonorrhoeae* (Ng), *Chlamydia trachomatis* (Ct), or no infection are given in table 22. Results from both symptomatic and asymptomatic men are included in the table, but seven men with mixed infections have been excluded.

Men with positive Tv culture were significantly older than men with Ng infection (p=0.003). Eighty three per cent (19/23) of Tv infections which were Ng culture and Ct enzyme immunoassay negative were asymptomatic. However, 37% (7/19) of these men had urethritis, defined as $\geq$ 5 polymorphonuclear cells (PMNs) per high power field, and 42% (8/19) were LED test positive (greater than trace). Both of these findings were significantly higher than asymptomatic men with no urethral pathogen isolated, of whom 16% (66/419) met the criteria for urethritis (odds ratio [OR] 3.1: 95% confidence interval [CI] 1.4-8.2, p=0.02), and 17% (72/419) were LED test positive (OR 3.5: 95% CI 1.4-9.0, p<0.01). Only one of the four men with

symptomatic unmixed urethral Tv infection met the criteria for urethritis, but all four were LED positive.

Table 22: Age, presence of urethritis and LED status of men with urethral infection

Pathogen Isolated (Unmixed Infections Only)	Number of men	Mean Age (95% CI)	Urethritis on gram stain (≥5 PMNs per high power field)	Urine LED positive (> trace)
T. vaginalis	23	32.3 (28.4-35.7)	8 (35%)	13 (50%)
N. gonorrhoeae	15	24.7 (22.7-27.0)	12 (80%)	13 (93%)
C. trachomatis	13	29.2 (23.8-31.6)	3 (23%)	9 (69%)
No pathogen	446	29.9 (29.3-30.5)	73 (16%)	83 (19%)

Thirty three percent (5/15) of unmixed Ng infections were asymptomatic with 60% (3/5) meeting the criteria for urethritis while 80% (4/5) were LED positive, and 77% (10/13) of single Ct infections were asymptomatic with 20% (2/10) positive for urethritis and 70% (7/10) LED positive.

T. vaginalis accounted for about 45% of all unmixed urethral infections. It was largely asymptomatic, as only 4 of 23 unmixed *T. vaginalis* infections were associated with discharge and/or dysuria. Men with *T. vaginalis* were older than men without the organism. *C. trachomatis* accounted for about 25% of all unmixed infections, and was also largely asymptomatic. Conversely, *N. gonorrhoeae*, accounting for about 30% of all unmixed infections, was mainly symptomatic and associated with younger age.

Study 7: HIV-1 vaccine acceptability survey

The demographic and behavioural characteristics of respondents are described in Table 23.

Table 23. Demographic and Behavioral Characteristics of Participants on the Vaccine Acceptability Survey

	Mean / median or proportion (%)
Male Trucking Company Employees (n = 201)	
Age (mean, years)	30 (range 17-54)
Years of formal education	
None	3 (2%)
0 - 7	53 (26%)
≥8	145 (72%)
Occupation	
Truck drivers or assistants	41 (20%)
Mechanics	73 (36%)
Ancillary workers	87 (43%)
Religion	
Protestant	76 (38%)
Catholic	66 (33%)
Moslem	50 (25%)
Marital status	
Married	121 (60%)
Unmarried	75 (37%)
Widowed or divorced	5 (3%)
History of sex with a FSW	105 (52%)
History of condom use	102 (51%)
Sexual behavior past year	
2 - 5 sex partners	95 (47%)
>5 sex partners	23 (11%)
Unprotected sex with a FSW	44 (22%)

Sixty percent of the men were married. Fifty eight percent reported two or more sex partners and 22% gave a history of unprotected sex with a FSW in the year prior to enrolment. One hundred and forty five men (72%) had completed primary education (8 years). The only statistically significant difference between the cohort as a whole and the sample participating in the vaccine acceptability survey was in the

percentage of truckdrivers and their assistants. These more mobile occupations made up 36% of the total male cohort, but only 20% of the sample surveyed ($p<0.001$).

Knowledge of respondents regarding vaccines currently in use in Kenya is shown in Table 24. Eighty six percent (86%) of the men knew that vaccines are used to prevent disease and 88% were able to name a vaccine preventable disease. However, less than half knew that vaccines were not 100% efficacious and less than one third knew that vaccines could have adverse effects. There were no statistically significant differences in any of the responses by educational level.

