Epidemiology and natural history of human papillomavirus around the time of sexual debut

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I, Catherine Houlihan confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Table of Contents

Acknowlegments **7**

Abstract **8**

List of tables **9**

List of figures **11**

List of acronyms **12**

Role of the candidate **13**

1 Background **14**

1.1 Human papillomavirus (HPV): infection, natural history and immune response 14

1.2 HPV epidemiology in sub-Saharan Africa 17

1.3 Risk factors for HPV 18

1.4 Collecting risk factor data for HPV research 19

1.5 Cervical cancer screening and HPV vaccination 19

1.6 Research aims and objectives 20

1.6.1 Study rationale 20

1.7 Objectives of the study 22

1.8 Thesis structure 22

1.9 Ethical approval 23

1.10 Funding 23

1.11 References 24

2 Methods **32**

2.1 Study design 32

2.2 Ethical issues 33

2.3 Study Procedures 34

2.3.1 Specimen collection 35

2.3.2 Laboratory testing 36

2.3.3 Sexual behaviour questionnaires and pilot testing 38

2.3.4 Data management 39

2.3.5 Data analysis 39

2.3.6 Clinical follow up 41

2.4 Sample size 42

2.5 Comparision of Audio Computer Assisted Self Interview (ACASI) with face-to-face interviews 44
2.5.1 ACASI sample size calculations 45
2.6 References 47
3 Manuscript 1: Prevalence of human papillomavirus in adolescent girls before reported sexual debut 50
3.1 Preamble 50
3.2 Cover sheet: manuscript 1 51
3.3 Copyright clearance certificate 52
3.4 Manuscript 1: Prevalence of human papillomavirus in adolescent girls before reported sexual debut 55
4 Manuscript 2: The incidence of human papillomavirus in Tanzanian adolescent girls before reported sexual debut 78
4.1 Preamble 78
4.2 Coversheet: manuscript 2 79
4.3 Manuscript 2: The incidence of human papillomavirus in Tanzanian adolescent girls before reported sexual debut 80
5 Manuscript 3: HPV incidence around the time of sexual debut in adolescent girls in Tanzania 100
5.1 Preamble 100
5.2 Cover sheet: manuscript 3 101
5.3 Manuscript 3: HPV incidence around the time of sexual debut in adolescent girls in Tanzania 102
6 Manuscript 4: Comparison of Audio Computer-Assisted Self-Interview with face to face interview for disclosure of sexual behaviour in adolescent girls in Tanzania 129
6.1 Preamble 129
6.2 Cover sheet: manuscript 4 130
6.3 Manuscript 4: Comparison of Audio Computer-Assisted Self-Interview with face to face interview for disclosure of sexual behaviour in adolescent girls in Tanzania 131
7 Discussion 155
7.1 Summary of results 155

7.1.1 Manuscript 1 and 2: Prevalence, incidence and clearance of HPV in adolescent girls before reported sexual debut 155
7.1.2 Manuscript 3: HPV Incidence around the time of sexual debut in adolescent girls in Tanzania. 156
7.1.3 Manuscript 4: Comparison of Audio Computer-Assisted Self-Interview with face-to-face interview for disclosure of sexual behaviour in adolescent girls in Tanzania 157
7.2 Methodological challenges 158
7.2.1 Strengths 158
7.2.2 Limitations 159
7.2.3 Sample size, study duration and missed visits 159
7.2.4 Study retention 161
7.2.5 Unmeasured variables 162
7.2.6 Outcome misclassification 163
7.3 Future research 164
7.3.1 Fomite transmission 164
7.3.2 Persistent infection with intermittent detection 165
7.3.3 Extending the age of vaccination in Tanzania 165
7.4 Conclusion 166
7.5 References 167
8 Annexes 172

Annex 1. Ethical approval from MRCC Tanzania 172
Annex 2. Ethical approval from LSHTM 173
Annex 3. Enrolment questionnaire 174
Annex 4. Follow-up questionnaire 184
Annex 5. Informed consent (parent) 193
Annex 6. Informed assent (participant) 198
Annex 7. Nurse-assisted self-administered vaginal swab pictures 203
Annex 8. Nurse-assisted self-administered vaginal swab instructions 206
Annex 9. Colloquial terms for sexual behaviours 207

Annex 10. Clinical outcomes.....212

Annex 11. ACASI questions.....213

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2 Abstract

Human papillomavirus (HPV) is a sexually transmitted virus associated with cervical cancer. The East African region has one of the highest incidences and mortality rates from cervical cancer but limited studies on HPV are available.

Research aims were to describe: HPV genotypes, risk factors and rate of acquisition of prevalent and incident HPV in girls before and after reported first sex; rate and risk factors associated with HPV clearance, and to examine sexual behaviour reporting in face-to-face (FtF) interviews compared to Audio Computer-Assisted Self-Interviews (ACASI).

A total of 503 girls aged 15 and 16 years in Mwanza, Tanzania, were enrolled and followed 3-monthly for 18 months with FtF-interviews and self-administered vaginal swabs. At enrolment, 474 girls reported no previous sex, and HPV was detected in 40/474(8.4%). During follow-up of girls who reported sex, new HPV incidence was 225/100 person-years(pys). Reporting sex in the past 3 months, and knowing the most recent sexual partner for a longer period before sex were associated with HPV acquisition. Median time from reported sexual debut to first HPV infection was 5 months, and median duration of infection 6 months. No factors were associated with HPV clearance. In girls who reported not having sex, HPV incidence was 29.4/100pys.

ACASI was compared to FtF-interview in 203 girls at the 12-month visit. Although ACASI was feasible and acceptable, there was no increase in reporting of sex or other sexual behaviours, with the exception of kissing, compared to FtF-interviews.

A very high incidence of HPV was seen in girls following sexual debut, and a higher than expected HPV prevalence and incidence were seen in girls who reported no previous sex. This emphasises the importance of HPV vaccination well before sexual debut. ACASI did not lead to increased reporting of vaginal sex and should be evaluated further in different settings.

3 List of tables

Table 2:1. Interpretation of cross-reactive probe 52/33/35/58 in the Linear Array HPV genotyping test (Roche, CA) 38

Table 2:2. Criteria for referral for clinical follow up of persistent HPV at study completion 42

Table 2:3. Power calculations for the detection of factors associated with HPV among those reporting sex 43

Table 2:4. Power calculations for the comparison of disclosure in ACASI and face to face interview 46

Table 3:1 Analysis of factors associated with detection of HPV in adolescent girls who reported never having had sex (n=474) 67

Table 3:2. Intravaginal cleansing and HPV prevalence 71

Table 4:1. Prevalence of reported behaviours among all girls in the study who did not report sex at enrolment or during follow-up (n=396), and among those randomly selected for the HPV incidence study (n=119) 89

Table 4:2. HPV genotype prevalence, incidence, duration and clearance among 119 girls who did not report sex at enrolment or during up to 18 months follow-up 90

Table 4:3. Incidence and point prevalence in adolescent girls who did not report sex at enrolment or during 18 months follow-up 92

Table 4:4. Selected risk factors for any new HPV infection among 119 girls who did not report sex at enrolment or during 18 months follow-up 93

Table 5:1. HPV genotype prevalence, incidence, duration and clearance among 105 sexually active girls during follow-up 114

Table 5:2. Incidence and point prevalence of HPV in adolescent girls who reported sex 116

Table 5:3. Association of selected potential risk/protective factors[1] with any new HPV infection among 105 adolescent girls who reported previous sex 117

Table 5:4. Clearance of new HPV infections and associated factors among girls who reported having had sex at any time during follow-up (unit of analysis is the infection) 120

Table 6:1. Socio-demographic characteristics of 203 girls in Tanzania in the ACASI versus FtF-interview sub-study 145

Table 6:2. Reporting of sexual behaviours in FtF-interview and ACASI in 203 girls in Tanzania aged 16 and 17 years 146

Table 6:3 Perceptions of ACASI and FtF-interview in 203 Tanzanian girls aged 16 and 17 years 148

Table S6:4 Reporting during FtF-interview in those who completed the FtF-interview first (without prior ACASI) and reporting during FtF-interview in those who completed the FtF-interview after a prior ACASI 150

Table S6:5. Reporting during ACASI in those who had not completed a prior F2F-interview and reporting in F2F-interview in those who had not completed a prior ACASI 151

Table 7:1. Distribution of selected variables at baseline in girls among those who attended and those who did not attend the final study visit at 18 months 161

4 List of figures

Figure 1:1. Cervical HPV infection natural history 15

Figure 2:1 Map illustrating Mwanza, Tanzania 32

Figure 2:2. Study nurse explaining how to collect a nurse-assisted self-administered vaginal swab to a study participant 35

Figure 2:3. Linear Array HPV genotyping test 38

Figure 2:4. A study participant completing an ACASI 45

Figure 3:1 Flow diagram of cohort enrolment 70

Figure 3:2. HPV genotypes detected in girls reporting never having had sex (n=474) 72

Figure 4:1. Enrolment of study participants 95

Figure 4:2. HPV genotype point prevalence (95% confidence interval), and number of infections at all visits including enrolment among 119 girls who did not report sex at enrolment or during follow-up 96

Figure 5:1. Enrolment and follow-up of study participants 122

Figure 5:2. HPV genotype point prevalence (95% confidence interval), and number of infections at all visits after reported first sex in 105 adolescent girls 123

Figure 5:3. Time from sexual debut to first infection with any HPV, any HR HPV or any LR HPV, among 41 girls who reported sexual debut during follow-up and were HPV-naïve at time of reported sexual debut 124

Figure 6:1. Flow diagram of inclusion in the ACASI versus FtF-interview sub-study 149

5 List of acronyms

ACASI	Audio computer-assisted self-interview
AIS	adeno-carcinoma in situ
aRR	adjusted rate ratio
BV	bacterial vaginosis
CI	confidence interval
CIN	cervical intra-epithelial neoplasia
FtF-interview	Face to face interview
GUD	genital ulcer disease
HIV	human immuno-deficiency virus
HPV	human papillomavirus
HR	high-risk (oncogenic)
HSV2	herpes simplex virus type 2
IARC	The International Agency for Research on Cancers
ICO	Catalan Institute of Oncology
ID	identification
IQR	inter-quartile range
LR	low-risk (non-oncogenic)
LSHTM	London School of Hygiene and Tropical Medicine
MITU	Mwanza Interventional Trials Unit
MRCC	Medical Research Coordinating Committee
PAP	papanicolaou
PID	pelvic inflammatory disease
Pys	person-years
RR	rate ratio
RTI	reproductive tract infection
SSA	sub-Saharan Africa
STI	sexually transmitted infection
VCT	voluntary counselling and testing
VDS	vaginal discharge syndrome
VIA	visual inspection with ascetic acid
VILI	visual inspection with lugol's iodine
WHO	World Health Organization

6 Role of the candidate

I was involved in conceptualising this study, with Dr Deborah Watson-Jones and Professor Richard Hayes. I wrote the funding application, designed the data collection tools, conducted the pilot studies and coordinated, from Tanzania, the study. I performed the data analysis for all studies, with the exception of the study of HPV epidemiology in girls who reported no previous sex, for which I was involved in parts of the analysis. Kathy Baisley performed the main analysis for this and parts of the analysis for the study of HPV epidemiology in girls who reported previous sex. The HPV genotyping tests were performed by laboratory staff under the guidance of Nacho Bravo at the Catalan Institute of Oncology. I visited the Catalan Insitute of Oncology to understand and review the testing procedures.

1 Background

1.1 Human papillomavirus (HPV): infection, natural history and immune response

Over 120 different genotypes of HPV have been described, of which 40 infect human epithelial cells lining the ano-genital region and orophaynx[1]. A causal relationship has been established between HPV and both adeno and squamous cell cancer of the cervix, and HPV has been implicated in the pathogenesis of other ano-genital and oro-pharyngeal cancers[2–4]. The virus initially infects epithelial basal membrane cells, which are usually accessed through micro-abrasions in the superficial epithelial layer[5]. Once infected, the HPV viral genome is incorporated into that of the basal membrane cell, and HPV avoids the host immune response by replicating in small numbers in these cells without causing cell lysis or viraemia. Active viral replication occurs later, in the superficial epithelial cells of the mucosa which are distant from immune surveillance, and which slough off. This method of host immune response avoidance, combined with replication in the superficial epithelial layers of mucosal tissue, particularly those of the genital tract, allows HPV to be a highly efficient sexually transmitted infection (STI).

HPV genotypes have been classified by the International Agency for Research on Cancers (IARC) into those with clear evidence that they are associated with cancer in humans (HPV types -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58 and -59, referred to as Group 1), genotypes **probably** associated with cancer in humans (HPV68, referred to as Group 2A), genotypes which are **possibly** associated with cancer in humans (HPV types -26, -53, -66, -67, -70, -73, -82, Group 2B), and other genotypes which are sufficiently similar in their viral structure and grouping to those associated with cancer (types -30, -34, -69, - 85 and -97, also called Group 2B)[6]. HPV genotypes with a strong association with cervical cancer are referred to as oncogenic, or high-risk (HR) and in epidemiological studies usually encompass genotypes in Group 1; or Group 1 and 2A; or Group 1, 2A and 2B. HPV genotypes not associated with cervical cancer are termed non-oncogenic or low-risk (LR). LR types include HPV6 and -11 which cause over 90% of cases of genital warts[7]. HPV has been associated with other cancers: cancer of the penis, vulva, vagina, anus and an emerging epidemic of HPV-associated mouth and oropharyngeal cancers[3]. A recent review of cases of invasive oropharyngeal squamous cell carcinomas detected HPV in 72%[4].

The natural history of HPV infection and the development of cervical cancer are illustrated in Figure 1.1. A range of histological abnormalities are seen at the cervix after HR HPV infection, ranging from mild dysplasia (cervical intra-epithelial neoplasia type 1 (CIN-1)), to moderate dysplasia (cervical intra-epithelial neoplasia type 2 (CIN-2)) which can spontaneously resolve,

to severe dysplasia or squamous-cell carcinoma in situ (cervical intra-epithelial neoplasia type 3 (CIN-3)). HPV is also associated with adeno-carcinoma, for which the precursor is adenocarcinoma in situ (Figure 1.1). Although infection with HPV can lead to the development of cervical cancer or genital warts, the majority of HR and LR HPV genotype-specific infections will be cleared[8]. The reported median duration of HPV infections with an individual genotype ranges from 8-31 months, and is longer for HR types than LR types[9–11].

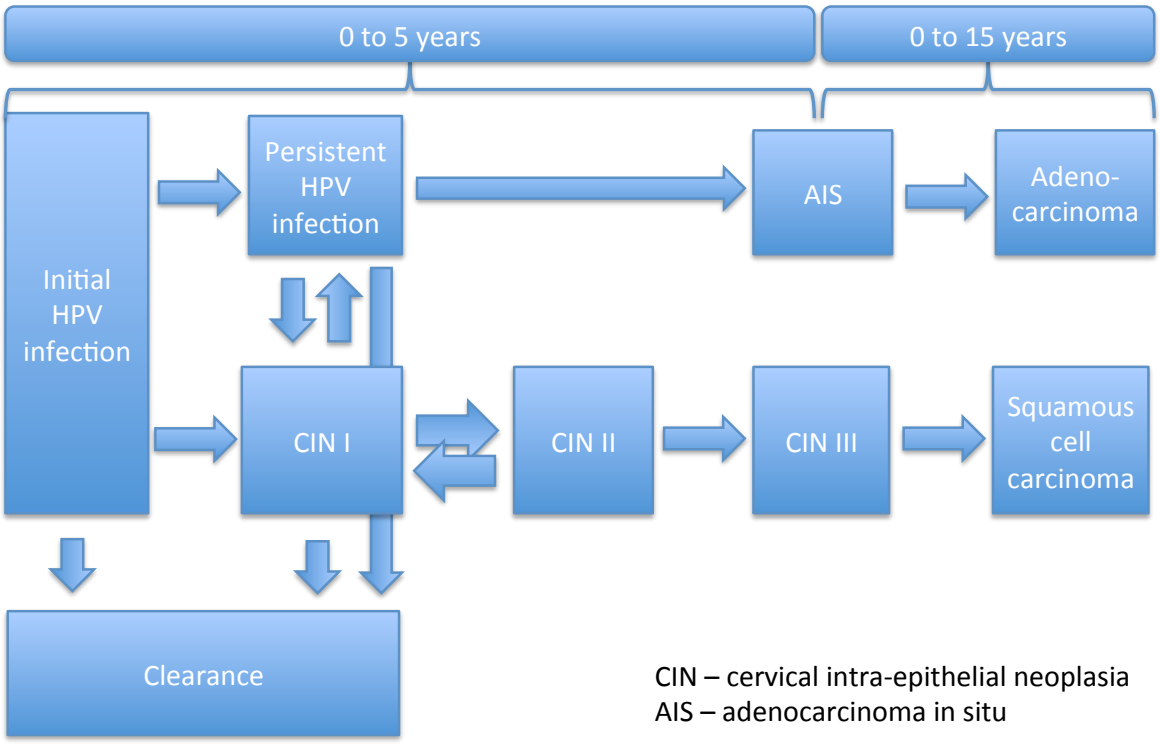

Figure 1:1. Cervical HPV infection natural history

Adapted from[12,13].

Longer duration of detection of HPV infection at the cervix, termed persistence, is associated with the development of pre-cancerous and cancerous changes[14]. Persistent detection of HPV has been defined in varying ways in epidemiological and HPV vaccine studies. A systematic review identified that 78% (of 41 studies) described persistent infections as those in which HPV genotype specific DNA was detected at two or more time points[14]. Persistent HPV has been used as an end-point in HPV vaccine studies because of its association with malignant transformation at the cervix (CIN-3 or greater)[14,15]. The use of cervical cancer as an end-point for HPV vaccination would be un-ethical and un-feasible because of the time required (Figure 1.1). Defining persistent HPV as the detection of the same HPV genotype at two consecutive time points appears straightforward. However, the frequency of testing is variable

in studies with the median time between sample collection ranging from 2 months to 6 years[14,15]. Thus an infection classified in one study as persistent, could be classified as transient in another if, for example, a long testing interval is employed and an infection clears and is then re-acquired, or is latent and then reactivates. A number of HPV vaccine trials have used end-points of both 6-month and 12-month persistence [16,17]. The IARC recently clarified this for use in vaccine studies by recommending a definition of persistence of cervical HPV as detection of the same genotype in two samples taken at least 6 months apart[12].

Clearance of HPV infection predominantly occurs through cell-mediated immune responses whereby cytotoxic CD8, and to a lesser extent CD4 T-cells, destroy HPV-infected cells[18]. Antibody-mediated responses, which are genotype-specific, are detectable in less than 60% of women who have evidence of incident cervical HPV DNA[19,20]. There are conflicting data on whether re-infection with the same genotype in women with genotype-specific antibodies is possible. No protection from incident HPV infection was shown in a large population-based cohort study of older women in Costa Rica who had antibodies to HPV16, -18 or -31[21]. However, young women in the USA who had antibodies to HPV16 detectable for at least one year had a lower incidence of genital HPV16[22]. A possible explanation for these conflicting results is that effective antibody responses reduce with age, allowing acquisition of a new infection. An alternative explanation is that HPV can behave as a latent infection (i.e. remain dormant in basal membrane cells without actively replicating) and can begin replication as immune-mediated protection wanes with age[23,24].

The state of an individual's immune system has a strong influence on the outcome of genital HPV infection. Human immunodeficiency virus (HIV) infection leads to increased prevalence of HPV, duration of cervical HPV detection, and higher rates of disease progression to HPV-related pre-cancerous lesions, and invasive cervical cancer[25]. The level of immunosuppression, estimated by the level of CD4 cells, is inversely correlated with persistence of HPV and the risk of development of cervical pre-cancerous lesions[25,26]. The prevalence and incidence of genital warts are also increased in the presence of HIV infection, with reduced rates of regression[27,28]. However, a recent study has demonstrated that treatment with anti-retroviral therapy does not lead to regression of HPV-associated cervical lesions[29]. Other immunosuppressive states, such as systemic lupus erythematosis and being the recipient of a solid organ transplant, have been shown to lead to increased prevalence of HPV infection and its consequences, including invasive cervical cancer[30].

World-wide, cervical cancer is the second most common cancer in women, and the second most common cause of cancer related mortality[31]. Over 85% of the world's cervical cancer deaths

occur in developing countries and the highest cervical cancer incidence and mortality occur in the East African region where these rates are over twice as high as the world average (east Africa 42.7/100,000person years (pys), world 14.0/100,000pys)[31].

1.2 HPV epidemiology in sub-Saharan Africa

Systematic reviews have summarised published data on the prevalence of HPV in women with normal cytology in different world-regions suggest and found that the highest prevalences are seen in Africa[24,32]. Within Africa, countries in East Africa have the highest reported prevalences[32,33]. However, in these world-wide summaries, women in Africa are less well represented than women in America or Europe: in one review, 8,568 women in Africa contributed data, compared to 692,964 from America, or 229,628 from Europe[32]. This review attempted to take into account the age of included women, the type of HPV test used, the type of population sampled, and the year of the study, and suggested that the prevalence of HPV in East Africa in women with normal cytology was 33.6% (95% CI:30.2-37.1), almost triple the overall world prevalence of 11.7% (95% CI:11.6-11.7). It is unclear why the overall HPV prevalence was found to be higher in the East African region compared to other regions in SSA which have higher HIV prevalences and a similar lack of national HPV vaccination campaigns[34]. Possible explanations include differing sexual behaviour[35], viral or host genetics[36,37]. HPV genotype distribution has been examined in women randomly selected from the general population[38]. In women with normal cytology, HPV16 was the most common type detected, followed by HPV42, -58, -31 and HPV18. In SSA, HPV positive women were significantly less likely to be infected with HPV16 than HPV positive women from Europe and South America; however HPV16 remained the most common genotype detected[38].

Few studies have described HPV incidence, clearance and persistence in SSA. HPV incidence has been reported in studies which followed HIV negative women annually for 12-24 months, and reported incidences of HPV ranging from 28.7 per 100pys in women in Uganda with median age 37 years[39], to 76.0 per 100pys in women with a median age of 18 years in Tanzania[40]. These are higher than reported incidence rates from Columbia of 6.2 per 100pys in women with a median age 32.3 years[41] and 2.5 per 100pys in women with a mean age of 24.6 years in the USA[42]. Incidence rates are difficult to compare between studies, since the number of genotypes tested for, sensitivity of the test used, frequency of follow-up visits, and statistical methods for incidence analysis vary.

The proportion of genotype-specific infections which have cleared varies between studies in SSA, and with other studies in different world regions. In Uganda, HPV clearance (defined as lack of detection of all genotypes) was 31% after a median follow up time of 18.5 months[43]. In

Tanzania, 64% of genotype specific infections had cleared (not detected at one follow-up visit) at 12 months[40]. This was similar to a study in women in women in Brazil in which clearance was similarly defined: 65% of women had cleared infections at 12 months[9]. In studies from SSA which have examined clearance separately for HR and LR HPV genotypes, the proportion of HR HPV infections cleared was less than that of LR HPV infections[39,40,43], consistent with studies in the USA, Canada, South America and Europe[9,44–46]. Again, studies examining clearance are challenging to compare since the duration of follow-up varies, as does the definition of clearance, which varies from requiring one negative test (for the same genotype, or all genotypes), to requiring 2 or 3 negative tests for the same genotype, and finally, whether only new infections are examined or prevalent infections are also included.