Table 24. Knowledge of Vaccines Currently in Use in Kenya

	Men (n = 201)
Knew that vaccines are used to prevent disease	173 (86%)
Were able to name a vaccine preventable disease	176 (88%)
Knew that vaccines were not 100% efficacious	98 (49%)
Knew that vaccines could have side effects	58 (29%)

One hundred and ninety one (95%) men thought that HIV infection and AIDS was a major problem in Kenya, and only 29 (14%) men felt at no personal risk of HIV infection.

Replies to questions regarding acceptance of a safe and efficacious HIV-1 vaccine, acceptance of the likely conditions of a vaccine trial, and acceptance of participation in a vaccine trial are given in Table 25.

Table 25. Acceptance of Conditions of an HIV-1 Prophylactic Vaccine Trial

	Men (n = 201)		
	Yes	**Perhaps**	**No**
Would accept an HIV vaccine of known high safety and efficacy	186 (93%)	8 (4%)	7 (4%)
Would accept a double blind, placebo controlled design	137 (68%)	30 (15%)	34 (17%)
Would accept a follow-up of 3-5 years	129 (65%)	34 (17%)	34 (17%)
Would accept a "false positive" HIV serologic result	132 (66%)	34 (17%)	33 (16%)
Would participate in trial of a candidate HIV vaccine	133 (67%)	35 (18%)	32 (16%)

Only 4% of said they would refuse an HIV-1 vaccine of known high safety and efficacy. The reasons given for refusal included the fear of acquiring HIV-1 infection from the vaccine (7 men), no perceived risk of infection (2 men). Sixty seven per cent of men said they would definitely agree to participate in a trial of a candidate HIV-1 vaccine given the conditions specified, and an additional 18% said they would possibly participate. Concerns regarding participation included fear of a positive HIV blood test result (27 men), fear of HIV infection from the vaccine (11 men), prolonged follow-up period (2 men), and fear of injections (1 man). There was no significant effect of educational level on vaccine trial.

Anticipated sexual behaviour changes as a result of participation in an HIV-1 vaccine trial are shown in Figure 6. Seventeen (9%) men said that they would decrease condom use, and 18 (9%) would increase numbers of sex partners. Two men stated that they would do both. Anticipated higher risk behaviour was not significantly associated with age, years of education, history of unprotected sex with FSWs.

Section 6: Discussion and Conclusions

Discussion

The research which is the subject of this thesis has been carried out to contribute to efforts to mitigate the effects of the HIV-1 pandemic in the developing world. The sexual risk reduction work (Studies 4 and 5) has been chosen as one of only two selected examples of evaluated interventions to prevent HIV-1 in male groups in developing countries, in a World Bank Policy Research Report aimed at policymakers "who shape the public response to HIV/AIDS to design an effective strategy for confronting the epidemic" [118]. The urethral infection data (Study 6) contributed to a meeting of international public health policymakers, chaired by WHO, on the future shape of the STD algorithms, held at the Eleventh Meeting of the International Society for STD Research in New Orleans[119]. Specific aspects discussed were the use of symptoms rather than signs of male urethral infection and the potential inclusion of *Trichomonas vaginalis* therapy in the male urethritis algorithms. A list of publications in international peer-review journals from this cohort is included before the reference section.

Many of the subjects which have been explored have inter-linked issues. An attempt is made to try to separate these issues for the purposes of discussion.

Circumcision Issues

It is biologically plausible and consistent with current knowledge, that uncircumcised men are at increased risk of acquisition of HIV-1 infection during sexual intercourse. There was insufficent statistical power to show a significant difference in HIV-1 acquisition between circumcised and uncircumcised men, because of insufficient numbers of uncircumcised men and insufficiently high HIV-1 seroincidence. With an adjusted hazard ratio of 2.0, an HIV-1 incidence of 6.0% in uncircumcised men, and only 13% of the study population uncircumcised, a total study population of 3,344 would have been required to have 80% power to show a significant difference, at the 5% level ($p<0.05$) within the study timeframe. This would have required a different scale of operations and funding structure.

Although the study did not have the power to show a significiant difference in HIV-1 acquisition between circumcised and uncircumcised men in 990 person years of follow-up, it provides evidence which is supportive of male circumcision as an intervention against HIV-1 infection. Even if it does not become statistically significant in the final analysis, it will add to the body of knowledge on this subject, by contributing to databases from different centres and being used in meta-analyses.

The principal tribe in Kenya which does not practice circumcision are the Luo. The tribal area of the Luo is on the banks of Lake Victoria, in Nyanza Province, western Kenya [3]. This means that a higher percentage of uncircumcised men in Mombasa are migrant workers, as the coastal tribes and ethnic groups all practice circumcision. The women who become the sex partners of the men who work away from home are often bar workers [4]. The man will give some money to help look after the womans' children or pay the rent. Sex is being traded, but the man may not

view the woman as a sex worker but as a girlfriend, because no straight cash-per-sex-act transaction is entered into. In this way, more uncircumcised men may be at risk of HIV-1 infection, despite not reporting FSW contact. This will be discussed further in the appropriate section.

For that reason, it may be that Mombasa was not, geographically, the best place to examine the relationship between circumcision and HIV-1 infection. The probability is that the majority of uncircumcised men had poorer access to their wives and families than the majority of circumcised men [44]. Also, if a man returns home to Eastern Province and has sex with a girlfriend, his level of risk is less than a man who returns to Nyanza, where the background seroprevalence is more than double [22]. In this way, it is probable that lack of male circumcision contributes to a positive feedback loop in which increased individual susceptibility of a significant proportion of the population gives overall increased transmission rates, which leads to increased background seroprevalence, and consequently higher transmission rates.

The extent to which any increased susceptibility to HIV-1 is mediated through other genital ulcer disease is unknown, and has not been answered by this cohort, due to the factors outlined above and relatively low GUD incidence (4.3/100 py).

Migration and Sex Partner Issues

Men who travel in the course of their work follow regular trade routes and may have "girlfriends" in many towns, and the women may, in turn, have more than one regular sex partner who helps them with living costs. In this way men may have very high risk contacts without telling us, and without attempt to deceive. Supporting evidence for this exists in the relationship between confirmed *H ducreyi* infection, chancroid, and unprotected FSW contact (Table 9). Chancroid is a painful genital ulcer disease of relatively short infectious duration (see introduction). For this reason, high rates of partner change are required to sustain the infection in the community, and infection is characteristically associated with recognised FSW contact [120]. In one Nairobi study, 66% of men with chancroid reported a paid sex partner as the source of infection [121]. Another potential reason for occupational travel being an independent risk factor is that the prevalence of STDs in towns which are truckstops is very high, increasing the risk involved in any sexual encounter [104]. Mgalla and Pool examined the structure of informal sex work female bar workers in north-west Tanzania [4]. They found that the distinction between regular and casual partners was in the nature and extent of financial support, and the women stated that they could demand condom use from casual partners, but not from regular partners. These regular partners were often itinerant workers and truck drivers.

In this cohort, men who reported sex with their wives in the previous three months were less likely to seek other partners, and almost five times less likely to become infected with a symptomatic STD. Adult male rural to urban migration has become a feature of life in many developing countries. While the economic root causes of this migration cannot be addressed by health or education sectors alone, the

structure of adult male interventions should incorporate specific strategies to access migrant labourers, as they often are clients of FSWs, an important urban male STD core group, and a source of spread of STDs and HIV-1 to rural areas [105].

Sexual Behaviour and STD Issues

Several limitations of the STD incidence and time-trend studies should be noted. The time-trend studies included STD evaluation only for symptomatic men, and hence provided an underestimate of STD incidence. As we have seen in the appropriate section, asymptomatic urethral infection is common. It is unclear the extent to which these asymptomatic infections increase susceptibility to HIV-1 infection, or transmission of HIV-1 to sex partners, but in future studies it would be valuable to monitor changes in both symptomatic and asymptomatic STDs. This study included only HIV-1 seronegative men, but the evaluation of behavioural interventions among HIV-1 seropositive men would be of great public health importance. Also, the reported changes apply to a selected group of HIV-1 seronegative men, as there were men who were screened but not enrolled, and almost 30% of men who did not contribute to follow-up. Although men who did and did not return for follow-up were similar for baseline characteristics except for occupational travel, it is possible that our results suffered from bias.