1.3 Risk factors for HPV

Clear differences in HPV prevalence are seen when examined by age, with the highest prevalence in women and girls under 25 years old across the world[32,47,48]. The high prevalence in young girls is thought to be due to initial infection after onset of sexual activity in an HPV-naïve host since individuals without previous exposure have no HPV-specific immune response. Further explanations include increased cervical ectopy in the immature cervix (cervical ectopy occurs when columnar epithelium which line the uterus and cervical canal extends to the surface of the cervix), or inadequate levels of protective cervical mucous[49]. In some, but not all, world regions, a second peak of high prevalence is seen in older age-groups[32,47]. Explanations for this second peak in older age are either behavioural i.e. changes in sexual behaviour in later life, or immunological i.e. waning immunological suppression of a latent infection or increased susceptibility to a new infection[23,50]. Risk factors for prevalent HPV appear similar across geographical regions and are predominantly related to sexual behaviour and partner characteristics. These include lifetime number of sexual partners, older age of sexual partner, having a sexual partner who has other partners[51–56] and being HIV-infected[57–59]. Other risk factors include cigarette smoking[52,60,61], and an inverse relationship with parity is seen[52]. A recent cross-sectional study in women in the USA found that cleansing the vagina, called 'douching', was associated with prevalent HPV infection[56].

Cohort studies find similar risk factors for HPV to those identified in cross-sectional studies. Incident HPV has been strongly and consistently associated with lifetime and recent number of sex partners[41,42,45,63–66] and HIV infection[29,67]. Additionally incident HPV infection is associated with inconsistent condom use[68], herpes simplex virus[66], pregnancy[41]. Hormonal contraception has shown inconsistent results; it been associated with increased risk of incident HPV in some studies[63,65], and lower risk in others[66]. Finally, a study in Uganda

demonstrated that having a male partner who was circumcised compared to an uncircumcised partner was clearly associated with a lower incidence of cervical HPV infection in women[39].

1.4 Collecting risk factor data for HPV research

In order to establish which behaviours and practices are risk factors for cervical HPV acquisition, sexual behaviour and vaginal practice information must be collected along with HPV DNA samples. The validity of research into risk factors for STIs relies heavily on the accuracy of the sexual behaviour data gathered. Certain populations pose a challenge for accurate sexual behaviour data collection. Women and adolescent girls are strongly influenced by social norms and expectations around sexual behaviour[69]. In populations such as those in SSA where there is a substantial burden of HPV infection, several studies have illustrated the inaccuracy of disclosure during FtF-interviews of previous sexual intercourse when validated against biomarkers of sexual activity[70], coital diaries[71] or direct observation[72].

Alternative methods of data collection which may address the under-reporting of sexual behaviours have been tested in SSA including self-completed questionnaires, FtF-interviews with responses placed in confidential boxes, and interviews using ACASI[73]. The use of ACASI has led to increased disclosure of lower age at first sex, higher number of partners and increased disclosure of sensitive behaviours in studies in the USA, Zimbabwe and Thailand[63-66].

1.5 Cervical cancer screening and HPV vaccination

In most countries, cervical cancer prevention involves screening for cervical cellular changes using the Papanicolaou (PAP) smear or liquid based cytology, in which cervical cells are examined under light microscopy. Screening programmes have proven successful at increasing the detection of early pre-cancerous changes and invasive cervical cancer in high-income countries with national programmes[74]. However, throughout sub-Saharan Africa screening coverage is extremely low at 0.4-20.2%[75]. The development of low-cost tests such as visual inspection of the cervix with acetic acid (VIA) or visual inspection with Lugol's iodine (VILI) have improved access to screening, although these tests are less sensitive and specific than cytological examination[76]. A battery-powered portable HPV detection kit with pre-prepared reagents called *care*HPV, has been developed. This test detects HR HPV and has a sensitivity for the detection of pre-cancerous and cancerous lesions (CIN grade 2 or higher) of 86%, higher than a conventional PAP smear (53%)[77]. HPV screening has demonstrated a significant impact in cervical cancer detection. A large randomised trial in the Nethrelands (POBASCAM) demonstrated that cervical HPV DNA testing detected an additional 79 pre-cancers per 100,000 women and 30 cancers per 100,000 women compared to standard cytology alone[78]. In rural

India, a similar trial of a single round of cervical cancer screening by HPV DNA testing led to a significant reduction in incidence of cervical cancer and cancer-related mortality during follow-up compared to VIA or cytological testing[79]. The IARC now recommend that screening in low-income setting settings should be based on either VIA or HPV-DNA testing[80].

Despite these advances in screening methodologies, the infrastructure does not exist in many developing countries to support routine screening and the treatment and follow-up of women with pre-cancerous cervical changes[75]. Thus, the World Health Organization (WHO) recommends an integrated programme for cervical cancer control in low and middle-income countries, combining HPV vaccination and screening [81].

There are two currently available HPV vaccines; the bivalent vaccine (Cervarix®) which protects against infection with HPV16 and -18, and the quadrivalent HPV vaccine (Gardasil®) which protects against HPV6, -11, -16 and -18. Large randomised controlled trials have indicated that both vaccines offer more than 95% protection against pre-cancerous cervical lesions (CIN grade 2 or greater), caused by vaccine HPV genotypes[82–84]. Long-term follow-up has demonstrated protection up to 8 years for both vaccines[85,86]. These vaccines are effective if administered prior to the acquisition of vaccine-related HPV genotypes[82,87]. The HPV vaccine has shown only 50% efficacy against incident infection with vaccine genotypes in those with serological evidence of previous infection[88]. The lack of effective protection in some individuals who were seropositive pre-vaccination is possibly explained by latent infection. In viral latency the virus persists in tissue, sometimes at a level below the detection threshold of tests. It has been shown that, in those with detectable HPV vaccine genotype DNA at the time of vaccination, the vaccine has no effect on viral clearance (lack of detection at 6 or 12 months after initial detection)[89].

Since there is clear evidence from high-income countries that HPV vaccination is less effective after HPV has been acquired, and further evidence from high-income countries that HPV prevalence is very low prior to first sex, HPV vaccination administration is recommended before first sex in these countries. There are few data on the prevalence of HPV before reported sexual debut in SSA and on how quickly HPV is acquired once sexual debut is passed.

1.6 Research aims and objectives

1.6.1 Study rationale

Given the high rate of cervical cancer in East Africa, it is essential to understand the HPV genotype-specific prevalence, incidence and risk factors for acquisition of HPV in women in this region. Most studies examining risk factors in East Africa have been cross-sectional[57,90–93].

Detailed examination of HPV incidence and sexual behaviours (including non-penetrative sex behaviours) and vaginal practices before and after sexual debut will help to clarify risk factors for HPV and provide data on how quickly HPV is acquired after initial sexual intercourse.

This study will also clarify whether HPV infection is detected prior to reported sexual debut in Tanzanian girls. This is important since the HPV vaccine is being primarily targeted at pre-adolescent and adolescent girls who are sexually naïve[81] and age for vaccination is based on the assumption that most girls will be HPV negative prior to sexual debut. Studies in the USA and Canada have demonstrated that a low proportion (0-1.7%) of girls have detectable vaginal HPV before first sex[61,94]. However similar evidence is not available for high HPV prevalence regions in Africa. Similarly, it is assumed that the natural history of HPV, in terms of initial acquisition, clearance and persistence of the virus, is the same in sub-Saharan African adolescent girls as it is in girls in high-income countries. This may not be the case; a higher proportion of girls may acquire HPV before reported sexual debut and/or a higher rate of persistent HPV infections in this population may partially explain the higher rate of cervical cancer (in addition to lack of screening services), since persistent infection is associated with malignant change at the cervix[95]. HIV infection has a significant effect on HPV incidence and duration of infection, as well as risk of progression to pre-cancerous and cancerous cervical changes[25,26]. However, national data on HIV prevalence in the proposed age group is less than 1%, and therefore HIV is unlikely to influence the HPV incidence and duration estimates in the study population[34]. Differences in the epidemiology and natural history of HPV in adolescents in sub-Saharan Africa, relative to those in higher income countries from where the majority of natural history data originate, may lead to inaccurate modelling of the impact and cost-effectiveness of screening and prevention programmes. In countries such as Tanzania, where resources are limited, the accuracy of predictive models around the relative benefits of cervical screening programmes versus vaccination programmes is paramount.

Which HPV genotypes girls catch at the time of first sex is also unknown in SSA. Rapid clearance of initial infection often occurs, and since some individuals do not develop antibodies to a specific genotype, it is not possible to know which genotypes girls have previously been infected with[20]. Identifying genotypes acquired early after sexual debut in girls may provide further information on the epidemiology of commonly circulating genotypes in specific populations of young women. Documentation of the genotypes circulating in a population prior to introduction of HPV vaccination will also be important for monitoring post-vaccination genotype-replacement, a WHO research priority for HPV[81]. This phenomenon occurs when vaccination removes a circulating genotype from the population, and that opening is filled by another genotype. Since infection with multiple genotypes is common and occurs irrespective of

genotype involved, it is unlikely that this phenomenon occurs[96]. A follow-up study examining the possibility of type replacement four years after vaccination found no evidence of this in Finland[97]. None the less, careful observation for possible type replacement is necessary since this may lead to the prevention of a smaller proportion of cancers than expected.

Finally, an evaluation of the tools used in sexual behaviour research in adolescent girls in Tanzania is necessary in view of the problems with face-to-face data collection techniques and the emerging technologies aimed at improving them[98].

1.7 Objectives of the study

Primary objectives

The primary objectives of the study were to:

1. Describe the genotype-specific prevalence of HPV at baseline in girls who report not having passed sexual debut, and factors associated with baseline HPV infection.

2. Identify the rate of HPV infection, clearance of genotype-specific HPV, the duration of infection after initiation of sexual activity, and the rate of infection in those who do not report initiation of sexual activity.

3. Evaluate sexual behaviour, vaginal practice and demographic factors associated with the incidence of genotype-specific HPV infection in those who report and do not report the initiation of sexual activity.

4. Identify risk factors associated with HPV genotype-specific clearance.

Secondary objectives

1. A comparison of face-to-face interview with audio computer assisted self-interview (ACASI) for the disclosure of sexual behaviour and intra-vaginal practices.

1.8 Thesis structure

The format of this thesis is that of a 'research paper style' thesis. The thesis consists of manuscripts that have been published, submitted or are ready for submission, alongside background and methods chapters, additional data and explanatory sections, and a concluding chapter. Manuscripts included this thesis are:

- HPV prevalence in adolescent girls in Tanzania before reported sexual debut (published)

- HPV incidence around the time of sexual debut in adolescent girls in Tanzania (ready for submission)
- HPV incidence in girls who report no previous sex in Tanzania (ready for submission)
- Audio-computer assisted self-interview compared to face-to-face interview for the reporting of sexual behaviours in adolescent girls in Tanzania (submitted)

1.9 Ethical approval

The HPV Epidemiology study protocol and consent forms were submitted for ethical approval to Medical Research Coordinating Committee (MRCC) Tanzania, and the London School of Hygiene and Tropical Medicine (LSHTM). Ethical approval was granted on 13th November 2011 by the Ethics Committee of the Medical Research Coordinating Committee (MRCC) in Tanzania (Ref: NIMR/HQ/R.8a/Vol. IX/1249, Annex 1) and on 3rd October 2011 by the London School of Hygiene and Tropical Medicine (LSHTM) (Ref: 6040, Annex 2). Amendments were submitted to MRCC Tanzania and LSHTM on 16th August 2012 and approved on 20th September 2012 by LSHTM and 22nd January 2013 by MRCC Tanzania.

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2 Methods

2.1 Study design

In order to describe HPV genotype-specific prevalence before reported sex, and HPV incidence and duration around the time of sexual debut, a cohort study (the HPV Epidemiology Study) was nested within a cluster-randomised trial of two HPV vaccination strategies in schools: age-based (girls born in 1998) and class-based (girls in primary school class 6)[1]. This trial was called the HPV Vaccine Project, and was conducted in 2010–2011 in rural and urban schools in the Lake Zone of northern Tanzania (Figure 2.1). The aim of the trial was to determine the feasibility, uptake and acceptability of each vaccination strategy. The study area included the two districts of Mwanza City (Ilemella and Nyamangana), which both have urban and peri-urban areas, and the neighbouring rural district of Misungwi. In these three districts in 2010, all private and government primary schools (N=242) were mapped and lists of female pupils born in 1998 or enrolled in class 6 were made. A total of 134 primary schools were randomly selected and allocated to the age-based or class-based vaccination strategy. Three doses of the quadrivalent HPV vaccine were given at 0, 2 and 6 months between August 2010 and June 2011. A total of 4684 of 5532 eligible girls (84.7%) received at least one dose of the vaccine, with three doses received by 76.1% overall[1].

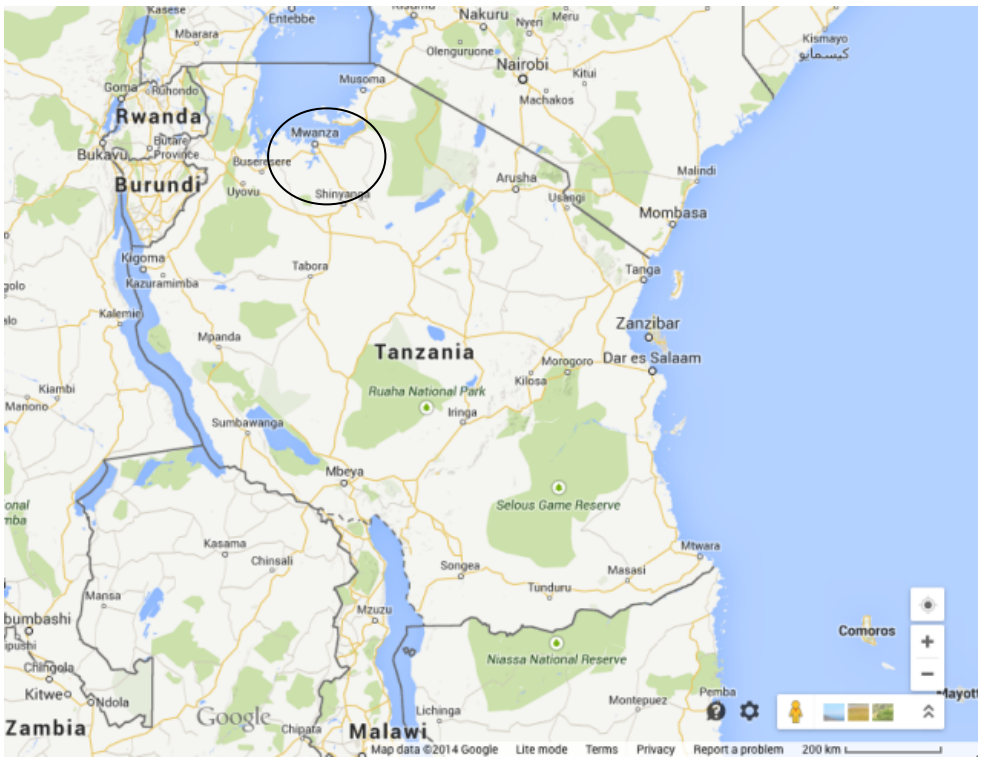


Figure 2:1 Map illustrating Mwanza, Tanzania

The remaining 107 primary schools that were not randomly selected for vaccination included 82 government primary schools, 24 private schools and one specialist school for albino children. The HPV Epidemiology Study was conducted in girls who were attending one of the 82 government schools. The aim was to enrol a random sample of approximately 526 girls, aged 15 and 16 years old, comprising 500 girls who denied having passed sexual debut and an additional 26 girls who reported having passed sexual debut within the last year. Enrolling girls who reported having passed sexual debut within the past year was conducted in order to prevent stigmatization of girls who reported, or who did not report, previous sex. This was done by randomly selecting 26 of the 82 government schools, and enrolling the first girl from that school who reported previous sex, if she reported that the first episode of sex had occurred within the last year.

Eligibility criteria for study enrolment included being aged 15 or 16 years; being enrolled in class (standard) 6 in 2010 in one of the 82 selected schools; self-reporting not being pregnant; planning to stay in the study area for 18 months or able to travel to the study area for study appointments; self-reporting never having had vaginal sex or, if randomly selected, reporting having passed sexual debut within the last year; and being willing to self-administer vaginal swabs.

Study participants were followed every 3 months for a total of 18 months. At each visit, participants underwent a face-to-face interview with one of three trained and experienced study nurses. Interviews covered socio-economic status, demographics, smoking, alcohol and drug use, sexual behaviour (including non-penetrative sex behaviours), female circumcision, having accessed the internet or viewed pornography, and menstrual and vaginal hygiene practices (Annex 3 and 4). At each visit, one nurse-assisted self-administered vaginal swab was collected.

2.2 Ethical issues

Since study participants were under 18 years old at the time of enrolment, consent was required from parents/guardians prior to requesting informed assent from participants. Guardians were defined as the named responsible adult on the school register or the head of household in which a girl resided. Parents/guardians of girls aged 15 or 16 years were identified from school lists and were visited at home by a study nurse, using contact information collected at schools during the HPV Vaccine Project. If the girl was age-eligible, the study was explained to the parent/guardian in Swahili and comprehension was verbally checked, followed by a request for signed informed consent (Annex 5). If the parent/guardian was illiterate, an

impartial literate witness was requested to provide a signature, and the parent/guardian was asked to place a thumb-print on the form. Informed consent included permission for the participant to be offered voluntary counselling and testing (VCT) for HIV at completion of the study, if she had passed sexual debut, without disclosure of results to parent/guardian.

After informed consent had been obtained from the parent/guardian, informed assent was requested from the potential participant. Similar procedures to those mentioned above were employed if the potential participant was illiterate. Assent was requested (Annex 6) in private at home, at a later school visit, or in the research clinic at Sekou Toure Regional Hospital. Assenting participants were assessed for eligibility using a short questionnaire. Inclusion criteria are documented above. Ineligible girls, and girls who reported previous sex who were either not randomly selected for inclusion or who had passed sexual debut longer than 1 year ago did not continue with further study procedures.

Confidentiality of participant's data was maintained to a high standard throughout the study. Each study participant was given an identification (ID) number consisting of 6 letters and numbers which were used on all questionnaires and which did not change during follow-up. Questionnaires were stored in locked filing cabinets in a locked office at the Mwanza Interventional Trials Unit (MITU) and submitted weekly to the MITU data team who signed receipt of them. Data were stored on a secured server. HPV genotyping results, once received from the Catalan Institute of Oncology (ICO), Barcelona, were stored on the same MITU server under the relevant specimen laboratory ID number. The participant's name and address as well as study ID numbers were required by the study team at every visit for that participant in order that they could be located and identified, and the ID number applied to the study questionnaire. The documentation listing the participant's name and address was stored in a separate locked cabinet from the questionnaire until they were submitted to the data team. The study coordinator held lists that matched the participant's name and address, study ID number and lab ID number. Confidentiality agreements were signed by each staff member working on the HPV Epidemiology Study, with intermittent confidentiality spot-checks performed by the MITU statistician (e.g. checking that filing cabinets were locked).

2.3 Study Procedures

Enrolment and follow-up visits took place at the participant's home, at school, at the MITU research clinic in Sekou Toure Regional Hospital in Mwanza city, or at a local health facility depending on the participant's preference, and the study nurses and participant's assessment of the level of privacy available. Follow-up appointments were scheduled every three months from

the date of enrolment. Participants were informed of the date of their next visit when they were seen, since these were pre-determined from enrolment to study completion. Depending on the participant's availability, study nurses made modifications to the appointment date. Participants could be seen from 2 weeks before until 4 weeks after their appointed date, after which time they were assumed to have missed an appointment. Since participants were under 18 years old, financial compensation for attending study appointments was not appropriate. Participants were offered a free health/hygiene-related item at each follow-up visit. These included; soap, toothbrush and toothpaste, cotton underwear, a comb and mirror set, and at the final visit, a tub of petroleum jelly (Vaseline).

2.3.1 Specimen collection

Participants in the study predominantly comprised girls who reported that they had never had vaginal sex. For this reason, a speculum examination was not performed, and nurse-assisted self-administered vaginal swabs were used to collect HPV samples. The procedure was carefully explained (Figure 2.2) and a diagrammatic instruction sheet, with written explanation in Swahili, was used (Annex 7 and 8). A Dacron tipped swab was inserted by the participant 1.5 inches into the vagina and rotated 3 times. The nurse observed the quality of sample collection, and requested that the participant repeat the procedure if it was felt to be inadequate. She would also assist girls who did not know their genital anatomy by showing them where the introitus was.

Figure 2:2. Study nurse explaining how to collect a nurse-assisted self-administered vaginal swab to a study participant (written consent from the participant for this photograph was obtained).

Self-administered vaginal swabs in girls who had not engaged in penetrative vaginal sex had been used in a previous study in the Mwanza region and were found to be acceptable, and provided adequate sampling of vaginal cells[2]. Studies have demonstrated that, when compared to clinician collected cervical specimens, self-administered swabs have similar sensitivity for HPV detection by PCR[3], and have good HPV genotype concordance[4].

Once collected, specimens were placed dry into cryotubes in the field, and transported in cold boxes with ice packs. They were submitted daily to the laboratory at MITU where they were stored at -20^{o}C until they were shipped to ICO, Barcelona, Spain for HPV genotyping.

2.3.2 Laboratory testing

HPV cannot be cultured and therefore accurate detection and identification of the virus depends predominantly on molecular techniques. Molecular techniques for HPV genotyping currently comprise nucleic acid-hybridization assays, signal-amplification assays and nucleic-acid amplification assays.

Nucleic acid-hybridization assays (Southern blot, *in situ* hybridization) have the disadvantage that a large amount of DNA is required in order for detection to be successful. Newer nucleic acid detection kits such as *care*HPV™ (QIAGEN Inc., Gaithersburg, MD, USA) use magnetic beads bound to antibodies which capture specific HPV nucleic acid sequences and use luminescence technology to produce a signal[5]. This test has high sensitivity for pre-cancerous or cancerous changes associated with HPV (CIN 2 or greater), combined with low cost, ease of use and rapidity of result, allowing them to be used in low-income settings for cervical cancer screening[5]. However, these nucleic acid-hybridization assays test only HR HPV genotypes (*care*HPV™) or have a lower sensitivity and involve cumbersome laboratory methods (Southern blot), limiting their use in epidemiological studies[6].

Signal-amplification assays include the *digene*® HPV Genotyping PS test (QIAGEN, Hilden, Germany), and the Cervista® HPV Assay. Although these tests have reasonable sensitivity and specificity for the detection of abnormal cervical cytology (CIN II or greater), they either only detect some of the HR types and are therefore also not appropriate for epidemiological studies where individual genotype results may be required[6,7].