The application of time-dependent covariates was unhelpful in identifying any relationship between incident HIV-1 infections and incident STDs. Potential reasons for this are that only men with symptoms who were available to attend the clinics were sampled, and the information collected on the timing of any STD between clinic visits was insufficiently precise. The fact that STDs were positively associated with HIV-1 acquisition on univariate analysis may merely be acting as a marker for higher risk sexual behaviour.

No baseline HIV-1 risk perception was done, so no information was available on personal assessment of risk of HIV-1 infection before enrolment and counselling,

but the vaccine acceptability survey carried out during follow-up revealed that 86% of men felt at some degree of personal risk of AIDS. This compares with only 8% of men with genital ulcer disease who were surveyed at an STD clinic in Nairobi in 1991 [121]. In that study, the factor most significantly associated with perception of personal risk was acquaintance with a person with AIDS. It is probable that the high perception of personal risk of HIV-1 infection in our cohort played a significant role in decreasing risk behaviour [122]. As the effects of the epidemic become more widespread, the proportion of the general population with personal knowledge of AIDS through illness in a family member or friend will increase. This is likely to increase the receptivity of men to education and risk reduction counselling, potentially leading to behaviour change.

The absence of reported increases in condom use in any group in the cohort, despite active condom promotion and open access to unlimited free supplies, is a cause for concern, and a subject requiring further examination. Condom use varied significantly with different categories of sex partner in this study. Our results are consistent with those of a study of truck drivers in Tanzania which found that men were less likely to use condoms with regular partners, whom they classified as "safe" [123]. This sense of security in men decreases the power of women to negotiate condom use, as they may be economically dependent on regular partners, as outlined by Mgalla and Pool, referenced above. Another study of factory workers in Tanzania reported no increase in overall condom use, but increased use with casual partners [57]. Men in the Mombasa study were more likely to use condoms with sex partners they classified as sex workers than with partners they classified as girlfriends. While condom use did not rise over time, percentage use was higher at baseline than in

Tanzania, with around 30% of extramarital sexual contacts from this cohort covered with condoms, compared with under 10% in the Tanzanian cohort.

Reported condom use increased in studies of HIV-1 discordant couples in Rwanda and Zaire, after an intervention of HIV-1 serotesting and counselling [124,125]. It is likely that the HIV-1 screening itself played a role in behaviour change in our cohort but, as we did not follow the men before serotesting, it is not possible to quantify that role. Our results highlight the difficulty in changing sexual behaviour of individuals once patterns have been established. Providing adolescents with condom handling and negotiation skills could facilitate condom negotiation for both males and females at the commencement of sexual activity, and in the long term a generation of peer health educators and positive role models might be created.

This study shows that a climate favourable to behaviour change currently exists in this cohort of east-African men. How this climate can best be exploited to full benefit on a larger scale could be evaluated by randomised, controlled trials of programmes that include counselling, condom promotion, and service delivery systems in different occupational and community groups. The extent of study effects, cohort attrition, and return to high risk behaviour could also be fully addressed in that context. In addition to behavioural intervention efficacy trials, identification of the most effective points of access and examination of cost-effectiveness issues should be carried out in order to ensure replicability. Exploration of partnerships with industry and private sector employers might be possible, with intervention programmes paid for by industry, if they could be persuaded that a programme would be economically advantageous in the long term.

Even if a safe and efficacious HIV-1 preventive vaccine were to be developed soon, it would be overly optimistic to expect that HIV-1 and STD transmission will be controlled in the near future. The need to effectively address the issue of sexual safety in both adults and adolescents in both urban and rural areas is urgent, especially in developing countries, where decisions on sexual relationships are often dominated by economic imperatives.

This was the first published study of urethral infection, including *T vaginalis*, in asymptomatic men in a workplace population, worldwide. Over 90% of men were asymptomatic, as were two-thirds of the infections identified, and these would have been missed by passive case finding. There was clearly an enormous burden of sexually transmitted infections in this population, despite the fact that the urethral infection study directly followed a documented decrease in risk behaviour and symptomatic STDs.

T. vaginalis has generally not been given much consideration as a urethral pathogen among men in developing countries. However, as *T. vaginalis* has been detected in vaginal specimens from upwards of 40% of general populations of women in Africa [126,127], it should not be surprising to find it in high levels in men as well.