Nucleic-acid amplification assays involve polymerase chain reactions (PCR). During an HPV PCR, oligonucleotide primers which bind to the part of the HPV DNA genome that codes for the L1 capsid protein are usually chosen, since this region is highly conserved[8]. After 30 cycles of amplification, one billion copies of HPV DNA can (theoretically) be generated and detected using

one of the following methods: restriction-fragment length polymorphism; linear probe assay; direct sequencing or genotype-specific primers. Since HPV infection can include multiple genotypes in one specimen[9], false negative HPV PCR results can be seen for a genotype if it is present at a low concentration, since the genotype(s) at higher concentration may be more likely to be amplified, and subsequently detected. The sensitivity and specificity of a genotype-specific HPV PCR assay further depends on the primers used, the reaction conditions (since these influence the DNA polymerase enzyme which is key to the amplification stage) and the presence of inhibitors to the reaction, or contamination with DNA that can lead to a false positive reaction[10]. In spite of this, HPV PCR assays are considered the most sensitive and specific method for HPV DNA detection and genotyping for epidemiological studies[6,11,12].

Several different HPV DNA PCR kits are available and in-house assays can be developed. The Linear Array HPV genotyping assay (Roche, CA) was selected for the HPV Epidemiology study since it detects a high number of HPV genotypes (N=37; HPV6,-11,-16,-18,-26,-31,-33,-35,-39,-40,-42,-45,-51,-52,-53,-54,-55,-56,-58,-59,-61,-62,-64,-66,-67,-68,-69,-70,-71,-72,-73,-81,-82,-83,- 84, IS39, and CP6108) and has a high sensitivity compared to other HPV PCR tests[13]. This test uses primers that amplify a segment of the L1 region of the HPV genome, which is 450 oligonucleotide base pairs long, and simultaneously amplifies a region of the human β-globin gene, which is 268 base pairs long. Amplification of DNA from the human β-globin gene allows validation of the test, and of sampling since detection of the human β-globin gene confirms the presence of human cells. Specimens collected during the HPV Epidemiology study were shipped to ICO in Barcelona, Spain for the Linear Array HPV genotyping assay to be performed, since HPV genotyping had not yet been established in Mwanza, and no other laboratory in Tanzania offered this.

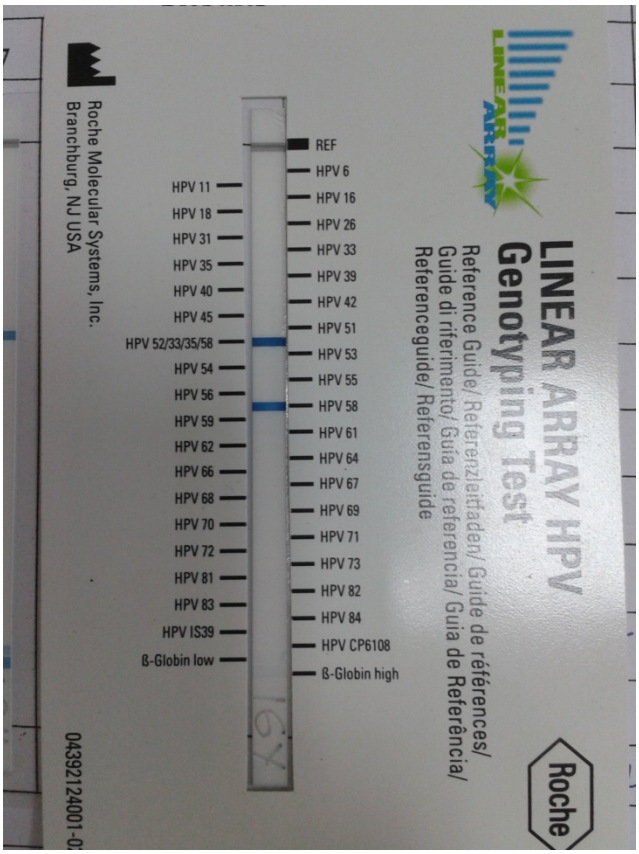
Steps in HPV genotype identification using the Linear Array HPV Genotyping Test include: specimen preparation, polymerase chain reaction (PCR), hybridization of the amplified products to the oligonucleotide probes and finally, detection of the amplified products which are bound to the probes by colorimetric determination. Colorimetric determination (visible bands as in Figure 2.3), permits identification of individual HPV genotypes. A reference guide is placed over these strips so that the reference line (the black line at the top of each strip in Figure 2.3) is in line with the reference line on the guide. One potential issue for interpretation with the Linear Array HPV Genotyping Test is that the strip contains a cross reactive probe that hybridizes with HPV genotypes 33, 35, 52 and 58 together. Table 2.1 summarises how a positive signal from this probe should be interpreted. Interpretation depends on whether this probe (indicated by a line on the strip) is positive alone, or positive in the presence of a positive signal for other probes. When a band is seen at the marker for 52/33/35/58, along with a positive band for HPV33 for

example, it is not possible to rule out co-infection with HPV52 since the sample is interpreted as positive for HPV33 alone. This may lead to under-estimation of HPV52 prevalence.

Table 2:1. Interpretation of cross-reactive probe 52/33/35/58 in the Linear Array HPV genotyping test (Roche, CA)

Band Result	Interpretation
33, 52/33/35/58	HPV 33
35, 52/33/35/58	HPV 35
58, 52/33/35/58	HPV 58
52/33/35/58 (without 33, 35 or 58)	HPV 52

For quality control and to reduce false negatives or false positives, control strips are included. At least one negative and one positive control are included for every 22 specimens processed.



This is an example result from one participant. The top black band is the reference band, blue bands indicate a positive result for one probe, and the corresponding genotype or group of genotypes

Figure 2:3. Linear Array HPV genotyping test

2.3.3 Sexual behaviour questionnaires and pilot testing

Sexual behaviour language, such as casual terms for oral sex, is different in different generations and also evolves over time[14,15]. In order to incorporate colloquial terminology into the sexual behaviour questionnaire, an informal focus group discussion (FGD) was held with similar aged

girls to those eligible for enrolment into the study. This was carried out on the 9th November 2011 in Mwanza City, and was coordinated by one of the study nurses. The purpose and project were explained to a local street leader who then approached girls and invited them to informally participate in the FGD. A total of 14 girls were approached, and all agreed to participate. Girls were aged between 14 and 17 years old. Colloquial terms are summarised in Annex 9 and were included in the enrolment and follow-up questionnaires (Annex 3 and 4).

All study questionnaires (enrolment, follow-up, STI screening and eligibility screening), consent forms and the explanation guide for nurse-assisted self-administered swabs were written in English and translated into Swahili by two study nurses. These were then back-translated into English by non-clinical tracers and data entry staff, who were blinded to the original English version. Modifications were made to the Swahili version and the process was repeated with different staff members. The sexual behaviour questionnaire was further reviewed by the International Language School in Mwanza[16]. An initial pilot study was carried out using only the enrolment questionnaire and study consent forms. Two further pilot studies were carried out to check all study procedures, including the consent process for parents/guardians, eligibility screening of participants, explanation and procedures for collecting and storing nurse-assisted self-administered swabs, submission of dummy samples to the laboratory for logging and storage, and submission of questionnaires and data entry.

2.3.4 Data management

Questionnaires were submitted weekly to the data manager at MITU, and entered by two data entry clerks into OpenClinica LLC (Akaza Research, Waltham, MA). Data checks were performed weekly for consistency (e.g. to check that the reported number of sex partners could not decrease over time), for completeness (e.g. to check that a STI screen was completed for all participants at every visit if they had reported sex at that visit or an earlier visit), and to ensure that numerical data were within expected ranges. Queries were referred to the research team on printed sheets. Corrections were made onto these sheets and directly onto the questionnaires using standardized procedures (e.g. dated and initialed corrections were made in blue ink). These were submitted to the data manager and the checks were re-run until they had been resolved.

2.3.5 Data analysis

Statistical methods are described in each publication in this thesis. Overall, incidence of any HPV was considered to be the detection of a new HPV genotype during follow-up. Since risk of acquisition of another HPV genotype is not thought to be influenced by previous or current infection with a different HPV genotype[17], girls were considered to be continually at risk and

could acquire more than one genotype during follow-up. Incidence and clearance of individual genotypes was calculated. When analyzing results from samples taken every 3 months (in girls who reported sex before or during the study), clearance of a genotype was considered to be two consecutive negative tests, or one negative and one missing sample for that genotype.

For the calculation of the median duration of infection, if a girl tested negative for a genotype between two visits with positive samples for the same genotype, the intervening negative sample was considered a false negative. This is supported by studies that have sequenced HPV genotypes to determine whether intervening negative samples between two positive samples for the same genotypes are due to re-infection or false negative[18,19].

When analyzing results from samples tested every 6 months (for girls who reported never having had sex), clearance of a genotype-specific infection was defined as a single negative sample for that genotype, since we only had 3 follow-up samples (6, 12 and 18 month samples), For duration of infection in the analysis of girls who never reported previous sex, two positive samples with one intervening missing sample (e.g. positive at month-6 and month-18, but with a missing sample at month-12), was considered an infection of unknown duration, and the individual censored at the date of the first positive sample.

Risk factors for prevalent HPV were evaluated using logistic regression. Risk factors for incident HPV were examined using Poisson regression in order that girls could contribute more than one incident HPV genotype at each visit, and random effects were used to account for correlation of events within the individual. Cox regression was used to analyse risk factors for clearance since this allowed the time to clearance to vary by genotype, which is likely[21]. The assumption of proportional hazards (i.e. that the rate in the exposed and unexposed would vary over time but remain proportional to each other) was tested. Risk factors for incident HPV (in those reporting and not reporting sex) and clearance of HPV were evaluated using a hierachical framework analysis with three levels, with age included throughout due to the strong association between age and HPV[21,23]. Socioeconomic status was measured using an asset index, created by combining data on ownership of common household items using principal component analysis. The first level of the hierarchical framework included age-adjusted sociodemographic variables measured at enrolment into the cohort study. These were retained in a core model if associated with HPV prevalence or incidence at $p<0.10$. At the second level, time-varying (measured at each follow-up visit) sociodemographic variables were included and retained if associated at $p<0.10$ and finally, time-varying behavioural factors were included and retained if associated at $p<0.1$. Since sociodemographic factors associated with HPV incidence or clearance were likely to directly or indirectly influence all other groups of risk factors it was logical that these were

included first, and retained if associated, before introducing behavioural risk factors in the model. Sociodemographic variables at baseline were also likely to influence those reported during follow-up and should therefore be included before time-varying sociodmographic factors were introduced. Behavioural factors included intravaginal practices (intravaginal cleansing or insertion), since these have previously been described in the Mwanza region[32] and have been associated with HPV[33]. Menstrual hygiene practices were also included since disposable sanitary pads or tampons are not usually available or affordable to the majority of adolescent girls in the region and the sharing or re-using of cloths or underwear for menstruation might theoretically increase risk of HPV acquisition or duration respectively. Sexual behaviours included the use of lubricants such as saliva during sex since oral HPV in a male partner may be a source of vaginal infection through this mechanism.

2.3.6 Clinical follow up

Prior to the study starting, study nurses were provided with accredited training on National Tanzanian Guidelines for the syndromic management of STI, and on HIV Voluntary Counselling and Testing (VCT), conducted by a regional accredited trainer in both. All participants who reported having had sex were verbally screened for symptoms of reproductive tract infections (RTIs) during the study visits. No testing for RTIs other than HPV were done due to budget constraints. Participants reporting symptoms of possible RTIs were examined by one of the study nurses in a clinic setting, either in the Sekou Toure Hospital research clinic, or one of the district health facilities if more convenient. Free syndromic treatment of RTIs was provided if required following the latest National Tanzanian Guidelines (2007)[22]. The study nurses carried a portable box containing necessary equipment and medications to diagnose and treat RTIs in district clinics. At the final study visit, VCT for HIV was offered to study participants who reported that they had ever had sex. Any participant testing HIV positive was referred to their local HIV Treatment and Care Centre with a referral letter.

HR HPV has a median duration of only 8-9 months[21,23,24], and the majority of HPV infections in women under the age of 25 resolve spontaneously[18,24]. Although early cytological changes often resolve without treatment in young women[25,26], since persistent HR HPV has been strongly associated with cervical cancer and girls in this study population may not be able to get screened later in life, we elected to offer girls with persistent high-risk HPV referral for screening and treatment. Participants with persistent HR HPV diagnosed at their final visit were referred to the regional oncologist at Bugando Medical Centre, a tertiary referral teaching hospital in Mwanza, for VIA and cryotherapy of any visible lesions.

Due to the high cost of testing an individual participant's sample using the Linear Array HPV Genotyping Test, all samples from all follow-up visits from every participant were not tested. Since we did not test samples from all visits for some participants, the definition of which girls required referral to Bugando Medical Centre varied.

All enrolment samples from all participants were tested. Girls who reported that they had had sex prior to enrolment, or during follow-up had all their samples tested (i.e. samples collected at month 3, 6, 9, 12, 15 and 18 of follow-up) (Table 2.2, Group 1). Of the 397 girls who reported never having had sex by their final study visit, 120 were randomly selected, (this was not based on attendance at any visit), and samples collected at the 6, 12 and 18-month visits were tested (Table 2.2, Group 2). Finally, in girls who did not report sex at any time, and who were not randomly selected for 6-monthly sample testing (Table 2.2, Group 3) but who had high-risk HPV at enrolment, the sample from their last attended visit was tested for HPV. Those who had the same HR HPV genotype detected at enrolment and at the end of the study were referred for clinical follow-up. Clinical outcomes from these groups are summarised in Annex 10.

Table 2:2. Criteria for referral for clinical follow up of persistent HPV at study completion

Participant (number)	**Follow-up samples which were tested for HPV**	**Criteria for clinical referral at study completion**
Reported sex either before or during study (106). **Group 1**.	All samples; 3, 6, 9, 12, 15, 18 months	Three consecutive positive samples for the same HR HPV genotype where the final sample was from the final visit attended
Did not report sex either before or during the study but randomly selected (120). **Group 2.**	6 month samples; 6, 12, 18 months	Two consecutive positive samples for the same HR HPV genotype where the final sample was from the final visit attended
Did not report sex either before or during the study and **not** randomly selected (277). **Group 3.**	If the enrolment sample was positive for a HR HPV genotype, the final (attended) visit sample was tested	Sample at final visit was positive for the same HR HPV genotype as enrolment

2.4 Sample size

Based on the age of sexual debut in MEMA kwa Vijana, a community randomised trial of an adolescent sexual and reproductive health intervention in schools in the Mwanza Region, it was estimated that 40% (N=200) of 500 girls aged 15 and 16 years who reported no previous sex at enrolment would pass sexual debut during the 18 months of follow-up[27]. Assuming a 30% cumulative loss-to-follow up, 158 participants were therefore expected to have reported ever having had sex at 18 months. We therefore aimed to enrol 500 girls who reported not having

passed sexual debut, of whom 200 would report sexual debut by 18 months of follow up, and of whom 140 would remain in follow-up. Further, we aimed to enrol 26 who reported sexual debut within the year prior to enrolment, of whom 18 would remain in follow-up at 18 months.

There are no data from sub-Saharan Africa on HPV incidence after initiation of sexual debut. A study in Washington, USA, observed a cumulative incidence of any HPV type of 28.5% during the first year after sexual debut[28]. Assuming a similar incidence, a sample size of 150 participants reporting ever having had sex by 18 months would allow an estimate of the cumulative incidence of any HPV type with a precision of ±7.2%.

Further analysis from the cohort of young women in Washington, USA, identified a cumulative incidence of HPV16 and HPV18 of 10.4% and 4.1% respectively[29]. The sample size of 150 participants reporting sex at 18 months would allow an estimation of these genotype-specific incidences with a precision of ±4.9% and ±3.2% respectively.

Collection of behavioural data was planned in order to investigate risk factors for HPV acquisition. The MEMA kwa Vijana trial found that 15% of girls aged 15-30 years reported a casual partner in the past 12 months, and 10% had more than 1 partner[27]. The proportion using a condom at last sex in the past 12 months was 15%. With a sample size of 150 reporting sex at 18 months, risk factors with a prevalence of 15% would provide 77% power to detect a relative risk of 2.75 or above (Table 2.3).

Table 2:3. Power calculations for the detection of factors associated with HPV among those reporting sex

Significance	Total enrolled	Proportion who reported sex	Number who reported sex	Proportion HPV positive at 18 months	Overall prevalence of risk factor	Relative risk	Prevalence of risk factor in HPV negative	Prevalence of risk factor in HPV positive	Power
More than 1 partner									
0.95	500	0.30	150	0.285	0.10	2.25	0.07	0.17	**0.40**
0.95	500	0.30	150	0.285	0.10	2.50	0.07	0.17	**0.49**
0.95	500	0.30	150	0.285	0.10	2.75	0.07	0.18	**0.58**
0.95	500	0.40	200	0.285	0.10	2.25	0.07	0.17	**0.50**
0.95	500	0.40	200	0.285	0.10	2.50	0.07	0.18	**0.61**
0.95	500	0.40	200	0.285	0.10	2.75	0.07	0.18	**0.70**
Casual partner in past 12 months									
0.95	500	0.30	150	0.285	0.15	2.25	0.11	0.25	**0.57**
0.95	500	0.30	150	0.285	0.15	2.50	0.10	0.26	**0.68**
0.95	500	0.30	150	0.285	0.15	2.75	0.10	0.27	**0.77**
0.95	500	0.40	200	0.285	0.15	2.25	0.11	0.25	**0.70**
0.95	500	0.40	200	0.285	0.15	2.50	0.11	0.26	**0.80**
0.95	500	0.40	200	0.285	0.15	2.75	0.10	0.28	**0.88**
Concurrency									
0.95	500	0.30	150	0.285	0.07	2.25	0.05	0.12	**0.29**
0.95	500	0.30	150	0.285	0.07	2.50	0.05	0.12	**0.36**
0.95	500	0.30	150	0.285	0.07	2.75	0.05	0.13	**0.42**
0.95	500	0.40	200	0.285	0.07	2.25	0.05	0.12	**0.36**
0.95	500	0.40	200	0.285	0.07	2.50	0.05	0.12	**0.45**
0.95	500	0.40	200	0.285	0.07	2.75	0.05	0.13	**0.53**

2.5 Comparision of Audio Computer Assisted Self Interview (ACASI) with face-to-face interviews

In order to assess alternative methods of sexual behaviour data collection, a cross-sectional comparison of different interview methods of sexual behaviour data collection was nested within the cohort study. An amendment to the protocol, including an explanation of this comparison, was submitted to the LSHTM and MRCC ethics boards and was approved on 20th September 2012 by LSHTM (A354), and 22nd January 2013 by MRCC Tanzania (NIMR/HQ/R.6c vol I/241). Further consent was not considered necessary.

Tablet computers were programmed with the assistance of the senior data manager at MITU and allowed participants to listen to audio questions in Swahili, and read them on the screen. The questions were recorded by an interviewer not employed on the study, and were a subset of those included in the standard FtF-interview. A total of 200 participants were randomly selected to be invited to complete an ACASI as well as a FtF-interview, the order of which was also randomly allocated (FtF-interview first or ACASI first). The random selection of participants for this comparison was drawn from those who had attended the 3-month visit, and who were resident in one of the two districts in Mwanza city (Nyamangana and Ilemela) at that visit. Participants resident in the district of Misungwi, or other districts but were travelling for appointments, were not included for logistical reasons. At the 12-month visit, randomly selected participants were invited to complete an ACASI if they were currently (at the 12 month visit) resident in one of the two Mwanza city districts.

Answers provided by the participant using the tablet computer were directly compared to those obtained by traditional interview. An initial set of 8 test-questions were also included to help the participant practice using the tablet computer and which were used as a final competence check at analysis. These 8 training questions, delivered before the sexual behaviour questions, covered non-sensitive topics, including ever having made a trip to the market, the number of people living in the house, and the person the respondent last spoke to before the ACASI. A non-clinical research assistant was trained to assist the participant with this interview. The participant could choose to repeat the 8 questions any number of times before commencing the rest of the interview. Questions included in ACASI are summarised in Annex 11. An example of a study participant completing an ACASI is shown in Figure 2.4.

Figure 2:4. A study participant completing an ACASI (written consent from the participant for this photograph was obtained)

2.5.1 ACASI sample size calculations

A randomized trial of ACASI in 15-23 year-olds in Zimbabwe, showed that 4%, 6% and 6% more participants using ACASI reported having had sex, had kissed and not having used a condom at last sex compared to those who reported these using informal confidential voting interviews[30].

A sample size of 200 girls undergoing two interviews (ACASI and FtF-interview) would allow >85% power to detect an increase of 5% in the disclosure of sexual debut (Table 2.5). These calculations are based on methods for matched case control studies[31] which examine discordant pairs. In this power calculation assumption, 1% report "No" to an answer on sexual behaviour in ACASI but "Yes" in the FtF-interviews and 6% report "Yes" in ACASI and "No" in the FtF-interviews.

Table 2:4. Power calculations for the comparison of disclosure in ACASI and face to face interview

Prevalence in face to face interviews	Prevalence in ACASI	% yes on both	Discordant		Associated OR[1]	Power		
			% yes in ACASI / no in FTF	% no in ACASI / yes in FTF		N=150	N=200	N=250
5%	7.5%	5.0%	2.5%	0	-	**27%**	**>99%**	**>99%**
5%	7.5%	4.9%	2.6%	0.1%	26.0	**40%**	**69%**	**88%**
5%	7.5%	4.5%	3.0%	0.5%	6.0	**32%**	**46%**	**59%**
5%	7.5%	4.0%	3.5%	1.0%	3.5	**27%**	**36%**	**45%**
10%	15%	10%	5%	0	-	**>99%**	**>99%**	**>99%**
10%	15%	9%	6%	1%	6.0	**69%**	**85%**	**93%**
10%	15%	8%	7%	2%	3.5	**54%**	**68%**	**79%**
10%	15%	7%	8%	3%	2.7	**45%**	**58%**	**68%**
20%	25%	20%	5%	0	-	**>99%**	**>99%**	**>99%**
20%	25%	19%	6%	1%	6.0	**69%**	**85%**	**93%**
20%	25%	17%	8%	3%	2.7	**45%**	**58%**	**68%**
20%	25%	15%	10%	5%	2.0	**34%**	**44%**	**53%**

2.6 References

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3 Manuscript 1: Prevalence of human papillomavirus in adolescent girls before reported sexual debut

3.1 Preamble

This manuscript describes the genotype-specific and overall prevalence of HPV at baseline in girls who report not having passed sexual debut, and factors associated with baseline HPV infection to address objective 1 of the research.

3.2 Cover sheet: manuscript 1

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I was involved in conceptualising the study. I wrote the data collection tools and coordinated the study, carried out the analysis, wrote the first draft of the manuscript and finalised it for publication.

NAME IN FULL (Block Capitals) CATHERINE F HOULIHAN

STUDENT ID NO: 280304

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3.4 Manuscript 1: Prevalence of human papillomavirus in adolescent girls before reported sexual debut

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Abstract

Background

Human papillomavirus (HPV) vaccines are recommended for girls prior to sexual debut since they are most effective if administered before girls acquire HPV. Little research has been done on HPV prevalence in girls who report not having passed sexual debut in high HPV-prevalence countries.

Methods

Using attendance registers of randomly-selected primary schools in the Mwanza region of Tanzania, we enrolled girls aged 15-16 years who reported not having passed sexual debut. A face-to-face interview on sexual behaviour and intra-vaginal practices, and a nurse-assisted self-administered vaginal swab were performed. Swabs were tested for 13 high-risk and 24 low-risk HPV genotypes.

Results

HPV was detected in 40/474 (8.4%;95%CI:5.9-11.0) girls. Ten different high-risk and 21 different low-risk genotypes were detected. High-risk genotypes were detected in 5.3% (95%CI:3.5-7.8). In multivariable analysis, only intra-vaginal cleansing (practiced by 20.9%) was associated with HPV detection (aOR=2.19,95%CI:1.09–4.39).

Conclusion

This cohort of adolescent Tanzanian girls had a high HPV prevalence prior to self-reported sexual debut, and this was associated with intravaginal cleansing. This most likely reflects under-reporting of sexual activity, and it is possible that intravaginal cleansing is a marker for unreported sexual debut or non-penetrative sexual behaviours.

Key words: Human papillomavirus, sub-Saharan Africa, sexual debut, prevalence

Introduction

Cervical cancer is the most common form of cancer in women in sub-Saharan Africa and the highest age-standardized incidence of cervical cancer in the world is found in East Africa at over 30.0 per 100,000 person-years(1). This is compared to approximately 15.0 per 100,000 women worldwide, and 6.0 per 100,000 in North America(1). Almost all cases of cervical cancer can be attributed to infection with one of 13 high-risk oncogenic genotypes of the human papillomavirus (HPV)(2). Although limited in number, studies in women indicate that East Africa also has one of the highest global prevalences of HPV infection(3,4). Worldwide data consistently show that the prevalence of HPV is highest in younger women, most of whom will clear the infection within 10 months(5). The early peak of HPV prevalence by age is explained by a rapid acquisition of HPV around the time of first sex in a previously unexposed, or immune-naïve individual(6,7).

The two available HPV vaccines, Gardasil® (MSD) and Cervarix® (GlaxoSmithKline Biologicals), cover the two most common cancer-causing HPV genotypes HPV-16 and -18. Additionally, Gardasil® covers HPV-6 and -11, which, though low-risk for cervical cancer, are common causes of genital warts. HPV vaccination is most effective if administered prior to acquiring infection with these vaccine-related genotypes(8). Data from North America and Europe support the assumption that most girls and women are HPV naïve prior to first sex and that HPV is acquired quickly after sexual debut and with changes of sexual partner(7,9). However, there are no data on HPV prevalence in young women prior to reported sexual debut from high HPV prevalence countries in sub-Saharan Africa, where sexual behaviour, vaginal hygiene practices and rates of under-reporting of sexual debut may differ. Such data are important because they may identify modifiable risk factors for HPV infection, and because any planned national vaccination campaign will target young girls who are assumed to be HPV naïve. Further, WHO has recommended vaccination catch-up campaigns in older girls if a significant proportion can be assumed to be naïve to vaccine genotypes(10).

To address the gaps in knowledge, we present baseline cross-sectional results from a cohort study in Tanzania that enrolled adolescent girls who reported that they had not passed sexual debut, and who were tested for vaginal HPV DNA. This study is the first to describe HPV prevalence in girls in sub-Saharan Africa who report no previous sexual intercourse, and the associations between reported sexual behaviours, vaginal practices and HPV infection.

Methods

Cohort enrolment

The cohort was enrolled between January and August 2012 from previously-prepared attendance lists of randomly-selected government primary schools in 3 districts of the Mwanza Region in Tanzania. These lists, covering all primary schools the districts, had been drawn up in 2010 to prepare for a trial of delivery methods for HPV vaccine(11). The lists contained the pupil's name, date of birth and which class they were enrolled in. Tracing information had also been collected on all girls. We enrolled eligible girls who had attended one of the 82 schools not selected to receive the vaccine.

Eligibility criteria included being aged 15 or 16 years at enrolment; enrolled in class 6 in 2010 in one of the selected schools; not pregnant (self-reported); planning to stay in the study area or able to travel to appointments; self-reporting never having had vaginal sex; and being willing to self-administer vaginal swabs. A sub-sample of 26 of the 82 schools were randomly selected and, in order to prevent stigmatization of girls who reported sex, at each of these 26 schools the first girl who reported ever having had sex was enrolled. An additional eligibility requirement for these girls was having passed sexual debut within the past 12 months.

Ethical Issues

The London School of Hygiene and Tropical Medicine Ethics Committee and the Medical Research Coordinating Committee, Tanzania approved the study protocol. Since all potential participants were considered minors in Tanzania (under 18 years old), written informed consent was required from a parent/guardian with subsequent participant informed assent. Consent for enrolment was taken before any assessment of previous sexual debut. The consent procedure involved separate face-to-face explanations of the study to parents and daughters by a study nurse, provision of written information and time to ask questions. Individuals unable to read were consented in the presence of an independent witness who provided an additional signature. Individuals unable to write provided a thumbprint. Parents/guardians were compensated for their time with Tsh5,000 (approximately $3), and participants were provided with a toothbrush and toothpaste.

Study procedures

After enrolment, girls had a face-to-face interview with a female study nurse. Interviews were carried out in private at the participant's home, school or local health centre, depending on participant preference. Interviews were carried out in Swahili using a structured paper questionnaire, which had been translated and back-translated from English. Questions covered demographic and socio-economic details, menstrual and vaginal hygiene-practices,

non-penetrative sexual behaviours, and details of previous penetrative-sex frequency and partners. Interviews included colloquial terms for sexual behaviours that had been collected during focus group discussions with similar-aged girls. One self-administered vaginal Dacron swab was obtained after instructions from a study nurse, who remained in the room and provided verbal and positional hand-guidance if necessary. Girls are being followed every 3 months for 18 months. We report results from the enrolment visit.

HPV detection and genotyping

Immediately after collection, swabs were stored dry in cryotubes and placed into cold boxes with ice-packs. They were submitted daily to the reference laboratory in Mwanza and stored at -20^{0}C until they were shipped to the Catalan Institute of Oncology, Barcelona, Spain. HPV detection and genotyping were performed using the Linear Array HPV genotyping assay (Roche, California, USA) which detects 37 HPV genotypes (HPV-6,-11,-16,-18,-26,-31,-33,-35,-39,-40,-42,-45,-51,-52,-53,-54,-55,-56,-58,-59,-61,-62,-64,-66,-67,-68,-69,-70,-71,-72,-73,-81,-82,-83,-84, IS39 and CP6108). For this study, 13 HPV genotypes HPV-16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-68 were classified high-risk (2). Other genotypes were considered low-risk.

Briefly, DNA was extracted from the specimens by silica-gel based methods (AmpliLute Liquid Media Extraction kit, Roche). Extracted material was amplified with PGMY PCR system, and the generated amplicons were detected and typed by reverse-line blot reaction (Linear Array HPV Detection Kit and Genotyping Test, Roche). PCR reaction in this assay is based on a multiplex system, including human b-globin amplification primers. This provides an internal quality control for sample material. Specimens consistently negative for b-globin amplification were excluded since it was assumed that vaginal sampling was unsuccessful. All protocols were performed according to manufacturer's instructions: each step was performed in separate rooms and negative controls were used.

Data management and statistical methods

Questionnaire data were double-entered into OpenClinica LLC (Akaza Research, MA, USA), and analysed using STATA V12.0 (StataCorp LP, Texas, USA). Baseline cohort characteristics were examined, and the prevalence of any HPV infection, and prevalence of individual HPV genotypes were calculated. Household wealth was estimated using a score based on the number of possessions owned by the head of the household in which the participant currently resided. Median age of menarche in the cohort was calculated using Kaplan-Meier survival methods to account for those who had not reached menarche.

Factors associated with prevalent HPV infection (treated as a binary outcome for any HPV genotype versus none) were identified using logistic regression models. Age was considered an a-priori potential confounder and factors reaching $p<0.1$ after adjustment for age were included in a multivariable model. Vaginal cleansing was investigated as a binary term for ever having cleansed, and as frequency of cleansing categorised as never, ≤2 and ≥3 times per day. All p-values are from likelihood ratio tests.

Results

Of 1,555 potentially-eligible girls on the original school attendance lists, 1,177 (76.5%) were located. From these, 801 (68.1%) were confirmed to be the appropriate age, and 628 (78.4%) consented to be screened for enrolment (Figure 1). Of those screened, 503 (80.1%) were eligible, selected and enrolled, of whom 481 (95.6%) reported not having had sex. Those not enrolled either reported having had sex and were not randomly selected for inclusion (N=102, 16.2%), or said they were planning to move out of the study area (N=23, 3.7%). b-globin was detected in 495 (98.8%) of specimens. We excluded 6 participants from the analysis who provided b-globin negative vaginal swabs, and one who did not provide a swab. We present HPV prevalence and associated factors from the remaining 474 girls who reported never having had sex, and the HPV prevalence in girls who reported having had sex within the past year.

Cohort description

Of the 474 participants who reported never having had sex, 225 were aged 15 (47.5%) and the remainder (52.5%) were 16 years old (Table 3.1). Only 11 (2.3%) reported ever having kissed, and 34 (7.2%) reported allowing a boy to touch their breasts. One girl reported touching a boy's penis, and another reported allowing a boy to touch her vagina. None reported oral or anal sex. The majority had passed menarche (N=376, 79.3%). Median age of menarche in the cohort was 15 years (inter-quartile range 14-15). One-fifth (N=99, 20.9%) reported ever having cleansed inside their vagina; 78.8% (N=78) using fingers and the remainder using a cloth (Table 3.2). Approximately half (53.5%) of those reporting cleansing used soap and water; the other half used water alone. One participant reported inserting a substance, tobacco powder, into her vagina.

HPV DNA prevalence

HPV DNA was detected from vaginal swabs in 40 of 474 girls who reported not having passed sexual debut, giving a prevalence of 8.4% (95% confidence interval(CI) 5.9-11.0). In total, 24 (5.1%, 95%CI 3.1-7.0) girls had a high-risk HPV genotype. Multiple genotype infections were detected in 20 (4.2%, 95%CI 2.4-6.0) girls, comprising 50.0% of those with HPV DNA. The two most commonly detected genotypes were HPV-42 and HPV-58, with a prevalence of 1.9% (N=9) and 1.1% (N=5), respectively (Figure 2). The prevalence of infection with any of the vaccine genotypes HPV-6,-11,-16 or -18 was 1.6% (95%CI 0.5-2.7).

A total of 22 eligible girls who reported having passed sexual debut within the last year were enrolled from 26 randomly-selected schools. HPV DNA was detected in 7 (31.8%, 95%CI 10.7-53.0) of these 22 girls, significantly higher than in those who reported never having had sex ($p<0.01$), and high-risk HPV was detected in 22.7% (95%CI 3.7-41.7, N=7). Though the numbers involved were small, their demographic profile was similar to that of girls who reported no previous sex (data not shown).

Associations with HPV

In univariable analyses of 474 girls who reported never having had sex, there was weak evidence that being age 16 years was associated with HPV detection (OR 1.76 95%CI 0.89-3.47, p=0.10). Only the reporting of ever having practiced intravaginal cleansing was associated with HPV DNA detection in univariable analysis, and this persisted after adjustment for age (adjusted OR(aOR) 2.19, 95%CI 1.09-4.39, p=0.03) (Table 3.1). Furthermore, there was strong evidence of a dose-response relationship between cleansing frequency and HPV DNA detection (per unit increase in cleansing frequency category after adjustment for age, aOR 1.54, 95%CI 1.17-2.03, p=0.002). However, among girls who reported cleansing, there was no evidence of an association of HPV detection with method (fingers versus cloth), or substance used (water alone versus soap)(Table 3.2).

Discussion

In the first study to examine the epidemiology of HPV in girls from sub-Saharan Africa who self-reported never having engaged in vaginal sex, the prevalence of HPV was surprisingly high (8.4%) based on the testing of self-administered vaginal swabs. This is higher than in two previous studies among women who reported no previous sex: one study of 130 Swedish women aged 10-25 years reported a prevalence of 1.5%, and one longitudinal study of American women aged 18-20 years reported that 1.7% of specimens were found to have HPV DNA(7,9). A further three small studies in Sweden, Denmark and Australia found no HPV DNA

in cervical or vaginal specimens from 15, 30 and 55 women respectively, aged between 13 and 41 years, who reported no previous sex(12–14).

Previous studies have confirmed vaginal sex as the predominant method for acquisition of cervical HPV infection in women, as demonstrated by rapid HPV acquisition after reported sexual debut, and a significant increase in the risk of HPV infection with increased number of partners and with high-risk partners(13,15). Lack of disclosure of previous sex is, therefore, the most likely explanation for the relatively high HPV prevalence observed in our study. Previous research in adolescents in different world regions consistently illustrates under-reporting of sexual behaviours(16,17) and studies conducted in the same geographical area of Tanzania as this study have demonstrated under-reporting of sex in girls of similar ages, and in older, sexually-active women(16,18). In young females in this region of Tanzania, in which the median age of reported sexual debut is 16(19), potential consequences of disclosure of sex under the age of 16 include expulsion from school, physical punishment, and social exclusion(20). However, irrespective of reporting errors, finding HPV in specimens collected from girls who report no previous vaginal sex remains important. Vaccine efficacy is highest in those who have not previously been exposed to the vaccine-related HPV genotypes, and age of vaccination is based on the assumption that girls are HPV negative prior to self-reported sexual debut(21). Our data reinforce WHO recommendations that vaccination should be targeted at younger girls(10) and suggest that the vaccination target age should be several years before the median age of self-reported sexual debut in the population.

Alternative explanations for the presence of vaginal HPV DNA in girls and women who report no previous sex include mother-to-child transmission (MTCT) of HPV, non-penetrative sex practices, or transmission via fomites(22). MTCT is the primary mechanism for HPV acquisition in cases of respiratory papillomatosis in children. However, in well-conducted prospective studies, MTCT of genital HPV types was infrequent and most often transient(23,24). Transmission through non-penetrative sex practices such as hand-genital or oro-genital contact has been described in exclusively homosexual women in the USA who report never having had sex with a man (4/21 women, 19%)(25). However, only two participants in our sample reported these practices, and neither had detectable vaginal HPV DNA.

In the age-adjusted analysis in our study, disclosure of ever having practiced intravaginal cleansing was associated with more than doubled odds of prevalent HPV. Furthermore, a positive dose-response relationship was seen with increasing frequency of vaginal cleansing. Commonly, women use intravaginal practices (IVP) to manage menstruation, as part of their

sexual practice (altering vaginal lubrication or tightness), and to improve genital hygiene. IVP includes both cleansing inside the vagina with water, soap or other products, and the insertion of products into the vagina (e.g. pulverized herbs)(26). IVP is a common practice in many parts of sub-Saharan Africa(27), and has been reported in 96% of women working in bars and guesthouses in Tanzania(26). In that population, many women reported initiating IVP at the time of menarche or when they were given instruction about sex or marriage(28), and in our study, 20.9% of girls reported intravaginal cleansing. IVP has been associated with increased risk of HIV acquisition in sex workers in Kenya, and with a doubled risk of acquisition of a new sexually transmitted infection (STI) in a prospective study of adolescent girls in the USA(29,30). With specific relevance to HPV, a cross-sectional study of 312 adolescent girls in the USA found that vaginal cleansing in the past 90 days was associated with a doubling of the odds of cervical HPV infection(31).

HPV has been detected in fingernail and fingertip specimens of young sexually active women in the USA, as well as on toilet seats in several European airports, and on surfaces in a sexual health clinic in the UK(32,33). HPV's viability to cause infection of animal cells after desiccation has been demonstrated in a UK laboratory setting(34). In East Africa, HPV infection in women is highly prevalent(3,35,36) and therefore an adolescent girl who performs intravaginal cleansing may theoretically self-infect with HPV either from her own external or extra-genital sites, or from objects such as cloth, taps or water buckets contaminated from other household members. Acquisition of HPV from household members may be direct, via the insertion of shared cloths, or indirect via contaminated fingers. A similar explanation has been postulated for the presence of vaginal *Trichomonas vaginalis* in Zambian girls aged 13-16 who reported no previous sexual contact(37). Intravaginal cleansing may additionally increase the risk of HPV infection through vaginal mucosal abrasions, allowing HPV to access target basal membrane cells, or through alteration of the vaginal microbiome, which has been associated with increased risk of other viral STIs including HIV(38).

An important and likely explanation for the observed association between intravaginal cleansing and HPV is that intravaginal cleansing may be a marker of unreported sexual debut or non-penetrative sex behaviours. Girls may be more likely to start vaginal cleansing after sexual debut because they believe that this may prevent pregnancy or STIs, relieve STI-related symptoms, or because they have been taught that this practice is appropriate for sexually-active women(27). Increased frequency of cleansing has been associated with higher number of sex acts and/or partners(26), as has HPV acquisition(7), which may explain the dose-response relationship between intravaginal cleansing and HPV infection seen in our study.

Multiple HPV-genotype infections were as common as single genotype infections in this study, consistent with observations in adolescent girls in previous studies in the USA(31,39). More than half of the girls with HPV were found to have a high-risk genotype, also consistent with the literature on sexually active adolescent girls or young women in Denmark, Canada and Tanzania(13,35,40). Of the 40 girls who had HPV, only 6 (15.0%) had one of the four HPV genotypes included the currently-licensed vaccines, and only 4 (10.0%) had one or both of the two high-risk genotypes in the vaccine.

Major strengths of this study include the interview method, in which the questionnaire included local age-specific colloquial terms for sexual practices, and was administered by trained nurses experienced in adolescent sexual behaviour research. In addition, vaginal swabs were self-administered but collection was directly observed by nurses. Self-administered vaginal swabs in a previous local study (median age 17) demonstrated collection of epithelial cells in 233/244(95%) of swabs (41), and 99% of our specimens contained b-globin, indicating successful sampling. Studies have shown a strong correlation for HPV detection between clinician-collected and self-administered vaginal swabs(42,43). Finally, the method used for HPV detection and genotyping, Roche Linear Array, has been proven in large studies to be highly sensitive(44,45).

One of the limitations of this study is that the enrolment list came from primary schools. Although primary school attendance is a legal requirement in Tanzania, and has been reported as 97%(46), it may be lower in rural populations and in upper primary school years (from which this sample was drawn), and may have affected the representativeness of the sample. Similarly, the refusal rate was 22%, which may have also affected sample representativeness, and the small sample size resulted in limited statistical power to detect associations. During screening for study enrolment a higher than expected proportion (80.3%) reported no previous sex. This may reflect under-reporting, and could relate to parental involvement in consent procedures. Biological markers would have allowed us to detect previous sex and mitigated some of the bias from relying on self-report. Unfortunately, currently available markers in vaginal fluids (Y-chromosome, semenogelin, prostate-specific antigen) do not reliably detect previous sex which occurred over 14 days prior to sample collection(47). A serological marker of sexual exposure, such as herpes simplex virus type-2(HSV-2) antibody(48) was not measured because of budget constraints, concerns that drawing a blood sample may have increased refusal rate, and because HSV-2 is not a gold standard for the detection of sexual debut(49). Finally, self-administered vaginal swabs were collected rather than physician-collected endo-cervical specimens. The HPV genotypes detected therefore may

not reflect cervical HPV genotypes(50). However it has been argued that vulvo-vaginal HPV infections may ascend to the cervix(7).

The proposed HPV vaccination programme in Tanzania will target girls in primary school class 4, where the median age is 10 years, and a catch-up campaign in older girls has not thus-far been proposed. This study provides useful evidence for policy makers in relation to the likely effectiveness of such a campaign. Overall, 80.3% of 15-16 year old girls reported no previous sex, 99.2% of whom did not have infection with either of the two high-risk HPV genotypes included in current HPV vaccines (HPV-16/18). The WHO recommend including older girls in catch-up campaigns if a significant proportion of girls are naïve to HPV vaccine types(10): our findings suggest that such a campaign in older girls may be efficacious in preventing infection with these HPV types.

The data we present strongly link prevalent HPV infection with reported intravaginal cleansing. Intravaginal cleansing may be a marker for undisclosed sexual activity in our population but could alternatively be a novel mechanism for the non-sexual transmission of HPV. If this is the case, the identification of this potentially modifiable risk factor would be highly relevant for a country with one of the highest rates of cervical cancer in the world. Further research to confirm and understand the link between intravaginal cleansing and HPV is required and is currently underway in Mwanza.

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Footnotes

Conflicts of interest: DWJ has received research grants from GSK Biologicals for HPV vaccine-related research. SdeS has received occasional travel assistance to attend conferences from Merk, GSK and Qiagen, and has received research grants from Merk and Qiagen. All other authors declare that they have no conflicts of interest.

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Previous presentation: The results from this study have been presented in part at the 28th International Papillomavirus Conference, November 30th to December 6th 2012, San Juan, Peurto Rico. Abstract number 3020.