Increased shedding of human immunodeficiency virus type 1 (HIV-1) in semen in the presence of urethral infection and urethritis has recently been described in Malawi [78]. In that study, men with Tv urethral infection were observed to have significantly increased HIV-1 shedding in semen. In light of the results from Malawi, there are several points worth making:

1. Absence of symptoms does not necessarly imply absence of urethritis, especially in the presence of Tv or Ct infection.

2. The association of higher prevalence of Tv infection with older age is unusual for a sexually transmitted disease, and suggests that infection may be of long duration.

3. Tv infection is associated with less severe symptoms than Ng infection and leads fewer men to seek treatment. In addition, even among symptomatic men, guidelines for syndromic management regimens for urethral discharge do not generally include treatment for Tv, so even if a man seeks treatment, he is unlikely to be adequately managed (see figure 3).

4. Tv infection has not featured highly in projections of population attributable risk fractions for HIV-1 infection associated with various STD pathogens. However, Tv infection is very common among women, as evidenced by a community study from Uganda which described a Tv infection prevalence of 47% in rural women[127]. Tv is probably more common among men than is generally appreciated, and long-standing, low-grade urethritis may lead to an increased risk of male-to-female transmission of HIV-1.

5. Further study into the role of Tv in HIV-1 transmission is warranted. If Tv does significantly enhance HIV-1 transmission, mass treatment strategies for Tv infection could potentially play a valuable part in HIV-1 prevention in developing countries, as an effective single-dose, low cost treatment for Tv (Metronidazole 2gm) is available worldwide.

Previous evaluations of the LED dipstick test in men have measured performance in detecting *N. gonorrhoeae* and *C. trachomatis* only. Sensitivities among asymptomatic men in North America have ranged from 41% to 100%, specificities from 83% to 96%, and positive predictive values from 20% to 58% [74,80-82]. The LED test in this study showed great promise. Used alone as a

predictor of urethral infection, it had a much higher sensitivity than did the presence of symptoms, albeit with a lower specificity. Among symptomatic men, it picked up 95% of infections, while excluding 59% of those without infection, consequently reducing the amount of unnecessary treatment that would occur if all men with symptoms were treated. It could thus be useful in the clinical management of symptomatic men in sub-Saharan Africa: with the high sensitivity and negative predictive value in symptomatic men, few infections would be missed, while the number of men treated unnecessarily and the subsequent drug costs would be reduced. Among asymptomatic men, the LED test had a sensitivity of over 50% and a specificity of over 80%, far from perfect performance, but easily the best performing screening method available. Perhaps the most practical overall approach in resource-poor settings would be to treat men with symptoms for all three pathogens, and screen and treat asymptomatic men (also for all three pathogens) on the basis of a positive LED test result.

As indicated above, the current WHO recommendations for the syndromic management of men with urethral discharge indicate that treatment should only be given to men whose complaint of discharge is confirmed on physical examination [58]. This study suggests that treatment on the basis of symptoms alone, or in combination with a positive LED test (without examination), would lead to substantial improvements in sensitivity, with only a small decline in specificity. Furthermore, current management guidelines do not include dysuria as an entry point for syndromic management in men. In this study, 6 of the 20 cases of symptomatic urethral infection would have been missed had men with dysuria (without discharge) been excluded. It

may be useful therefore to add dysuria as an entry point in management guidelines, although this would lead to some additional overtreatment.

It should be noted that no pathogen was identified in about two-thirds of all specimens positive by LED testing, and that about one-quarter of these met laboratory criteria for urethritis (five or more polymorphonuclear cells per high power field on urethral swab gram stain). This suggests that either the sensitivity of the diagnostic procedures used was less than 100%, or that individuals may have been infected with other pathogens. For example, detection of ureaplasma infection was not attempted, and it is known that EIA testing for *C. trachomatis* underestimates prevalence [128]. Thus, LED performance parameters obtained under the conditions in this study may have underestimated its true performance.

STD risk assessment scores, incorporating demographic, behavioural and other characteristics, have been developed for identifying women with cervical infection in Zaire [79] and Tanzania [129]. These are potentially useful in many developing country settings, where laboratory testing for sexually transmitted pathogens is generally unavailable. However, in this study, only recent non-cohabitation with a spouse was found to be an independent risk factor for *N. gonorrhoeae* and/or *C. trachomatis* infection. The utility of risk assessment to predict urethral infection in male populations in developing countries remains to be demonstrated.