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Table 3:1 Analysis of factors associated with detection of HPV in adolescent girls who reported never having had sex (n=474)

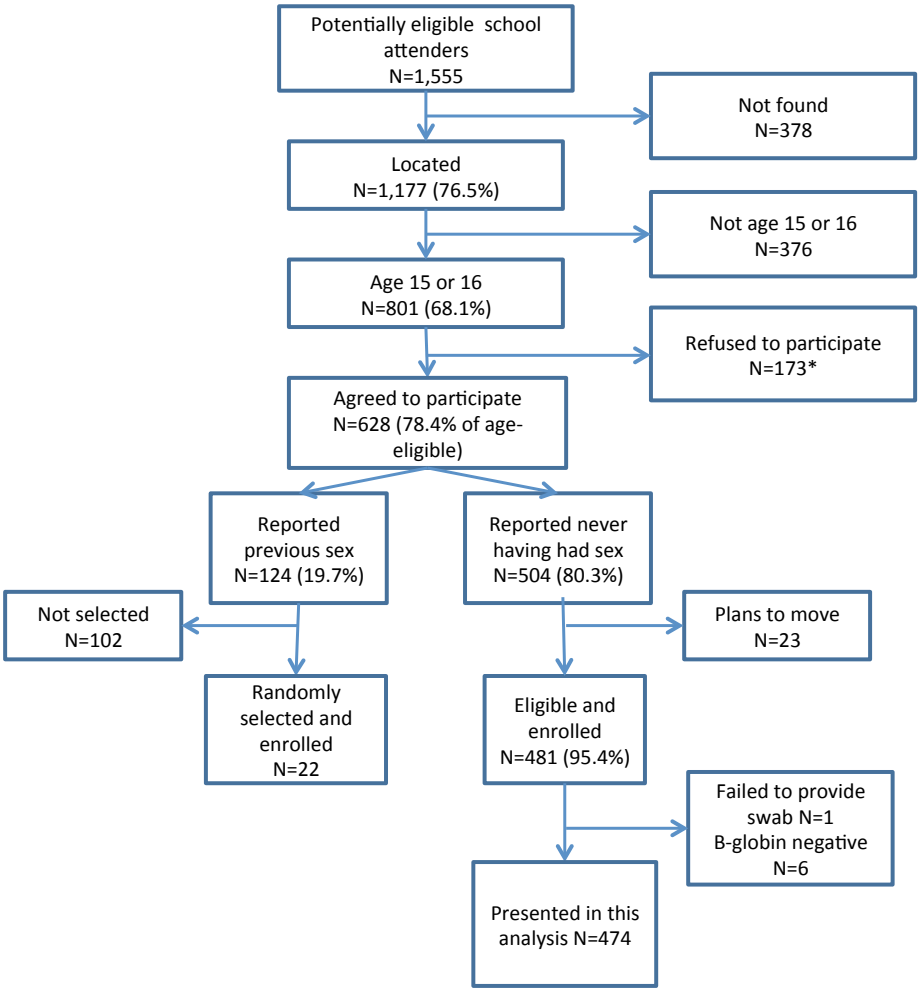
Characteristic		No. (%)	HPV-positive No. (%)	Age-adjusted analysis		Multivariable analysis	
				OR (95% CI) [a]	*p* [b]	aOR (95% CI) [c]	*p* [b]
Overall		474	40 (8.4)				
Socio-demographic							
Age (years)	15	225 (47.5)	14 (6.2)	1	0.10	1	0.10
	16	249 (52.5)	26 (10.4)	1.76 (0.89-3.47)		1.74 (0.88-3.43)	
Current residence	Urban	233 (49.2)	16 (6.9)	1	0.13	1	0.29
	Rural	241 (50.8)	24 (10.0)	1.47 (0.76-2.84)		1.43 (0.74-2.79)	
Lives with	One or both parents	359 (75.7)	31 (8.6)	1	0.23	1	0.63
	Other relatives or friends	115 (24.3)	9 (7.8)	0.84 (0.39-1.84)		0.83 (0.38-1.81)	
	Husband	0		-			
Composite measure of household wealth	High	113 (23.8)	11 (8.3)	1	0.40	1	0.91
	Medium	228 (48.1)	18 (7.9)	0.84 (0.38-1.85)		0.78 (0.35-1.73)	

	Low	133 (28.1)	11 (9.7)	0.91 (0.37-2.19)		0.87 (0.36-2.11)	
Current occupation	Schooling or vocational training	329 (50.4)	25 (7.6)	1	0.15	1	0.33
	Working	6 (0.8)	0	-		-	
	Not working, not schooling	139 (29.3)	15 (10.8)	1.44 (0.73-2.82)		1.40 (0.71-2.77)	
Religion	Christian	417 (88.0)	35 (8.4)	1	0.21	1	0.30
	Muslim	43 (9.1)	3 (7.0)	0.85 (0.25-2.88)		0.76 (0.22-2.63)	
	Other religion	8 (1.7)	2 (25.0)	3.28 (0.63-17.06)		4.15 (0.79-21.96)	
	None	6 (1.3)	0	-		-	
Alcohol, drugs or cigarettes (ever)	No	473 (99.8)	40 (8.5)	-		-	
	Yes	1 (0.2)	0				
Sexual Behaviour							
Kissed, ever	No	463 (97.5)	40 (8.6)	-	-	-	-
	Yes	11 (2.3)	0				
Breast touching, ever	No	440 (92.8)	38 (8.6)	1	0.53	1	0.63
	Yes	34 (7.2)	2 (5.9)	0.62 (0.11-2.72)		0.71 (0.16-3.10)	
Hand-genital contact with a boy, ever	No	472 (99.6)	40 (8.5)	-	-	-	-
	Yes	2 (0.4)	0				

Oral sex, ever	No	474 (100)	40 (8.4)	-	-	-	-
	Yes	0	0				
Menstruation							
Passed menarche	No	98 (20.7)	7 (7.1)	1	0.24	1	0.84
	Yes	376 (79.3)	33 (8.8)	1.73 (0.88-3.43)		1.09 (0.46-2.58)	
Sanitary item used during menstruation [d]	Cloth or paper	171 (36.1)	11 (6.4)	1	0.33	1	0.34
	Underwear	77 (16.2)	8 (10.4)	1.67 (0.64-4.33)		1.69 (0.65-4.41)	
	Sanitary napkin	128 (27.0)	14 (11.0)	1.90 (0.83-4.84)		1.78 (0.77-4.13)	
Intra-vaginal practices							
Ever cleansed	No	375 (79.1)	26 (6.9)	1	0.03	1	0.03
	Yes	99 (20.9)	14 (14.1)	2.19 (1.09-4.39)		2.19 (1.09-4.39)	
Ever inserted	No	473 (99.8)	40 (8.5)	-	-	-	-
	Yes	1 (0.2)	0				

[a] ORs adjusted for age as a-priori confounder. [b] p-value from likelihood ratio (LR) test. [c] ORs adjusted for age and ever cleansed. [d] Of those passed menarche

Figure 3:1 Flow diagram of cohort enrolment



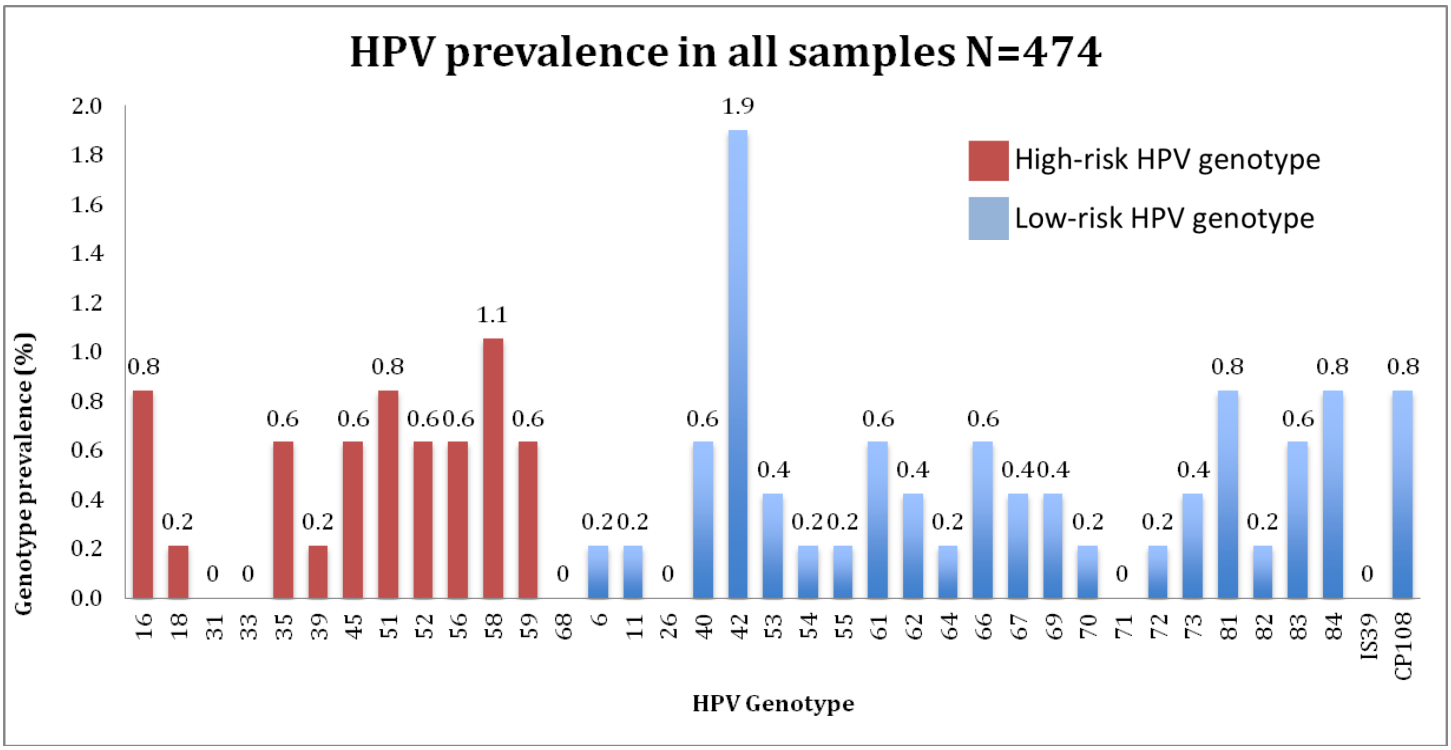
*146 (84.4%) were parents and 27 (15.6%) were girls

Table 3:2. Intravaginal cleansing and HPV prevalence

Characteristic		No. (%)	HPV positive No. (%)	aOR (95% CI)[b]	*p*[a]
Frequency of cleansing	Never	375 (79.1)	26 (6.9)	1	0.01
	≤2 per day	61 (12.9)	5 (8.3)	1.21 (0.44-3.28)	*p trend =0.002*
	≥3 per day	38 (8.0)	9 (23.7)	4.03 (1.72-9.45)	
Cleansed with	Water	46 (46.5)	5 (10.9)	1	0.39
	Soap + water	53 (53.5)	9 (17.0)	1.67 (0.51-5.39)	
Cleansed using	Fingers	78 (78.8)	13 (16.7)	1	0.13
	Cloth	21 (21.2)	1 (4.7)	0.25 (0.03-2.04)	

[a] p-value from likelihood ratio (LR) test. [b] adjusted for age as a-priori confounder.

Figure 3:2. HPV genotypes detected in girls reporting never having had sex (n=474)



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4 Manuscript 2: The incidence of human papillomavirus in Tanzanian adolescent girls before reported sexual debut

4.1 Preamble

This manuscript describes the genotype-specific and overall; rate, duration and clearance of HPV infection in girls before reported sexual debut, and factors associated with the rate of HPV before sexual debut. These address part of objectives 2 and 3 which are:

- Identify the rate of HPV infection, clearance of genotype-specific HPV, the duration of infection after initiation of sexual activity, and the rate of infection in those who do not report initiation of sexual activity.
- Evaluate sexual behaviour, vaginal practice and demographic factors associated with the incidence of genotype-specific HPV infection in those who report and do not report the initiation of sexual activity.

4.2 Coversheet: manuscript 2

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I was involved in conceptualising the study. I wrote the data collection tools and coordinated the study, carried out parts of the statistical analysis, drafted the manuscript and finalised it for publication.

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4.3 Manuscript 2: The incidence of human papillomavirus in Tanzanian adolescent girls before reported sexual debut

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Abstract:

Purpose: Acquisition of human papillomavirus (HPV) in women occurs predominantly through vaginal sex. However, HPV has been detected in girls who report no previous sex. We aimed to determine incidence and risk factors for HPV acquisition in girls who report no previous sex in Tanzania, a country with high HPV prevalence and cervical cancer incidence.

Methods We followed 503 adolescent girls aged 15-16 years in Mwanza, Tanzania with face-to-face interviews and self-administered vaginal swabs every 3 months for 18 months; 397 girls reported no sex before enrolment or during follow-up, of whom 120 were randomly selected. Samples from enrolment, 6, 12 and 18-month visits were tested for 37 HPV genotypes. Incidence, clearance, point prevalence and duration of any HPV and genotype-specific infections were calculated and associated factors evaluated.

Results Of 120 girls who reported no previous sex, 119 were included, contributing 438 samples. HPV was detected in 51(11.6%) samples. The overall incidence of new HPV infections was 29.4/100 person years(pys)(95%CI:15.9-54.2). The point prevalence of vaccine types HPV6,-11,-16 and -18 was 0.9%, 0.9%, 2.0% and 0%, respectively. Spending a night away from home and using the internet were associated with incident HPV, and reporting having seen a pornographic movie was inversely associated with HPV incidence.

Conclusion Incident HPV infections were detected frequently in adolescent girls who reported no previous sex over 18 months. This is likely to reflect under-reporting of sex. A low point prevalence of HPV genotypes in licensed vaccines was seen, indicating that vaccination of these girls might still be effective.

Introduction

Human papillomavirus (HPV) infects the mucosal surfaces of the female and male ano-genital tract[1]. The predominant mechanism of HPV acquisition in women is thought to be through penetrative vaginal sex. Historically, HPV has only detected in a small proportion of girls and women who report no previous sex[2,3], and HPV incidence and prevalence have been shown to increase rapidly in women after reported first sex[4]. Risk factors for incident HPV infection, identified in longitudinal data, include having a higher number of sex partners[5,6]. In addition, HPV genotypes have been shown to be concordant between couples in sexual relationships[7]. High-risk (HR) or oncogenic HPV genotypes have been associated with cervical cancer[8,9]. Vaccination against these HR HPV genotypes is recommended before first sex since the vaccine offers less protection once an HPV genotype has been acquired[10].

Tanzania has one of the highest rates of cervical cancer in the world and, to date, no national HPV vaccination programme[11] although the country is conducting a GAVI supported HPV vaccine demonstration project. In a previous cross-sectional analysis at enrolment of a cohort of adolescent girls in Tanzania, we identified a high prevalence (8.4%) of HPV in girls who reported no previous sex[12]. HPV detection was associated with reporting having cleaned inside the vagina, which may have been a marker for undisclosed sexual activity[13]. To further investigate the detection of vaginal HPV in girls who stated that they had not passed sexual debut, we present results from longitudinal follow-up of these girls in Mwanza, Tanzania. This study is the first to report the incidence of HPV in girls who report no previous sex in sub-Saharan Africa (SSA).

Methods

Study procedures

The cohort was enrolled between January and August 2012, as described previously[12]. Briefly, we enrolled eligible girls attending government primary schools in three districts in the Mwanza region of Tanzania that had been randomly selected in preparation for an HPV vaccine trial[14]. Eligibility criteria included having been in class 6 in 2010 in one of the non-vaccine schools, reporting never having previously had sex, being willing to undergo study procedures, able to attend appointments, and being 15 or 16 years old at the time of enrolment.

During the study, samples were collected every 3 months for 18 months as described previously[12]. At enrolment and each follow-up visit, girls underwent a face-to-face interview in Swahili using a structured questionnaire, which included questions on sexual behaviour and intravaginal practices. Girls were additionally asked to provide one self-administered vaginal swab under the supervision of a trained research nurse. In this paper, we present data from a randomly

selected sub-group of girls who reported never having had sex before the study or during study follow-up.

Ethical Considerations Permissions

The study protocol was approved by the Medical Research Coordinating Committee, Tanzania (Ref: NIMR/HQ/R.8a/Vol. IX/1249), and the London School of Hygiene and Tropical Medicine Ethics Committee (Ref: 6040). Since participants were under the age of legal consent, written informed consent was required from a parent/guardian before written assent was requested from the participant. Girls with persistent HR HPV at study completion (defined as the same HR HPV genotype detected at two consecutive 6-month visits) were referred for follow-up with the national cervical screening programme.

HPV detection and genotyping

Dry vaginal swabs were stored in cryotubes immediately after collection and transported in cold-boxes with ice-packs before submission to the Mwanza Intervention Trials Unit Laboratory in Mwanza, where they were stored at -20^{o}C until they were shipped to the Catalan Institute of Oncology in Barcelona. Samples from enrolment, month-6, month-12 and month-18 visits for girls included in this sub-study were tested using the Linear Array HPV genotyping assay (Roche, California, USA) which detects 13 HR and 24 LR genotypes[15]. We considered HPV genotypes categorised by the International Agency for Research on Cancer (IARC) as associated with cancer (group I) and probably associated with cancer (group IIa) as HR HPV genotypes[16]. All other genotypes (group IIb: possibly associated with cancer, and III, not associated with cancer) were considered LR types. Samples negative for β-globin were classified as inadequate samples for HPV genotyping.

Data management and statistical methods

Data were double-entered into OpenClinica LLC (Akaza Research, Waltham, MA, USA) and analysis performed using STATA V10.3 (Statacorp LP, College Station, Texas, USA.

Reported sexual behaviours at enrolment and over follow-up were tabulated in all girls who reported not having had sex, and in those girls whose samples were tested for the current study. Genotype-specific incidence was calculated using person-time at risk from the date of enrolment. A new (i.e. incident) infection was defined as a first positive test for the specific HPV type among those not infected with that genotype at enrolment. Where there were missed visits or missing HPV results leading to a gap in the observations, girls were censored at the last available result before the gap.

The duration of infection was calculated using person-time from the date of infection to the date of clearance, assumed midway between the last positive and first negative sample for that genotype. Clearance of a genotype-specific infection was defined as a single negative sample for that genotype. Infections that were not cleared were censored at the date of the last positive sample. If there were two positive samples with one intervening missing sample (e.g. positive at month-6 and month-18, but with a missing sample at month-12), duration was considered unknown and censored at the date of the first positive sample.

The overall incidence of all HPV infections (Tables 2 & 3) was calculated as the number of new infections (allowing multiple events at each time point) over person-years at risk, and girls were assumed continuously at risk. Gaps (explained above) where a visit was missed were removed, but girls were assumed at risk again after a gap. The overall incidence rate was estimated using Poisson regression with random effects to account for clustering of infections within the same girl. The HPV genotype-specific point prevalence was estimated as the number of visits where the genotype was detected, divided by the total number of visits, including the enrolment visit

Poisson regression with random effects was used to estimate rate ratios for factors associated with HPV incidence. A conceptual framework was used to examine risk factors, and age was considered an a priori confounder and included in all models. Socio-economic status was determined by an asset index which was created by combining data collected at enrolment from the entire cohort (i.e. including those who did and did not report sex during the study) using principal components analysis.

Results

A total of 1,555 listed school attendees were potentially eligible based on age criteria (Figure 4.1). Of those, 1,177 (75.7%) were located, and 503 met eligibility criteria and were enrolled, 481 (95.4%) of whom reported never having had sex. Of those, 397 (82.5%) reported never having had sex during the study, and at the final study visit confirmed that they had never had sex. We randomly selected 120 (30.2%) of these girls. One girl spontaneously reported that she had been HIV-positive from birth (girls were not explicitly asked this) and was excluded from the analysis. Table 4.1 summarises reported sexual behaviour, cigarette smoking, alcohol consumption and drug use in all 396 girls in the cohort who reported no previous sex, and in the randomly selected 119 girls, who did not report being HIV-positive.

At enrolment, 61 of the 119 (51.3%) girls were aged 16 years, and the remainder were aged 15 years. Approximately half (52.9%) lived in urban areas, 92.4% were Christian, 79.0% were in school, and 19.3% were neither working nor in school. The final visit of the study (18 months) was attended by 90.8% of girls. At this visit, girls were asked if they were circumcised (i.e. had experienced genital cutting), and of the 108 girls who were asked, none reported this. Girls contributed 438 valid specimens.

Overall, 10 of the 119 girls (8.5%) who never reported having had sex had HPV detected at enrolment. The 119 girls contributed a total of 152.8 person-years(pys) of follow-up, during which 44 new HPV infections were detected. The overall incidence of new HPV infections in girls who reported never having had sex was 29.4 per 100pys (95% Confidence Interval (CI):15.9-54.2), and for new HR HPV infections was 11.3/100pys (95%CI:5.8-22.1) (Table 4.3). Infection with at least one HPV genotype was detected at 11.6% of visits, and infection with at least one HR HPV genotype was detected at 6.6% of visits.

The HPV genotype with the highest incidence was HPV66 (2.7/100pys), followed by HPV59 (2.6/100pys), and HPV16,-51,-52 and -6 (2.0/100pys for each) (Table 4.1). The genotypes with the highest point prevalence were HPV16 (2.1%), HPV42 (1.8%), HPV61 (1.6%) (Figure 4.2).

The mean duration of any new HPV infection was 7.0 months; 8.1 for any HR HPV infection, and 6.6 for any LR infection (Table 4.2). The rate of clearance of LR HPV infections was more than three times that of HR HPV infections (103.3 versus 28.8/100pys).

In the unadjusted analysis, there was some evidence of an association between new HPV infections and living in an urban compared to a rural area, having spent a night away from home in the last 6 months and having cleansed inside the vagina in the last 6 months (Table 4.4). In the adjusted analysis, there was strong evidence that having spent a night away from home in the last 6 months (adjusted risk ratio (aRR) 5.47 (95%CI:1.72-17.4.), or having used the internet (aRR 3.90 (95%CI:1.05-14.50) were associated with new HPV infection. There was some evidence that having watched a pornographic movie was associated with a lower risk of HPV acquisition (aRR 0.18 (95%CI:0.03-1.03)). There was weak evidence that having cleansed inside the vagina with soap in the past 6 months, compared to not having cleansed inside the vagina in the last 6 months was associated with a new HPV infection (aRR 2.64 (95%CI:0.75-9.31)).

Discussion

We followed a cohort of 119 adolescent girls who reported never having sex for 18 months in Tanzania, a country with a high HPV prevalence and the world's highest cervical cancer incidence. We found that 11% of the samples provided during this period showed evidence of vaginal HPV infection, which likely reflects under-reporting of sex or, less likely, acquisition of HPV through non-penetrative sex or vaginal cleansing. Studies have compared self-reporting of sex with biomarkers of recent sex[17], compared different methods of interview[18,19], or carried out repeat surveys[20] in East Africa, and have shown that under-reporting of sex is common.

A number of cross-sectional studies have found that cervical or vaginal HPV was detected in 0-1.5% of self-reported virgins in Europe, Australia and USA[2,3,21,22]. Longitudinal studies, which sampled girls who consistently reported no previous sex at multiple time points during follow-up, have detected higher rates of HPV. One study in girls in the USA with a median age of 19 years detected HPV in 7.8% of girls who were followed for 24 months and consistently reported that they had never had sex[4]. Another study in the USA followed 14-17 year old girls every 3 months for a median of 5.2 years, and found HPV in 10 of 22 (45.5%) who reported never having had sex[23]. Both of these studies identified an association between non-penetrative sexual behaviours and HPV detection in girls. Non-penetrative sex practices including hand-genital contact and oral-genital sex have been identified as risk factors for HPV acquisition in heterosexual women[4,24], and in homosexual women who report never having had penile-vaginal sex[25]. In our study population, oral-genital sex is infrequently reported in adolescents[26,27]; oral-genital sex was not reported by any of our participants and hand-genital contact was only reported by 2 participants.

Use of smart phones and access to the internet is increasing in Tanzania [28], and increasing access to pornography may influence sexual attitudes and behaviours[29]. New HPV infection was associated with reporting having accessed the internet, but inversely associated with reporting having seen a pornographic movie. Reasons for the latter observation are unclear and this may be a chance finding. It may be possible that having seen a pornographic movie is associated with risky sex (i.e. without a condom), but is under-reported by those at increased risk. Reporting not having seen pornography may therefore be a marker for risky sex and consequently HPV risk.

Intravaginal cleansing was associated with prevalent HPV in girls who reported never having had sex at the time of enrolment into our cohort[12]. We have suggested that intravaginal cleansing was most likely to have been a marker for un-reported sex, but additionally commented that the practice of intravaginal cleansing, which involves inserting fingers or a cloth inside the vagina, may introduce HPV from external genitalia or fomites. In this current study, there was no strong evidence that recent intravaginal cleansing was associated with new HPV infection during

longitudinal follow-up, but there was weak evidence of an association with reported cleansing with soap compared to not cleansing in the past 6 months. Again, this is most likely to be a marker of unreported sex, since girls may be more likely to perform intravaginal cleansing if they believe this practice is appropriate or desirable before or after sex, or that it is effective in reducing the risk of STI or pregnancy[13]. However, since intravaginal cleansing has been associated with HPV in cross-sectional studies[30] and the acquisition of other STIs in longitudinal studies[31,32], and since HPV has been detected on fingers of women with genital warts and on surfaces in sexual health clinics in the UK[33,34], self-infection remains a theoretical possibility which should be further investigated.

We also observed a strong association between spending a night away from home in the past 6 months and HPV acquisition. Traveling or short time migration have been associated with HIV infection in previous studies in SSA[35,36], but not with HPV. It is likely that spending a night away from home is a marker for unreported sex and therefore HPV risk.

One of the limitations of this study was that we only tested samples from girls every 6 months rather than testing samples from 3-monthly visits and, as a result, it is likely that the incidence of HPV was underestimated since some short-lived infections may have cleared quickly between the 6-month visits. This may have been partially mitigated by using the highly sensitive HPV Roche Linear Array, reducing the risk of false negative tests. A further limitation is the relatively small number of events in the risk factor analysis, which will have resulted in imprecise effect estimates. The major strength of this study was the face-to-face interviews. These included current colloquial terms for sexual behaviours. The interviewers were experienced in sexual behaviour research with adolescents, and attempted to meet the same participant at every visit, establishing a trusting relationship and potentially reducing under-reporting. We nested a study of alternative interview methods within our cohort and found no increase in reporting of sex when comparing audio-computer assisted self-interview. Unfortunately, the interviews did not include questions about masturbation, which has previously been reported by adolescent girls in Tanzania[26], and which could potentially lead to transmission via fingers. Similarly, HPV could be acquired through fomite transmission during female genital cutting via unsterilized equipment or the fingers of the practitioner. Although only 1 participant in our cohort reported having undergone genital cutting this may have been an underestimate. In a study of girls and women aged 15-44 years in a different area of Tanzania, 73% had evidence of genital cutting, and only one-fifth of those with evidence of this on examination had reported it[37].

Our results demonstrate that some Tanzanian adolescent girls who report never having had sex have a relatively high incidence of vaginal HPV. HPV16 and HPV 18 are responsible for over 70% of cervical cancers and currently available HPV vaccines offer a high level of protection from these genotypes[38,39]. In our study, HPV16 was the most frequently detected genotype. However, 98% of these girls had no evidence of HPV16 or -18, suggesting that catch-up vaccination campaigns in girls older than the current target population recommended by WHO (9-13 years old [40]) may be effective at reducing cervical cancer. This may be particularly relevant since the Tanzania vaccination programme has proposed primarily targeting girls in standard (class) 4 if they are in school, or 9-year-old girls if out of school.

Table 4:1. Prevalence of reported behaviours among all girls in the study who did not report sex at enrolment or during follow-up (n=396), and among those randomly selected for the HPV incidence study (n=119)

	Number of girls reporting the behaviour among all (n=396) who did not report sex[1] (%)	Number of girls reporting behaviour among (n=119) who did not report sex[1] and who have HPV results (%)
At enrolment		
Ever drank alcohol		
Yes	1 (0.3%)	0 (0%)
Ever smoked		
Yes	0 (0%)	0 (0%)
Ever took drugs		
Yes	0 (0%)	0 (0%)
Ever cleansed vagina		
Yes	68 (17.2%)	20 (16.8%)
Ever kissed a boy		
Yes	0 (0%)	0 (0%)
Boy ever touched breasts		
Yes	22 (5.6%)	8 (6.7%)
Ever touched boy's genitals or boy touched girl's genitals		
Yes	2 (0.5%)	0 (0%)
Ever had oral sex		
Yes	0 (0%)	0 (0%)
During follow-up[2]		
Spent a night away since last visit		
Yes	272 (68.7%)	86 (72.3%)
Used internet ever[3]		
Yes	95 (24.0%)	35 (29.4%)
Ever watched a pornographic movie[3]		
Yes	62 (15.7%)	17 (14.3%)
Drank alcohol since last visit		
Yes	4 (1.0%)	1 (0.8%)
Practiced vaginal cleansing since last visit		
Yes	141 (35.6%)	42 (35.3%)
Kissed a boy since last visit		
Yes	6 (1.5%)	3 (2.5%)
Boy touched breasts since last visit		
Yes	12 (3.0%)	3 (2.5%)
Touched boy's genitals/boy touched girl's genitals since last visit		
Yes	2 (0.5%)	1 (0.8%)

[1]Girls who did not report sex at enrolment or during the study up to and including the final visit (18 months) [2]Girls were asked at every visit whether they had experienced any of these behaviours since they were last seen in the study [3]Girls were asked during follow-up if they had ever used the internet or seen a pornographic movie, but they were not asked when they had done that. After a girl reported ever having used the internet or having seen a pornographic movie, all subsequent visits are counted as 'yes'.

Table 4:2. HPV genotype prevalence, incidence, duration and clearance among 119 girls who did not report sex at enrolment or during up to 18 months follow-up

HPV type	Prevalent HPV infections (%)[1]	New infections/pys (rate/100 pys) [2]	New infections that were cleared (%)[3]	Number of new infections cleared / pys (rate/100 pys)	Mean (median) months duration (Kaplan-Meier) [4]
High risk genotypes					
HPV16	1 (1%)	3 / 149.7 (2.0)	0	0/ 1.7 (0)	9.2* (†)
HPV18	0	0 / 152.8 (0)	–	–	–
HPV31	0	1 / 152.6 (0.7)	0	0/ 0.3 (0)	‡
HPV33	0	0 / 152.8 (0)	–	–	–
HPV35	1 (1%)	0 / 151.4 (0)	–	–	–
HPV39	0	1 / 152.1 (0.7)	0	0/ 0.7 (0)	‡
HPV45	0	0 / 152.8 (0)	–	–	–
HPV51	1 (1%)	3 / 150.6 (2.0)	2 (67%)	2/ 1.3 (159.0)	5.9 (5.8)
HPV52	1 (1%)	3 / 150.6 (2.0)	0	0/ 0.8 (0)	3.2* (†)
HPV56	1 (1%)	1 / 152.1 (0.7)	0	0/ 0.3 (0)	‡
HPV58	2 (2%)	1 / 151.1 (0.7)	0	0/ 0.2 (0)	‡
HPV59	1 (1%)	4 / 151.3 (2.6)	0	0/ 1.0 (0)	3.1* (†)
HPV68	0	1 / 152.2 (0.7)	0	0/ 0.7 (0)	‡
All HR infections[5]	**8**	**18**	**2 (11%)**	**2 / 6.9 (28.8)**	**8.1* (†)**
Low risk genotypes					
HPV6	0	3 / 151.1 (2.0)	1 (33%)	1/ 1.5 (67.3)	7.5* (6.0)
HPV11	1 (1%)	1 / 150.2 (0.7)	1 (100%)	1/ 0.5 (194.3)	‡
HPV26	0	2 / 151.4 (1.3)	1 (50%)	1/ 0.7 (135.0)	5.8 (5.8)
HPV40	0	0 / 152.8 (0)	–	–	–
HPV42	2 (2%)	1 / 150.1 (0.7)	0	0/ 0.3 (0)	‡
HPV53	0	0 / 152.8 (0)	–	–	–
HPV54	0	0 / 152.8 (0)	–	–	–
HPV55	0	1 / 152.1 (0.7)	0	0/ 0.8 (0)	‡
HPV61	1 (1%)	2 / 150.4 (1.3)	1 (50%)	1/ 0.7 (136.5)	5.8 (5.8)
HPV62	1 (1%)	1 / 150.7 (0.7)	1 (100%)	1/ 0.5 (207.5)	‡
HPV64	0	2 / 151.9 (1.3)	1 (50%)	1/ 0.7 (137.6)	5.8 (5.8)
HPV66	0	4 / 150.9 (2.7)	1 (25%)	1/ 1.7 (58.0)	7.5* (5.8)
HPV67	0	2 / 150.9 (1.3)	2 (100%)	2/ 1.0 (207.5)	5.8 (5.8)
HPV69	0	0 / 152.8 (0)	–	–	–
HPV70	0	1 / 152.6 (0.7)	0	0/ 0.3 (0)	‡
HPV71	0	0 / 152.8 (0)	–	–	–
HPV72	0	0 / 152.8 (0)	–	–	–
HPV73	0	1 / 152.6 (0.7)	0	0/ 0.2 (0)	‡
HPV81	1 (1%)	0 / 151.4 (0)	–	–	–
HPV82	1 (1%)	1 / 152.1 (0.7)	0	0/ 0.3 (0)	‡

HPV83	0	0 / 152.8 (0)	–	–	–
HPV84	1 (1%)	2 / 150.3 (1.3)	1 (50%)	1/ 0.7 (135.3)	6.0 (6.0)
HPV CP-108	0	1 / 152.6 (0.7)	0	0/ 0.2 (0)	‡
HPV IS-39	0	1 / 152.1 (0.7)	1 (100%)	1/ 0.5 (208.1)	‡
All LR infections[5]	**8**	**26**	**11 (42%)**	**11 / 10.6 (103.3)**	**6.6* (5.8)**
All HPV infections	**16**	**44**	**13 (30%)**	**13 / 17.6 (73.9)**	**7.0* (6.0)**

[1]Positive for that genotype at the enrolment visit. [2]New infection defined as first positive test for the specific HPV type, among those not infected at enrolment. [3]Clearance defined as a negative sample for the specific genotype; denominator is total genotype-specific new infections. [4]Mean duration of new infections estimated using Kaplan Meier methods restricted by the longest follow-up time (i.e. duration). *Mean duration of infection for the genotype is underestimated because the individual with the longest observed duration was censored. †Median duration could not be estimated because survival curve does not drop below 50%. ‡One infection only, Kaplan-Meier survival function not estimated. [5]Total number of group (HR or LR)-specific infections among 119 girls.

Table 4:3. Incidence and point prevalence in adolescent girls who did not report sex at enrolment or during 18 months follow-up

Outcome	All girls (N=119) [1]	Negative for all HPV genotypes at enrolment (HPV naïve) (N=109) [2]
Incidence		
	New infections / person-years (rate / 100 person-years, 95% CI) [3]	
All HPV	44 / 152.8 (29.4; 15.9–54.2)	40 / 140.6 (27.9; 14.7–53.0)
All HR HPV	18 / 152.8 (11.3; 5.8–22.1)	18 / 140.6 (12.4; 6.4–24.0)
All LR HPV	26 / 152.8 (18.1; 9.3–35.1)	22 / 140.6 (15.8; 7.9–31.7)
Prevalence		
	Total infections (number of visits with at least one infection / all visits; % of all visits) [4]	
All HPV	87 (51 / 438; 11.6%)	50 (24 / 402; 6.0%)
All HR HPV	38 (29 / 438; 6.6%)	23 (16 / 402; 4.0%)
All LR HPV	49 (36 / 438; 8.2%)	27 (17 / 402; 4.2%)

[1]HPV incidence among all girls who did not report passing sexual debut during the study; includes 10 girls in whom prevalent HPV was detected at enrolment. [2]HPV incidence among girls who did not report passing sexual debut during the study and no HPV was detected at enrolment. [3]Rate estimated from random effects Poisson regression: point estimates and 95% CI take into account correlation of repeated events within girls. Girls assumed to be continually at risk and can acquire >1 infection at each visit. [4]Total number of genotype-specific infections and number of visits where at least one genotype was detected, at all visits including enrolment visit.

Table 4:4. Selected risk factors for any new HPV infection among 119 girls who did not report sex at enrolment or during 18 months follow-up

	Number of infections / person-years (rate/ 100 pys)	Crude RR (95% CI)	Adjusted RR (95% CI)[1]
Sociodemographic (enrolment)			
Age at enrolment		P=0.40	
15 years	29 / 73.9 (36.9)	1	
16 years	15 / 78.9 (21.7)	0.59 (0.17 -1.99)	
Religion		P=0.78	P=0.85
Christian	37 / 141.5 (27.2)	1	1
Muslim	5 / 8.9 (50.0)	1.84 (0.17 - 19.81)	1.70 (0.16 - 18.03)
Other	2 / 2.4 (69.9)	2.57 (0.04 - 176.57)	2.08 (0.03 - 137.78)
SES score (tertiles)[2]		P=0.56	P=0.76
Low	9 / 47.1 (19.3)	1	1
Middle	12 / 52.4 (25.0)	1.30 (0.27 - 6.13)	1.21 (0.24 - 6.02)
High	23 / 53.4 (42.4)	2.20 (0.49 - 9.81)	1.89 (0.32 - 11.12)
Sociodemographic (time varying)			
Current residence		P=0.07	P=0.34
Urban	34 / 84.7 (40.3)	1	1
Rural	10 / 68.1 (15.9)	0.39 (0.14 - 1.10)	0.57 (0.18 - 1.80)
Current occupation		P=0.24	P=0.41
School	1 / 7.8 (7.2)	0.27 (0.02 - 2.89)	0.70 (0.05 - 9.11)
Work/vocational training	28 / 117.5 (27.2)	1	1
Not working	15 / 27.6 (43.9)	1.61 (0.65 - 4.02)	1.83 (0.73 - 4.61)
Spent a night away since last seen		P<0.001	**P<0.001**
No	26 / 112.0 (20.0)	1	**1**
Yes	18 / 40.8 (109.6)	5.47 (1.72 - 17.4)	**5.63 (1.81 - 17.49)**
Behavioural (time varying)			
Current menstrual hygiene		P=0.12	P=0.12
Cloth	10 / 58.6 (22.5)	1	1
Underwear	8 / 21.6 (21.8)	0.97 (0.23 - 4.10)	0.41 (0.07 - 2.58)
Sanitary pad	26 / 60.3 (45.3)	2.02 (0.55 - 7.34)	1.18 (0.25 - 5.64)
Pre menarche	0 / 12.4 (0)	–	–
Used internet[3]		P=0.12	**P=0.03**
No	34 / 132.7 (25.9)	1	**1**
Yes	10 / 20.1 (58.0)	2.24 (0.79 - 6.36)	**3.90 (1.05 -14.50)**
Watched a pornographic movie[3]		P=0.20	**P=0.03**
No	42 / 137.9 (31.6)	1	**1**
Yes	2 / 15.0 (11.5)	0.36 (0.07 - 1.92)	**0.18 (0.03 - 1.03)**
Cleansed vagina in last 6 months[4]		P=0.03	P=0.32
No	24 / 118.4 (21.2)	1	1
Yes	20 / 34.4 (59.2)	2.79 (1.08 - 7.17)	1.79 (0.57 - 5.59)
Substance used for vaginal cleansing		P=0.01	P=0.12

Did not cleanse	30 / 126.8 (25.5)	1	1
Soap or soap and water	14 / 19.1 (103.4)	4.05 (1.30 - 12.6)	2.64 (0.75 - 9.31)
Water only	0 / 6.9 (0)	–	–
Kissed a boy in the last 6 months		P=0.56	P=0.34
No	43 / 151.4 (28.6)	1	1
Yes	1 / 1.5 (87.1)	3.05 (0.06 - 155.9)	6.59 (0.09 - 481.3)
Boy touched participant's breasts in last 6 months			
No	44 / 151.9 (29.6)	–	–
Yes	0 / 1.0 (0)	–	–
Boy touched participant's genitals or participant touched boy's genitals in the last 6months			
No	44 / 152.4 (29.4)	–	–
Yes	0 / 0.5 (0)	–	–

[1] Potential risk/protective factors were examined using a conceptual framework with three levels; age was considered an a priori confounder and included in all models. Age-adjusted sociodemographic factors at enrolment were retained in a core model if associated with HPV infection at $p<0.10$. Time varying sociodemographic factors were sequentially added and retained at $p<0.10$. Variables not presented include enrolment variables (tribe, who the girl lived with, reporting of ever having kissed, had a partner touch her breasts or genitals or whether she had touched a partner's genitals and whether she had cleansed inside the vagina) since none of these were associated in the unadjusted or adjusted analysis.). [2]SES: socio-economic status. This was determined using an asset index created by combining data collected from the entire cohort at enrolment, separated into tertiles. [3]Girls were asked during follow-up if they had ever used the internet or seen a pornographic movie, but they were not asked when they had done that. After a girl reported ever having used the internet or having seen a pornographic movie, all subsequent visits are counted as 'yes'. [4]Vaginal cleansing is cleaning inside the vagina with water, soap or other products using fingers or a cloth.

Figure 4:1. Enrolment of study participants

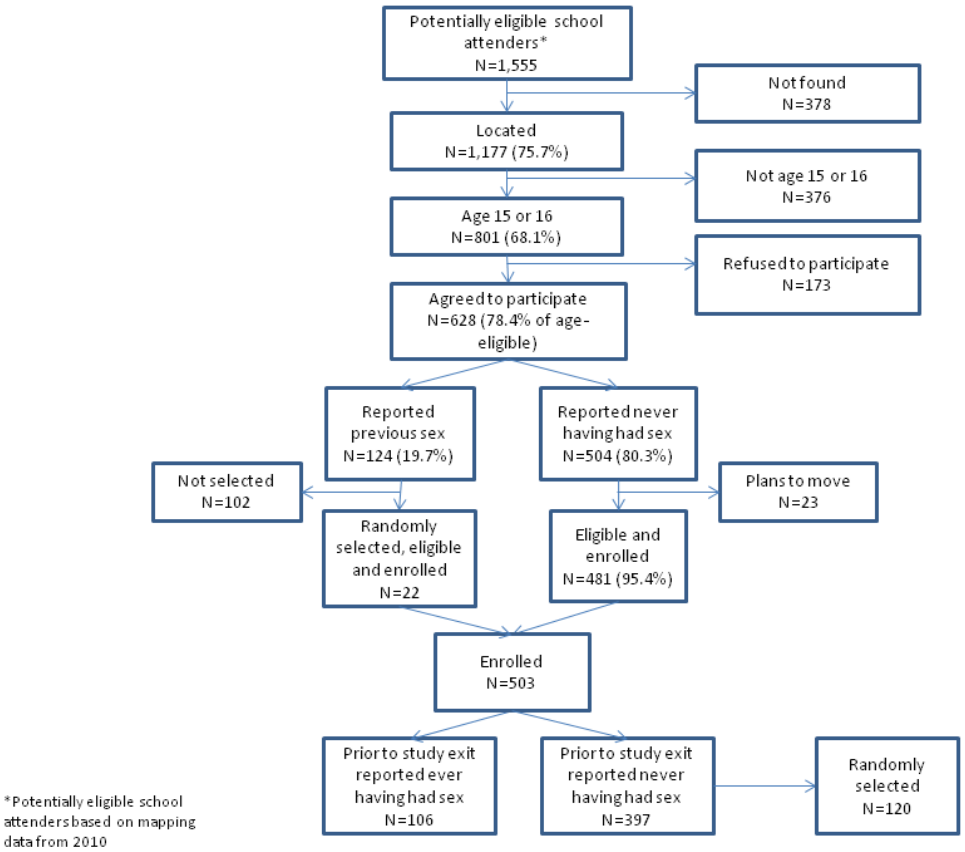
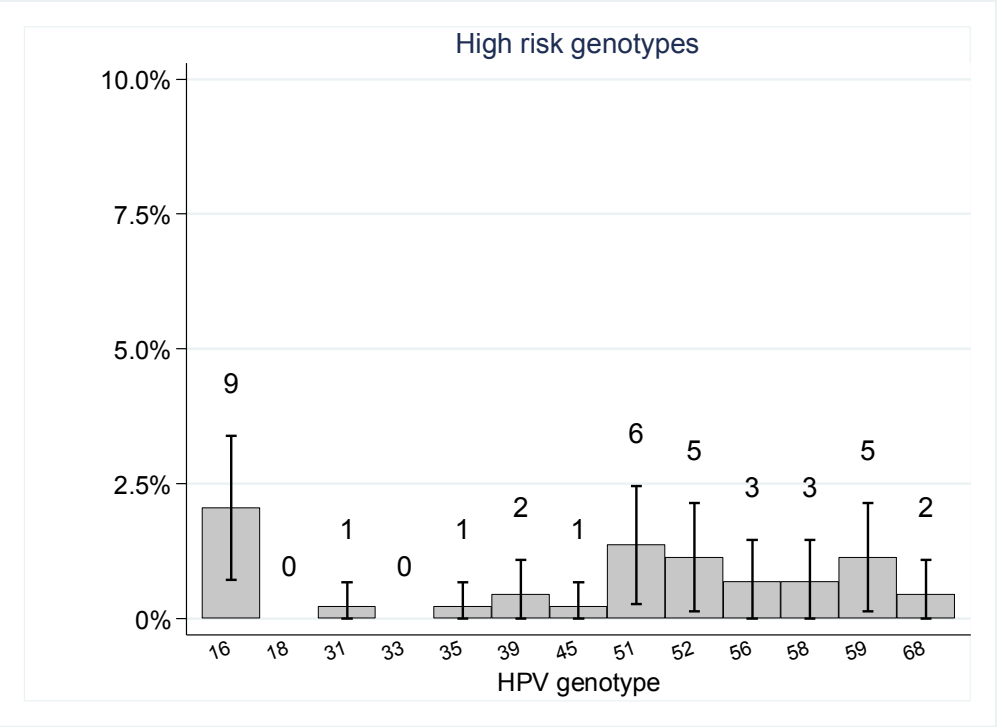
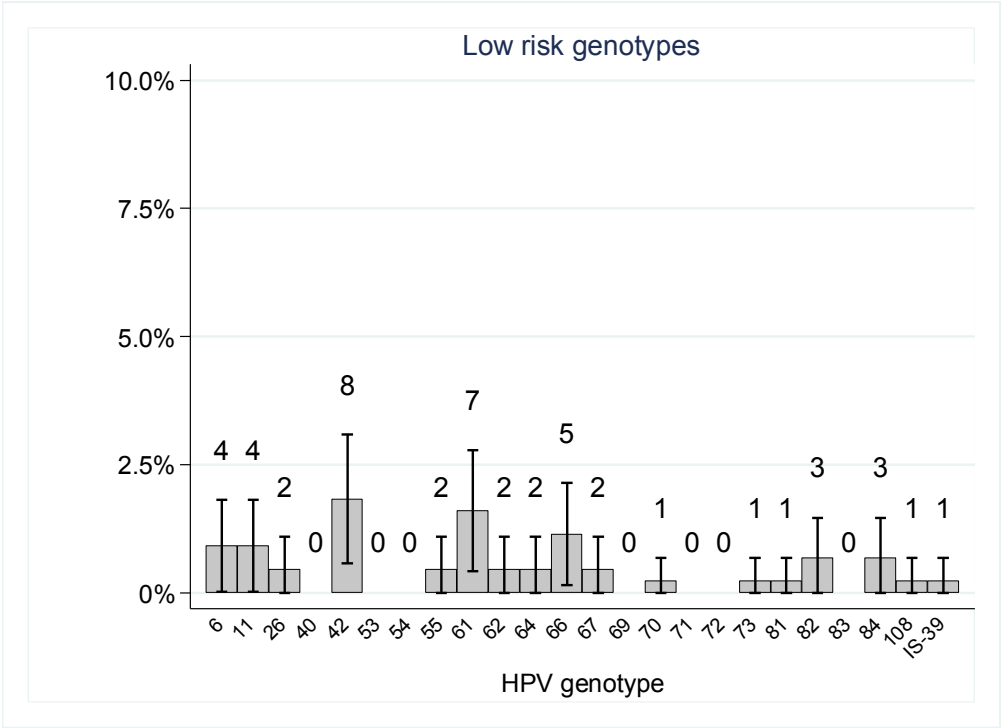


Figure 4:2. HPV genotype point prevalence (95% confidence interval), and number of infections at all visits including enrolment among 119 girls who did not report sex at enrolment or during follow-up





The HPV genotype-specific point prevalence was estimated as the number of visits where the genotype was detected, divided by the total number of visits attended including the enrolment visit. Visits with missing vaginal samples, or with samples that were β-globin negative, were excluded.