The key findings of the urethral infection study were first the high prevalence in general of symptomatic and asymptomatic urethral infection, as well as the unexpectedly high prevalence of *T. vaginalis*, in this population of men in a Kenyan workplace. Second, a complaint of urethral discharge may be a more useful predictor

of infection than the sign of discharge on physical examination, and the most useful entry point for syndrome-based treatment may be a complaint of discharge and/or dysuria. Third, the LED test appears to be a reasonably accurate predictor of infection among both symptomatic and asymptomatic men. Finally, risk assessment did not prove to be very useful in predicting urethral infection in this population.

The other main group of STDs are those which cause genital ulceration. Clinical diagnosis of genital ulcer disease by aetiological agent is unreliable, with one American study finding sensitivities of 31% for clinical diagnosis of primary syphilis, 34% for chancroid and 35% for genital herpes [130]. Another issue which would have interfered with the accurate diagnosis of chancroid is that *H ducreyi* will only survive for 2-4 hours on a swab, unless refrigerated, and it is difficult to culture with a sensitivity greater than 80% [131]. It is probable that the sensitivity achieved by the swabs taken in the field was lower. A sub-study of 501 HIV-1 seronegative men, supervised by Dr Rakwar, found that 26% had antibodies to H ducreyi at enrolment [132]. Poor genital hygiene in men is also thought to predispose men to chancroid infection [133]. This certainly applies to many of the men in this cohort, who live and work in very hot and dirty conditions with limited access to water and privacy for bathing and washing clothes. Syphilis was the only other genital ulcer aetiology which could be diagnosed in Mombasa, but 50% (65/130) of a sub-group of the men had antibodies to Herpes Simplex Type II which also causes genital ulcers, suggesting that this was a common infection [132].

Potential treatment strategies, including the use of simple screening tools, such as the LED test, need to be further evaluated and ultimately incorporated into effective STD control programmes.

HIV-1 Vaccine Acceptability Issues

A high rate of willingness to participate in candidate prophylactic HIV-1 vaccine trials was documented in this study of Kenyan trucking company workers. Eighty four per cent of men indicated interest in participation. Of concern, however, were the disturbing anticipated behaviour changes reported by a significant number of respondents. Seventeen percent of men said they would increase sexual risk behaviour if involved in a vaccine trial. This occurred despite an intensive individual HIV educational counselling programme included in the cohort study and despite having been informed in the context of the vaccine survey that half of trial participants would receive placebo and half a vaccine of unknown efficacy. This finding underscores the need for extensive education both before enrolment of individuals and throughout the duration of any vaccine trial.

The successful conduct of HIV-1 vaccine efficacy trials requires the availability of cohorts with high seroincidence rates. A number of articles have addressed the ethical dilemma which confronts HIV vaccine investigators [134-143]. While a high HIV-1 seroincidence permits a trial to be conducted with fewer subjects and shorter follow-up, the ethical principles of autonomy and beneficence demand that the well- being of the individual participants be maximised by risk reduction counselling aimed at reducing the likelihood of HIV-1 acquisition. In the extreme case, if counselling were fully effective in inducing behaviour change and the HIV-1 seroincidence rate fell markedly, an HIV-1 vaccine trial would not be feasible. Although the possibility that participation in a vaccine trial would result in reduced HIV-1 seroincidence has been extensively discussed [134-139], less attention has been given to the converse. Our study suggests that participation in an HIV-1 vaccine

trial might be associated with increased risk taking behaviour and therefore possibly increased risk of HIV-1 infection. In a study in Rwanda, women participating in focus group discussions expressed concern that their seropositive male partners may be less likely to use condoms if they knew that the woman was participating in a vaccine trial [144]. Females may be particularly vulnerable because of the absence of a practical and effective female controlled means of avoiding infection during heterosexual intercourse.

Although the process of obtaining consent of participants has become standard for biomedical research involving human subjects, special care must be taken to ensure that individuals participating in HIV-1 vaccine trials are fully informed of the implications of involvement. Counselling must be tailored to each community, directed by cultural and language considerations and the level of literacy of the cohort and the individual. The concept of a placebo-controlled double-blind trial is not a simple one, particularly for poorly educated individuals from communities in which the scientific method does not have widespread acceptance.