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5 Manuscript 3: HPV incidence around the time of sexual debut in adolescent girls in Tanzania

5.1 Preamble

This manuscript describes the genotype-specific and overall; rate, duration and clearance of HPV infection in girls after reported sexual debut, and factors associated with the incidence and clearance of HPV. These address part of objectives 2, 3 and 4 which are:

- Identify the rate of HPV infection, clearance of genotype-specific HPV, the duration of infection after initiation of sexual activity, and the rate of infection in those who do not report initiation of sexual activity.
- Evaluate sexual behaviour, vaginal practice and demographic factors associated with the incidence of genotype-specific HPV infection in those who report and do not report the initiation of sexual activity.
- Identify risk factors associated with HPV genotype-specific clearance.

5.2 Cover sheet: manuscript 3

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2.2. Please list the paper's authors in the intended authorship order
Catherine Houlihan, Kathy Baisley, Ignacio G Bravo, Saidi Kapiga, Silvia de Sanjosé, John Changalucha, David A Ross, Richard J Hayes Deborah Watson-Jones

2.3. Stage of publication – Not yet submitted / ~~Submitted / Undergoing revision from peer reviewers' comments / In press~~

3. For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)

I was involved in conceptualising the study. I wrote the data collection tools and coordinated the study, carried out the statistical analysis with Kathy Baisley, wrote the first draft of the manuscript and finalised it for publication.

NAME IN FULL (Block Capitals) Catherine F Houlihan

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SUPERVISOR/SENIOR AUTHOR'S SIGNATURE (3 above) .....

5.3 Manuscript 3: HPV incidence around the time of sexual debut in adolescent girls in Tanzania

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Keywords

Human papillomavirus, sexual debut, sub-Saharan Africa

Running title

Incidence of HPV around the time of first sex

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Abstract

Background

No reports exist on genotype-specific human papillomavirus (HPV) acquisition in girls after first sex in sub-Saharan Africa, despite high HPV-prevalence and cervical cancer incidence.

Methods

We followed 503 girls aged 15-16 years in Mwanza, Tanzania, 3-monthly for 18 months with face-to-face interviews and self-administered vaginal swabs. Swabs were tested for 13 high-risk and 24 low-risk HPV genotypes. Incidence, clearance and duration of overall HPV and genotype-specific infections were calculated and associated factors evaluated.

Results

106 participants reported first sex prior to enrolment (N=29) or during follow-up (N=77). One was HIV-positive at the final visit. The other 105 girls contributed 323 adequate specimens. Incidence of any new HPV genotype was 225/100 person-years(pys), and incidence of vaccine types HPV6,-11,-16 and -18 were; 12, 2, 2 and 7/100pys, respectively. Reporting sex in the past 3 months, and knowing the most recent sexual partner for a longer period before sex were associated with new HPV acquisition. Median time from reported sexual debut to first HPV infection was 5 months, and infection duration, 6 months.

Conclusion

HPV incidence was very high, including of some vaccine genotypes, in Tanzanian adolescent girls after first sex, and infection duration was short, highlighting the importance of vaccinating girls before sexual debut.

Introduction

A number of closely related human papillomavirus (HPV) genotypes are classified by the International Agency for Research on Cancers (IARC) as oncogenic (Group I) or probably oncogenic (Group IIA)[1], and are commonly referred to as 'high-risk' (HR) HPVs. Persistent infection (repeated detection over at least 6 months) with HR HPV, is associated with ano-genital cancers in men and women[2,3]. Infection with HR HPV genotypes is the primary cause of cervical cancer[4], and the highest age-standardised cervical cancer incidence and mortality worldwide are seen in sub-Saharan Africa (SSA), along with some of the highest HPV prevalences[5,6]. Worldwide data describing HPV prevalence by age show that the highest prevalence is in women under 25 years old[6]. From limited studies which have tested girls for HPV before and after fist sex, prevalence is high following sexual debut[7–9].

Current HPV vaccines are prophylactic, not therapeutic, and should be given before HPV acquisition[10]. Knowledge of the rates and timing of HPV acquisition is thus essential to inform HPV vaccination policy. To date, no studies have documented genotype-specific HPV incidence or overall HPV incidence in girls in SSA around the time of sexual debut. A national HPV vaccination programme for Tanzania, although in the planning stages, has not yet commenced. In order to examine initial HPV infection and natural history, we enrolled unvaccinated adolescent girls aged 15 and 16 years old and followed them every 3 months for 18 months in Mwanza, Tanzania.

Methods

Cohort enrolment

The cohort was enrolled as described previously[11]. Briefly, for preparation for an HPV vaccination trial, registration lists of girls enrolled in government primary schools in 3 districts of Mwanza region, northern Tanzania, were collected in 2010[12]. We enrolled girls who had been in class 6 in 2010 in one of the 82 government schools not randomly selected for vaccination. Additional enrolment eligibility criteria included: being aged 15 or 16 years, self-reporting never having had sex and currently not pregnant, being able to attend appointments, and being willing to self-administer a vaginal swab. In order to prevent stigmatization of girls enrolled or not enrolled, we randomly selected 26 schools from which we enrolled the first girl who reported ever having had sex, if her reported first sex was within the past year.

Ethical permissions

The London School of Hygiene and Tropical Medicine Ethics Committee and the Medical Research Coordinating Committee, Tanzania approved the study protocol in 2011. Since participants were under 18 years old at enrolment, written informed consent was required from a parent/guardian with subsequent informed assent from participants. Further consent procedures have been described previously[11].

Study procedures

Girls were enrolled between January and August 2012, and followed three-monthly for 18 months. At each visit, girls had a face-to-face interview with a female study nurse. All interviews were carried out in Swahili using a structured paper questionnaire. Enrolment questions and interview locations have been described previously[11]. Follow-up questions included sexual behaviour, vaginal practices and marriage since the last visit, and their current occupation. At each visit, one nurse-assisted, self-administered vaginal Dacron swab was obtained, irrespective of reported sex. At each follow-up visit, girls who reported previous sex (at any time) were offered a pregnancy test and asked about symptoms of reproductive tract infections. Those reporting symptoms were examined in the research clinic and offered syndromic treatment according to Tanzanian guidelines. At study completion, girls were offered a rapid test for HIV with appropriate referral if positive. In this paper, we present data from girls who reported having had sex prior to enrolment, or who passed sexual debut during follow-up.

HPV detection and genotyping

Swabs were placed dry into cryotubes immediately after collection, stored in cold boxes with ice-packs in the field, submitted daily to the laboratory in Mwanza and stored at -20^{0}C. They were shipped to the Catalan Institute of Oncology, Barcelona, Spain where HPV detection and genotyping were performed using the Linear Array HPV genotyping assay (Roche, California, USA). This test detects 37 HPV genotypes (HPV6,-11,-16,-18,-26,-31,-33,-35,-39,-40,-42,-45,-51,-52,-53,-54,-55,-56,-58,-59,-61,-62,-64,-66,-67,-68,-69,-70,-71,-72,-73,-81,-82,-83,-84, IS39 and CP6108). For this study, we classified HPV genotypes which were in IARC groups I (termed carcinogenic) and IIa (termed probably carcinogenic) as HR; these were; HPV-16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-68. We classified all remaining genotypes (group IIb, termed possibly carcinogenic and group III, no evidence of association with cancer) as LR[1]. Methods for DNA extraction, amplification and genotype

detection were described previously[11]. Specimens negative for β-globin amplification were excluded since vaginal sampling was assumed to be unsuccessful.

Data management and statistical methods

Questionnaire data were double-entered into OpenClinica LLC (Akaza Research, Waltham, MA, USA), and analyzed using STATA V13.0 (StataCorp LP, College Station, Texas, USA). Analyses were restricted to girls whose reported sexual debut was before enrolment or during follow-up ('sexually active'). Girls who were HIV-positive were excluded from all analyses.

For each HPV genotype, the number of prevalent infections (present at enrolment among those sexually active at entry), new infections (genotype not detected at enrolment or before reported sexual debut) and cleared infections (a new genotype infection that is no longer detected) were tabulated among all sexually active girls. The genotype-specific prevalence was estimated as the number of visits where the genotype was detected, divided by the number of sexually active visits.

Genotype-specific incidence was calculated; person-years (pys) at risk were calculated from enrolment (among girls whose reported sexual debut was before enrolment) or date of sexual debut (among girls who reported sexual debut during follow-up). Date of infection was defined as the midpoint between the last negative and first positive sample for that genotype. Since the incidence rate was high and infection duration short, girls with intervals of missing HPV results over 180 days were censored at the last available HPV result before the gap. Kaplan Meier methods were used to estimate time from sexual debut to first HPV infection among girls who reported sexual debut during follow-up and who were HPV-DNA negative at all visits before reported sexual debut ('HPV naïve').

The overall incidence of all new HPV, new HR HPV, and new LR HPV infections were calculated among: 1) all sexually active girls; 2) girls who reported sexual debut during follow-up; and 3) HPV-naïve girls who reported sexual debut during follow-up. In these analyses, girls were assumed to be continually at risk of infection with a new genotype, and could acquire more than one new infection at each visit. Periods longer than 180 days with missing HPV results were removed from the analysis; however, observation time after this gap contributed to the analysis. The overall incidence rate and 95%

confidence interval (CI) were estimated using random effects Poisson regression to account for clustering of multiple infections within the same girl.

Rate ratios (RR) for factors associated with HPV incidence among all sexually active girls were estimated using random effects Poisson regression. Potential risk factors were examined using a conceptual framework with three levels; age was considered an a priori confounder and was included in all models. Socioeconomic status was measured using an asset index, created by combining data on ownership of common household items in the entire cohort (i.e. including non-sexually active participants) using principal component analysis.

The genotype-specific clearance rate was calculated among all sexually active girls who had acquired a new genotype; pys at risk were calculated from the date of infection (midway between the last negative and first positive sample for the genotype). Clearance was defined as two consecutive negative samples, or one negative and one missing sample, for the genotype. When a girl tested negative for a genotype between two visits with positive samples for the same genotype, the intervening negative sample was considered a false negative. If that sample was missing, it was considered positive for the same genotype. The date of clearance was defined as the midpoint between the last positive and first negative sample. Girls who did not clear an infection were censored at the last sample date. Kaplan-Meier methods were used to estimate the median and mean genotype-specific duration, and the proportion cleared at 12 months.

Factors associated with clearance of new HPV infection were examined using methods for multiple failure-time data. HPV was the unit of analysis; therefore, girls infected with multiple genotypes could clear more than one infection. Failure (i.e. clearance) events were assumed to be unordered, so genotype-specific clearance was considered to be independent of clearing other genotypes. Cox regression was used to examine risk factors for clearance. The Cox model was stratified by HPV genotype and robust standard errors were used to adjust for correlation of repeated events among girls.

Results

Cohort screening, enrolment and follow up

We located 1,177 (75.7%) of 1,555 potentially-eligible girls on the original school attendance lists. Of these, 801 (68.1%) met the age criteria, of whom 628 (78.4%) consented to be screened (Figure 5.1). Of those screened, 503 (80.1%) were eligible and enrolled, and 416 (82.7%) attended the final visit. Overall, 106 (21.1%) participants reported sex, either at enrolment (N=29) or during follow-up (N=77). Among 29 girls whose reported date of first sex was before enrolment, median time from sexual debut to enrolment was 4.2 months (range 0.1-12.4). Among 106 girls reporting sex, 91 (85.8%) attended the final visit (18 months), and the median (IQR) follow-up time was 17.8 (17.4-17.9) months.

At the final visit, 49 of 91 (53.8%) participants accepted HIV testing; one (1.1%) was positive and excluded from the analyses. The remaining 105 girls contributed 437 "sexually-active visits" (visits after the reported date of sexual debut, including the enrolment visit) to the analysis; vaginal swabs were provided at 353 of these visits (80.8%) of which 323 (91.5%) were adequate specimens and were genotyped.

At enrolment, 71/105 (67.6%) participants were aged 16 years and the others were aged 15. Nearly two-thirds lived in rural areas (68, 64.8%), 92 (87.6%) were Christian, 7 (6.7%) were in school, and over half were neither working nor schooling (60, 57.1%). During the study, 71 (67.6%) reported ever having cleansed inside their vagina, and one (1.0%) reported ever having inserted a substance into the vagina. Only one girl reported that she was circumcised.

HPV prevalence and incidence

Of the 29 girls who reported ever having had sex at enrolment, 7 (24.1%) had at least one prevalent HPV infection at enrolment (Table 5.1).

A total of 28 new HR infections and 57 new LR infections were detected during follow up (Table 5.1). During the study, the most common HR genotypes were HPV51 (5.0% of visits), HPV58 (4.6%), HPV56 (4.0%) and HPV59 (4.0%) (Figure 5.2). The most common LR types were HPV84 (9.0% of visits), HPV83 (7.4%), HPV61 (5.3%), HPV66 (5.3%) and CP-108 (5.3%).

Genotype-specific incidence ranged from 2.2/100 person-years (pys) to 14.2/100 pys for each of the HR genotypes, and 0 to 24.1/100 pys for each of the LR genotypes (Table 5.1). The highest incidence rates (per 100 pys) of HR types were for HPV58 (14.2), HPV51 (9.6) and HPV18 (6.7). The highest incidence rates of LR types were for HPV84 (24.1), CP-108 (16.1), HPV66 (14.4), and HPV6 (12.2).

Among the 76 girls who reported first sex during follow-up, 35 (46.1%) had at least one HPV infection detected before the reported date of first sex. Among HPV-naïve girls, median time from reported sexual debut to HPV infection was 4.9 months (Figure 5.3), and to first HR-HPV, 9.3 months. Cumulative incidence of any HPV infection at 6 months was 52.8%; 35.8% for HR and 34.7% for LR genotypes.

The overall incidence rate (per 100 pys) of new HPV infections in sexually active girls (Table 5.2) was 225 (95%CI:166–305); HR-HPV incidence was 66 (95%CI:45–95) and LR-HPV was 157 (95%CI:111-222). Among girls who reported sexual debut during follow-up, the incidence rate for new HPV infections and for new HR-HPV infections were 209 (95%CI:146–299) and 63 (95%CI:40–99), respectively. Restricting to HPV-naïve girls, the incidence rate for new HPV infections was 193 (95%CI:118–316) and for new HR-HPV infections was 72 (95%CI:42–122). Overall, HPV was detected at 46% of "sexually active visits" in all girls (Table 5.2).

Risk factors for incidence of new HPV infection

In the adjusted analysis, there was evidence of an association with occupation (adjusted (a)RR=1.95, 95%CI:1.1-3.42 comparing those not working with those in work or vocational training)(Table 5.3). There was strong evidence of an association with the reporting of recent sex (aRR 2.48, 95%CI:1.40-4.37, comparing girls who reported sex once with those who reported no sex in the past 3 months). There was also evidence of a higher rate of new HPV infections among girls who had known their most recent partner for longer (aRR 3.15, 95%CI:1.32–7.50, comparing those who knew their partner for at least 6 months with those who knew the partner for <1 month). There was weak evidence of a higher rate of new HPV infections among girls reporting 3 or more partners compared with only 1 partner. There was also weak evidence of a lower rate among girls who reported vaginal cleansing (aRR 0.69, 95%CI:0.43–1.10).

HPV duration and clearance

Overall, 33 girls had at least one new HPV genotype during follow-up and contributed 85 new infections to analysis of HPV genotype-specific duration and clearance. In total, 26 of 85 (30.6%) new infections were cleared during follow-up. Median duration of new HPV genotype-specific infections was 6.1 months, and for new HR and new LR HPV genotypes 6.0 and 6.1 months, respectively. The overall rate of clearance (per 100 pys) was 90.4 for any HPV genotype, 106.7 for HR and 82.6 for LR genotypes.

In the unadjusted analysis, clearance of any HPV was associated with younger age of the girl, the most recent male partner being reported to have been circumcised, and the girl reporting that she had not practised vaginal cleansing in the past 3 months (Table 5.4). After adjusting for age, there was no evidence of a significant association with any of the factors examined.

Discussion

In this study, we demonstrated an extremely high incidence of vaginal HPV infection after first sex in adolescent Tanzanian girls. Acquisition was rapid in the initial months after first reported sex, and over half of the girls were positive for any HPV DNA in the first 6 months after reported sexual debut. These findings support current recommendations that adolescent girls should ideally be vaccinated before first sex[13].

Few studies have examined HPV incidence in young women after sexual debut. First acquisition of HPV (which predominantly occurs in the months after first penetrative sex) is a unique opportunity to document HPV genotypes to which young women are exposed and which may then become latent (and therefore un-detectable) until reactivation later in life. Current molecular testing cannot differentiate reactivation from first acquisition or reinfection and therefore all studies of HPV incidence in sexually active women can only record presumed incidence of HPV infections, since some apparent new infections may actually be reactivations. HPV84,-83,-61,-66 and CP-108 were the most common genotypes seen in our study. This is in contrast to global prevalence data in cytologically normal women that have reported HPV16,-18,-52,-31 and -58 as the most prevalent genotypes[6]. In our study the incidence rate of HPV vaccine genotypes was low; ranging between 2.4 and 13.6 per 100pys for each of the HPV types covered by the quadrivalent vaccine (HPV6,-11,-16 and 18); and between 1.3 and 13.6 per 100pys for each of the HPV types covered by the new nonavalent vaccine (HPV6,-11,-16,-18,-31,-33,-45,-52,-58). Incidence rates of HPV16 (2.3/100pys) and HPV18 (6.7/100pys) were lower relative to other genotypes. Our data could be used in modelling studies to explore whether catch-up vaccination campaigns in older girls (for example up to age 17 years) have additional impact on cervical cancer incidence.

The overall HPV incidence in our study (225/100 person-years; 187/1000 person-months) was far higher than that reported in already sexually active women. A cohort study of sexually active women in Brazil, median age 33 years, reported an incidence of 13.4/1000 person-months[14], and a study in women in Canada, median age 21, reported an incidence of 19/1000 person-months[15]. Cumulative incidence has been reported as 39-44% at 24 to 36 months after first sex in Brazil and the USA [7,8,14], lower than the 53% at the much shorter follow-up period of 6 months in our study. Young women are known to have a high incidence of infection but the particularly high incidence in our study may additionally be driven by a high HPV prevalence of infection in the male partners of these young women[6,7,16–18]. However, the incidence in our cohort is higher than other studies in young women in East Africa. A cohort study of sexually active women in Uganda (median age 20 years) found an HPV incidence of 30.5/100pys[19], and a previous study in Mwanza, Tanzania (median age 18) reported an incidence of 74/100pys[17].

Comparing the incidence of individual genotypes in our Tanzanian study with a study which reported incidence of HPV6,-11,-16 and -18 in women in the USA[20]; in our participants, the incidence of HPV6,-11 and -18 were three fold higher, but a lower rate was seen for HPV16 in our Tanzanian study (2.3/100pys vs 5.4/100pys in the study in the USA). This is in keeping with findings that HPV16 is less common in SSA than in other regions including the USA[6,21].

Not working was associated with increased incident HPV risk compared to being employed or in vocational training. Girls who are not working may be at increased risk of engaging in sex in exchange for gifts or money or forced sex, which are risk factors for HIV and other STIs[22], but have not clearly been identified as risk factors for HPV[23,24]. These behaviours were infrequently reported in our study, although they have been described in other studies in older women in the region[25]. Reporting sex once in the past 3 months was associated with increased risk of incident HPV compared to not reporting sex. A single episode of sex may be more likely to occur outside of the context of a stable relationship (although information on the nature of the sexual partner was not collected in our study). Knowing a partner for 6 or more months before sex was associated with a more than three-fold risk of incident HPV compared to knowing a partner for under one month. Girls may be more likely to be involved in risky sex (i.e. without a condom) and therefore be at increased risk of HPV[26], if a partner is well known to them. Contrary to that, however, reported condom use at last sex was not associated with lower HPV incidence, although numbers were small. Reported male

partner circumcision was similarly not associated with incident HPV, in contrast to a large study in Uganda[27]. However, girls in our study may not have always known whether their partners were or were not circumcised.

Limitations of our study include the use of self-administered swabs rather than clinician-collected cervical swabs. We used these since we anticipated that most girls would not have passed sexual debut at enrolment and therefore speculum examination was considered undesirable. Over 90% were β-globin positive, indicating adequate sampling[28]. Also, a previous study in Uganda had demonstrated good HPV-genotype correlation in self-administered and clinician-administered swabs[29].

We chose to remove un-observed intervals (without vaginal swab results) of over 180 days from the analysis. However, sensitivity analyses, where we assumed girls to be uninfected with a new genotype during those intervals, gave similar results (incidence among all "sexually active" girls was 158/100pys, (95%CI:123–203)). Samples that were negative or missing for a given genotype but had been taken between two samples that were positive for that genotype were classified as positive because studies demonstrating long-term persistence have shown that sporadic detection of the same genotype early in the course of the persistent infection is common[30]. We excluded one girl who was HIV positive at study completion, since HPV incidence is higher with HIV infection[31,32]. Only 46% of participants who attended the final visit accepted an HIV test and therefore we may have unknowingly included HIV positive girls in the analysis, although national estimates are that there is a very low HIV prevalence in 15-19 year old girls in Tanzania (1.3%)[33].

In our study, median time from first reported sex to acquisition of any HPV was 5 months. This is longer than the 2.4 months reported in college students in the USA who were tested every three months[34]. Differences in the types of relationships formed (marriage versus casual sex partner), recent sex and condom use may explain these differences since some of these have been identified as risk factors for acquisition in our or other studies[26,35]. Reporting bias may have influenced accurate assessment of these risks: participants in our study may have been less willing to report sex and had less accurate recall of dates of sex compared to the women in the USA study. The median duration of infection in our study was shorter (6 months) than in previous studies (reported range 8-31 months[14,15,36]). This may be an underestimate since the duration of follow-up was limited depending on the point at which girls reported sexual debut. A short duration of infection could also be due to cervico-vaginal immune

activation in Tanzanian girls, which has been shown to be higher in STI and HIV-uninfected young women in Kenya compared to the USA[37]. High levels of endo-cervical T-lymphocytes identified in the women studied in Kenya could have mediated HPV clearance[37]. Finally, higher cervical HPV viral load, age over 30 years, being HIV positive and having a high number of sex partners were associated with lower HPV clearance in women in Uganda[32]. We identified no associations with HPV clearance, potentially because our cohort displayed little variation in age or number of sex partners, and all those included in the estimates of the clearance rate were either HIV-negative or had unknown HIV status.

We report a rapid acquisition of HPV infection, extremely high incidence, and rapid clearance in young women after their first reported sex. This study was carried out in a region with one of the highest incidences of cervical cancer in the world, and our findings may help to explain these high rates of cervical cancer and the high HPV prevalence observed in East Africa[6] and support the current recommendation that HPV vaccination should be given to girls before their first sex[38].