Another difficult concept which must be conveyed during the consent process for HIV-1 vaccine trials is the potential change in serostatus. In a US survey, 84% of seronegative intravenous drug users initially said that they would participate in a phase III vaccine trial, but this dropped to 41% once it was explained that they would test seropositive as a result of participation [145]. In Bangkok, almost half of health care providers surveyed were concerned about participation in a vaccine trial if HIV-1 antibody screening were a routine part of job or health insurance applications[146]. HIV antibody tests are increasingly being used for employment or health care provision screening purposes in Kenya. Consequently, safeguards against potential

discrimination for HIV vaccine trial participants would be required. In addition, the means of differentiating vaccine induced seropositivity from HIV infection should ideally be accessible at the site of the vaccine trial, so that subjects can be informed promptly if they become infected.

The evaluation of candidate HIV vaccines will require multiple clinical trials in populations which differ according to mode of HIV acquisition, patterns of sexual behaviour, exposure to STDs and other transmission cofactors, viral strain type, and genetic and nutritional factors which may alter susceptibility. Before this can happen, the legitimate concerns of all study populations must be fully addressed.

Conclusions

The research outlined in this thesis has provided information on the HIV-1 and other STD risk in this occupational male cohort in sub-Saharan Africa, and the potential acceptance of HIV-1 prevention interventions. No clear answer to the question of whether male circumcision should be recommended as a long-term HIV-1 prevention strategy was obtained in the research time frame, but information was gathered which may assist other investigators. This information has been published in international peer-review journals (below), or will be published in the near future. The most important findings presented in this thesis were:

- The HIV-1 seroincidence of 4% per annum and high level of sexual risk behaviour.
- The failure to increase condom use over time, despite active condom promotion, high risk perception and a decrease in high-risk sexual contacts.
- The high prevalence of asymptomatic urethral infection , especially Trichomonas vaginalis.
- The acceptance of the conditions of an HIV-1 preventive vaccine trial of a high percentage of men, but the anticipated negative behaviour change of a minority.

Many of the men who participated in these studies when they were young and outwardly healthy are now dead. Their wives and children have lost their source of financial support. WHO estimated that six million new HIV-1 infections occurred in 1997 [11]. Now, at the end of 1998, the need for effective HIV-1 preventive interventions has never been greater.

Publications and presentation from this thesis

(included in envelope in back cover)

Jackson DJ, Rakwar JP, Richardson B, Bwayo JJ, Mandaliya K, Ndinya-Achola JO, Martin HL, Moses S, Kreiss JK. Decreased incidence of sexually transmitted diseases among trucking company workers in Kenya: results of a behavioral risk reduction programme. *AIDS* 1997, 11:903-909.

Jackson DJ, Rakwar JP, Chohan BH, Bwayo JJ, Mandaliya K, Ndinya-Achola JO, Martin HL, Kreiss JK, Moses S. Urethral infection in a workplace population of East African men: evaluation of strategies for screening and management. *Journal of Infectious diseases* 1997, 175:833-838

Jackson DJ, Rakwar JP, Bwayo JJ, Kreiss JK, Moses S. Urethral *Trichomonas vaginalis* infection and HIV-1 transmission. *Lancet* 1997, 350:1076 (peer-reviewed research letter).

Jackson DJ, Martin HL, Bwayo J, Nyange PM, Rakwar JP, Kashonga F, Ndinya-Achola JO, Kreiss J. Acceptability of trials of a candidate prophylactic HIV-1 vaccine in high risk heterosexual cohorts in Mombasa, Kenya. *AIDS*, 1995, 9: 1279-1283.

Jackson DJ, Rakwar JP, Bwayo J, et al: HIV / STD incidence among truck drivers in Mombasa, Kenya: role of circumcision status. *X International Conference on AIDS / HIV STD World Congress.* Yokohama, August 1994. (409C).

Other Cohort Publications

Rakwar J, Jackson D, MacLean I, et al. Antibody to Haemophilus ducreyi among trucking company workers in Kenya. *Sexually Transmitted Diseases*, 1997, 24, 267-271.

Martin HL, Jackson DJ, Mandaliya K, et al. Preparation for AIDS vaccine evaluation in Mombasa, Kenya: establishment of seronegative cohorts of commercial sex workers and trucking company employees. *AIDS Research and Human Retroviruses*, Volume 10, Supplement 2, 1994, S235-S237.

Section 7: References

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