Table 5:1. HPV genotype prevalence, incidence, duration and clearance among 105 sexually active girls during follow-up

HPV type	Prevalent (%)[1]	New infections/pys (rate/100 pys) [2]	New infections that were cleared (%)[3]	N cleared / pys (rate/100 pys)	Mean (median) months duration (Kaplan-Meier) [4]
High risk genotypes					
HPV16	0	1 / 44.0 (2.3)	0	0/ 0.1 (0)	‡
HPV18	0	3 / 44.6 (6.7)	1 (33%)	1/ 0.5 (211.7)	2.7 (2.7)
HPV31	0	2 / 43.6 (4.6)	1 (50%)	1/ 0.9 (113.8)	6.1 (6.1)
HPV33	0	2 / 44.5 (4.5)	1 (50%)	1/ 0.4 (268.6)	3.0 (3.0)
HPV35	0	2 / 44.5 (4.5)	1 (50%)	1/ 1.0 (99.5)	6.0* (4.9)
HPV39	1 (3%)	2 / 44.3 (4.5)	0	0/ 1.2 (0)	13.5* (†)
HPV45	0	1 / 43.1 (2.3)	1 (100%)	1/ 0.2 (403.6)	‡
HPV51	2 (7%)	4 / 41.7 (9.6)	3 (75%)	3/ 1.9 (158.7)	6.1 (6.1)
HPV52	0	1 / 44.3 (2.3)	1 (100%)	1/ 0.5 (198.5)	‡
HPV56	0	1 / 44.1 (2.3)	0	0/ 0.4 (0)	‡
HPV58	1 (3%)	6 / 42.1 (14.2)	0	0/ 1.8 (0)	13.2* (†)
HPV59	3 (10%)	2 / 42.7 (4.7)	1 (50%)	1/ 0.3 (289.9)	2.7 (2.7)
HPV68	0	1 / 44.9 (2.2)	0	0/ 0.1 (0)	‡
All HR infections[5]	**7**	**28**	**10 (36%)**	**10 / 9.4 (106.7)**	**6.9* (6.0)**
Low risk genotypes					
HPV6	1 (3%)	5 / 41.0 (12.2)	2 (40%)	2/ 1.4 (146.8)	5.0 (6.1)
HPV11	0	1 / 45.1 (2.2)	0	0/ 0.2 (0)	‡
HPV26	0	0 / 45.5 (0)	–	–	–
HPV40	0	2 / 43.8 (4.6)	0	0/ 0.3 (0)	1.7* (†)
HPV42	0	3 / 41.9 (7.2)	1 (33%)	1/ 0.8 (133.3)	6.1 (6.1)
HPV53	0	2 / 42.7 (4.7)	1 (50%)	1/ 0.6 (180.4)	3.3* (3.0)
HPV54	1 (3%)	4 / 41.8 (9.6)	1 (25%)	1/ 1.4 (73.6)	6.1* (†)
HPV55	0	4 / 43.8 (9.1)	1 (25%)	1/ 1.3 (78.4)	6.2* (4.9)
HPV61	1 (3%)	0 / 45.0 (0.0)	–	–	–
HPV62	0	3 / 43.6 (6.9)	1 (33%)	1/ 1.7 (59.2)	9.4* (2.7)
HPV64	0	1 / 45.4 (2.2)	0 (0 %)	0/ 0.1 (0)	‡
HPV66	1 (3%)	6 / 41.5 (14.4)	2 (33%)	2/ 3.2 (62.5)	11.3* (11.8)
HPV67	1 (3%)	1 / 44.6 (2.2)	1 (100%)	1/ 0.3 (299.4)	‡
HPV69	0	0 / 44.8 (0)	–	–	–
HPV70	0	0 / 44.5 (0)	–	–	–
HPV71	0	1 / 45.1 (2.2)	0	0/ 0.4 (0)	‡
HPV72	0	0 / 45.5 (0)	–	–	–
HPV73	1 (3%)	4 / 43.1 (9.3)	1 (25%)	1/ 0.9 (117.6)	3.5* (2.7)

HPV81	0	1 / 45.0 (2.2)	0	0/ 0.1 (0)	‡
HPV82	0	0 / 45.4 (0)	–	–	–
HPV83	0	3 / 40.7 (7.4)	1 (33%)	1/ 2.0 (50.6)	11.9* (†)
HPV84	2 (7%)	9 / 37.3 (24.1)	3 (33%)	3/ 3.1 (97.5)	6.0* (6.0)
HPV CP-108	0	7 / 43.6 (16.1)	1 (14%)	1/ 1.8 (55.6)	7.0* (4.9)
HPV IS39	0	0 / 45.5 (0)	–	–	–
All LR infections[5]	**8**	**57**	**16 (28%)**	**16 / 19.4 (82.6)**	**8.9* (6.1)**
All HPV infections	**15±**	**85**	**26 (31%)**	**26 / 28.8 (90.4)**	**8.4* (6.1)**

[1]Positive for that genotype at the enrolment visit, amongst 29 girls who were sexually active at enrolment. [2]New infection defined as first positive test for the specific HPV type, among those not infected at enrolment or before reported sexual debut. Girls with gaps >180 days in observation time are censored at the last available HPV result before the gap [3]Clearance defined as ≥2 consecutive samples negative for the specific genotype; denominator is total genotype-specific new infections. [4]Mean duration of new infections estimated using Kaplan Meier methods restricted by the longest follow-up time (i.e. duration) [5]Total number of group (HR or LR)-specific infections among 105 girls. *Mean duration of infection for the genotype is underestimated because the individual with the longest observed duration was censored. †Median duration could not be estimated because survival curve does not drop below 50%. ‡One infection only, Kaplan-Meier survival function not estimated ±15 infections within 7 girls. 5 had at least one HR HPV genotype at enrolment, 5 had at least one LR HPV genotype at enrolment, 7 had any genotype at enrolment.

Table 5:2. Incidence and point prevalence of HPV in adolescent girls who reported sex

Outcome	All girls who reported sexual debut before or during the study (N=105)	All girls who reported sexual debut during study (N=76) [1]	Girls who reported sexual debut during study and were HPV naïve (N=41) [2]	Reported sexual debut prior to enrolment (N=29)
Incidence				
	New infections / person-years (rate / 100 person-years, 95% CI) [3]			
All HPV	119 / 56.4 (225; 166–305)	62 / 30.1 (209; 146–299)	40 / 19.5 (193; 118–316)	57 / 26.3 (248; 144–425)
All HR HPV	37 / 56.4 (66; 45–95)	19 / 30.1 (63; 40–99)	14 / 19.5 (72; 42–122)	18 / 26.3 (71; 37–135)
All LR HPV	82 / 56.4 (157; 111–222)	43 / 30.1 (146; 97–218)	26 / 19.5 (127; 66–246)	39 / 26.3 (176; 95–327)
Point prevalence				
	Total infections (number of visits with at least one infection / sexually active visits; % of all visits) [4]			
All HPV	323 (148 / 323; 45.8%)	186 (87 / 172; 50.6%)	91 (40 / 106; 37.7%)	137 (61 / 151; 40.4%)
All HR HPV	108 (87 / 323; 26.9%)	57 (49 / 172; 28.5%)	32 (26 / 106; 24.5%)	51 (38 / 151; 25.2%)
All LR HPV	215 (118 / 323; 36.5%)	129 (70 / 172; 40.7%)	59 (29 / 106; 27.4%)	86 (48 / 151; 31.8%)

[1]HPV incidence among all girls who reported passing sexual debut during the study; includes 35 girls in whom HPV was detected before reported sexual debut (infections before reported sexual debut do not contribute to the incidence estimate in this column, but girls are not excluded from the analysis). [2]HPV incidence among 41 girls who reported passing sexual debut during the study and no HPV was detected before reported sexual debut. [3]Rate estimated from random effects Poisson regression: point estimates and 95% CI take into account correlation of repeated infections within girls. Girls assumed to be continually at risk and can acquire >1 infection at each visit. Observation time after gaps >180 days contribute to the analysis therefore total number of infections is different from that in Table 1. [4]Total number of genotype-specific infections and number of visits where at least one genotype was detected at all visits after reported date of sexual debut, including enrolment visit.

Table 5:3. Association of selected potential risk/protective factors[1] with any new HPV infection among 105 adolescent girls who reported previous sex

	Number of infections[2] / person-years (rate/ 100 pys)	Crude RR (95% CI)	Adjusted RR (95% CI)[3]
Sociodemographic (enrolment)			
Age at enrolment		P=0.45	
15 years	32 / 19.1 (189)	1	
16 years	87 / 37.3 (244)	1.29 (0.67–2.47)	
Religion		P=0.83	P=0.83
Christian	99 / 49.2 (213)	1	1
Muslim	16 / 5.5 (333)	1.56 (0.59–4.12)	1.57 (0.60–4.16)
Other	4 / 1.7 (219)	1.16 (0.11–12.30)	1.07 (0.10–11.30)
Socio-economic status score (tertiles)		P=0.57	P=0.63
Low	58 / 25.5 (246)	1	1
Middle	33 / 20.3 (174)	0.70 (0.35–1.43)	0.72 (0.36–1.48)
High	28 / 10.6 (256)	1.04 (0.48–2.23)	1.03 (0.48–2.21)
Sociodemographic (time varying)			
Current residence		P=0.80	P=0.53
Urban	50 / 20.7 (235)	1	1
Rural	65 / 34.6 (217)	0.92 (0.49–1.72)	0.82 (0.44–1.53)
Current occupation		P=0.05	**P=0.06**
School	5 / 2.9 (164)	1.13 (0.30–4.26)	**1.15 (0.30–4.36)**
Work|vocational training	29 / 23.1 (144)	1	**1**
Not working	85 / 30.4 (283)	1.96 (1.12–3.43)	**1.95 (1.11–3.42)**
Currently married?		P=0.66	P=0.79
No	90 / 43.9 (217)	1	1
Yes	29 / 12.5 (248)	1.14 (0.63–2.09)	0.92 (0.50–1.70)
Alcohol since last visit		P=0.42	P=0.48
No	116 / 55.2 (228)	1	1
Yes	3 / 1.2 (134)	0.59 (0.16–2.22)	0.62 (0.16–2.40)
Behavioural (time varying)			
Total partners ever		P=0.09	P=0.09
1	86 / 43.1 (225)	1	1
2	16 / 10.0 (155)	0.69 (0.35–1.34)	0.77 (0.38–1.54)
3+	16 / 2.5 (509)	2.26 (0.83–6.17)	2.76 (0.95–8.04)
Number of times had sex in past 3months		P=0.005	**P=0.008**
0	49 / 31.4 (159)	1	**1**

1	30 / 9.3 (400)	2.52 (1.44–4.42)	**2.48 (1.40–4.37)**
2+	39 / 15.5 (257)	1.62 (0.95–2.77)	**1.52 (0.88–2.63)**
Most recent male sexual partner circumcised		P=0.22	P=0.23
No	33 / 16.1 (182)	1	1
Yes	80 / 31.5 (263)	1.47 (0.79–2.72)	1.48 (0.77–2.85)
Don't know[4]	5 / 8.0 (109)	–	–
Most recent sexual partner was in a concurrent relationship		P=0.06	P=0.25
No	53 / 31.1 (171)	1	1
Yes	12 / 2.7 (402)	2.37 (0.99–5.68)	1.83 (0.66–5.07)
Don't know[4]	53 / 21.7 (287)	–	–
Age difference of most recent partner		P=0.11	P=0.10
≤2 years	8 / 8.9 (82)	1	1
3–5 years	26 / 11.0 (215)	2.65 (1.03–6.83)	2.58 (1.04–6.45)
>5 years	35 / 13.1 (237)	2.64 (1.04–6.73)	2.33 (0.94–5.77)
Don't know[4]	49 / 22.6 (316)	–	–
Used condom at last sex		P=0.93	P=0.90
No	105 / 46.5 (226)	1	1
Yes	13 / 9.1 (235)	1.04 (0.45–2.40)	0.95 (0.41–2.20)
Does partner put saliva on penis		P=0.58	P=0.26
No	78 / 33.5 (229)	1	1
Yes	7 / 1.7 (309)	1.34 (0.48–3.75)	1.87 (0.64–5.48)
Don't know[4]	2 / 0.5 (292)	–	–
Does partner use vaseline for sex		P=0.007	P=0.17
No	77 / 35.7 (208)	1	1
Yes	10 / 1.1 (717)	3.44 (1.47–8.04)	2.06 (0.76–5.63)
Time had known most recent partner before first sex (with that partner)		P=0.14	**P=0.03**
<1m	26 / 16.1 (143)	1	**1**
1-6m	64 / 28.0 (252)	1.76 (0.89–3.47)	**1.76 (0.90–3.46)**
6+m	28 / 11.0 (314)	2.19 (0.92–5.20)	**3.15 (1.32–7.50)**
Cleansed vagina in past 3months[5]		P=0.02	P=0.11
No	68 / 29.8 (300)	1	1
Yes	51 / 26.6 (169)	0.56 (0.35–0.90)	0.69 (0.43–1.10)

[1]Potential risk/protective factors were examined using a conceptual framework with three levels; age was considered an a priori confounder and included in all models. Age-adjusted sociodemographic factors at enrolment were retained in a core model if associated with HPV infection at p<0.10. Time-varying sociodemographic factors were added sequentially and retained if associated at p<0.10. Time-varying behavioural factors were then added sequentially, and retained at p<0.10. All p-values presented in the table are from the likelihood ratio test. [2]Girls are assumed to be continually at risk and can acquire >1 infection at each visit. Observation time after gaps >180 days contribute to the analysis; therefore, the total number of infections (119) is different from that in Table 1. [3]Sociodemographic factors at enrolment adjusted for age (a priori). Time-varying sociodemographic factors adjusted for age (a priori) and all independent sociodemographic predictors of HPV infection (at p<0.1) (occupation). Behavioural factors adjusted for age, occupation and all independent behavioural predictors of HPV infection (number of times had sex in past 3

months and time knew most recent partner before sex (variables in bold)). [4]Don't know' responses considered missing data and not included in analysis. [5]Vaginal cleansing is cleaning inside the vagina with water, soap or other products using fingers or a cloth.

Table 5:4. Clearance of new HPV infections and associated factors among girls who reported having had sex at any time during follow-up (unit of analysis is the infection)

	Number cleared / person-years (rate/ 100 pys)	Crude HR (95% CI)	Adjusted HR (95% CI)
Sociodemographic (enrolment)			
Age at enrolment		P=0.03	
15 years	8 / 6.1 (132.2)	1	
16 years	18 / 22.7 (79.3)	0.09 (0.01–0.74)	
Religion		P=0.08	P=0.21
Christian	25 / 23.1 (108.3)	1	1
Muslim	1 / 4.8 (20.8)	0.39 (0.13–1.13)	0.53 (0.20–1.43)
Other	0 / 0.9 (0.0)	–	–
Socio-economic status score (tertiles)		P=0.10	P=0.12
Low	16 / 15.3 (104.6)	1	1
Middle	7 / 8.0 (87.6)	0.81 (0.22–3.02)	0.73 (0.37–1.45)
High	3 / 5.5 (54.9)	0.35 (0.13–0.93)	0.40 (0.14–1.09)
Sociodemographic (time varying)			
Current residence		P=0.10	P=0.24
Urban	13 / 15.2 (85.4)	1	1
Rural	13 / 12.9 (101.0)	2.23 (0.85–5.85)	0.44 (0.11–1.71)
Current occupation		P=0.88	P=0.56
School	6 / 7.8 (77.0)	1	1
Work|vocational training	0 / 0.1 (0.0)	–	–
Not working	20 / 20.8 (96.0)	0.90 (0.24–3.38)	1.55 (0.34–7.09)
Currently married		P=0.25	P=0.46
No	21 / 23.8 (88.3)	1	1
Yes	5 / 5.0 (100.3)	0.44 (0.11–1.78)	0.60 (0.15–2.33)
Alcohol since last visit		P=0.35	P=0.35
No	24 / 27.5 (87.2)	1	1
Yes	2 / 1.2 (164.0)	2.45 (0.37–16.28)	2.45 (0.37–16.28)
Behavioural (time varying)			
Total partners ever		P=0.58	P=0.16
1	18 / 17.6 (102.6)	1	1
2	4 / 5.4 (73.8)	0.84 (0.17–4.05)	0.26 (0.04–1.53)
3+	4 / 5.7 (70.7)	0.60 (0.23–1.57)	0.84 (0.35–2.01)
Number of times had sex in the past 3months		P=0.14	P=0.14
0	13 / 11.0 (118.4)	1	1
1	0 / 6.2 (0.0)	–	–

2+	13 / 11.5 (112.7)	0.21 (0.02–1.69)	0.21 (0.02–1.69)
Most recent male sexual partner was circumcised		P=0.06	P=0.18
No	15 / 13.7 (109.7)	1	1
Yes	11 / 14.4 (76.3)	0.38 (0.14–1.04)	0.56 (0.24–1.30)
Don't know[2]	0 / 0.5 (0.0)	–	–
Most recent sexual partner was in concurrent relationship			
No	17 / 18.2 (93.3)	–	–
Yes	0 / 1.6 (0.0)	–	–
Don't know[2]	9 / 8.8 (102.1)	–	–
Used condom at last sex			
No	26 / 26.9 (96.8)	–	–
Yes	0 / 1.8 (0.0)	–	–
Partner put saliva on penis		P=0.75	P=0.75
No	18 / 22.0 (81.8)	1	1
Yes	3 / 2.0 (150.7)	0.88 (0.40–1.92)	0.88 (0.40–1.92)
Don't know[2]	–	–	–
Partner use vaseline for sex			
No	21 / 23.0 (91.3)	–	–
Yes	0 / 1.0 (0.0)	–	–
Time had known most recent partner before sex		P=0.13	P=0.89
<1m	7 / 7.4 (94.9)	1	1
1-6m	17 / 17.2 (98.6)	2.05 (0.64–6.53)	0.98 (0.30–3.15)
6+m	2 / 3.8 (52.1)	0.82 (0.17–4.01)	0.72 (0.09–5.54)
Cleansed vagina in past 3 months[3]		P=0.02	P=0.30
No	19 / 15.9 (119.4)	1	1
Yes	7 / 12.8 (54.5)	0.26 (0.08–0.83)	0.49 (0.13–1.87)

[1]New infection defined as first positive test for the specific HPV type, among those not infected at enrolment or before reported sexual debut. Girls with gaps >180 days in observation time are censored at last available HPV result before the gap. All P values are from likelihood ratio tests. [2] 'Don't know' responses considered missing data and not included in analysis [3]Vaginal cleansing is cleaning inside the vagina with water, soap or other products using fingers or a cloth.

Figure 5:1. Enrolment and follow-up of study participants

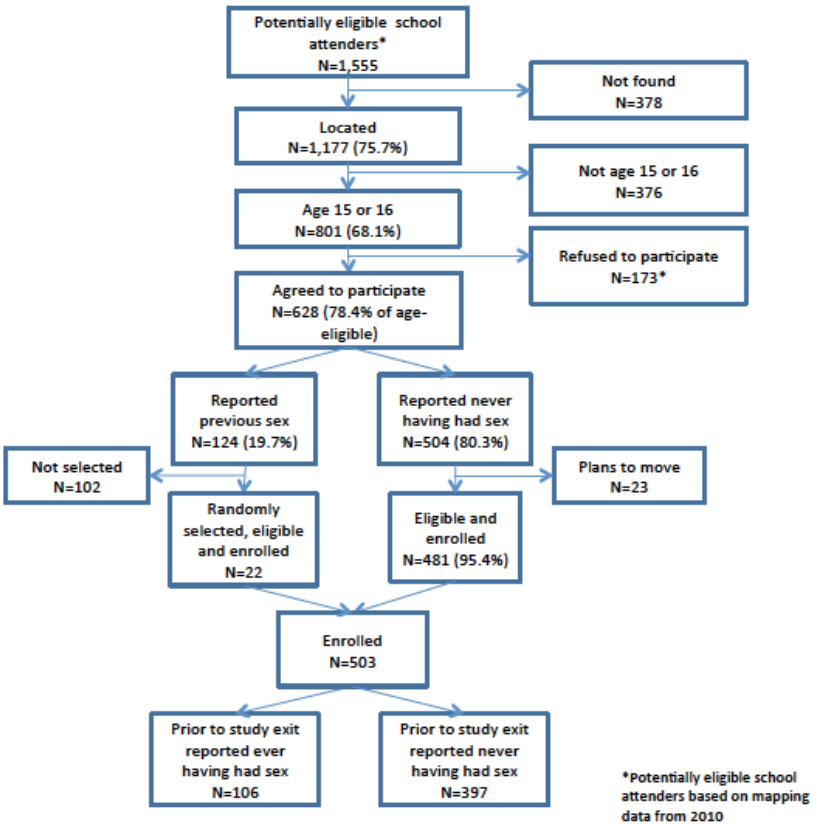
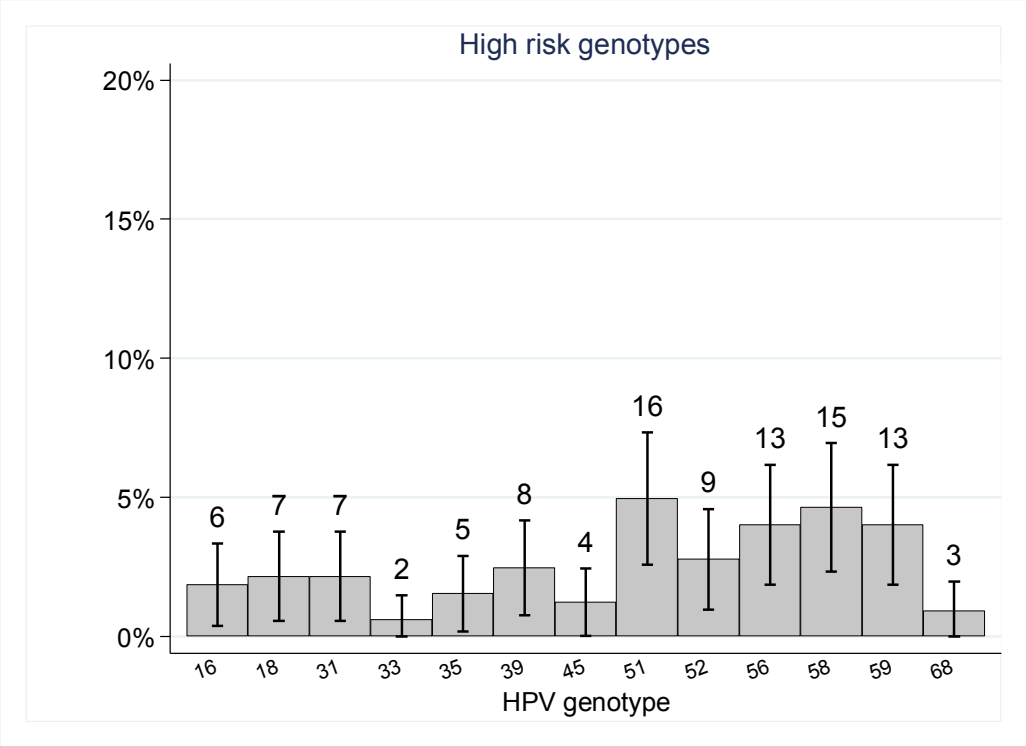
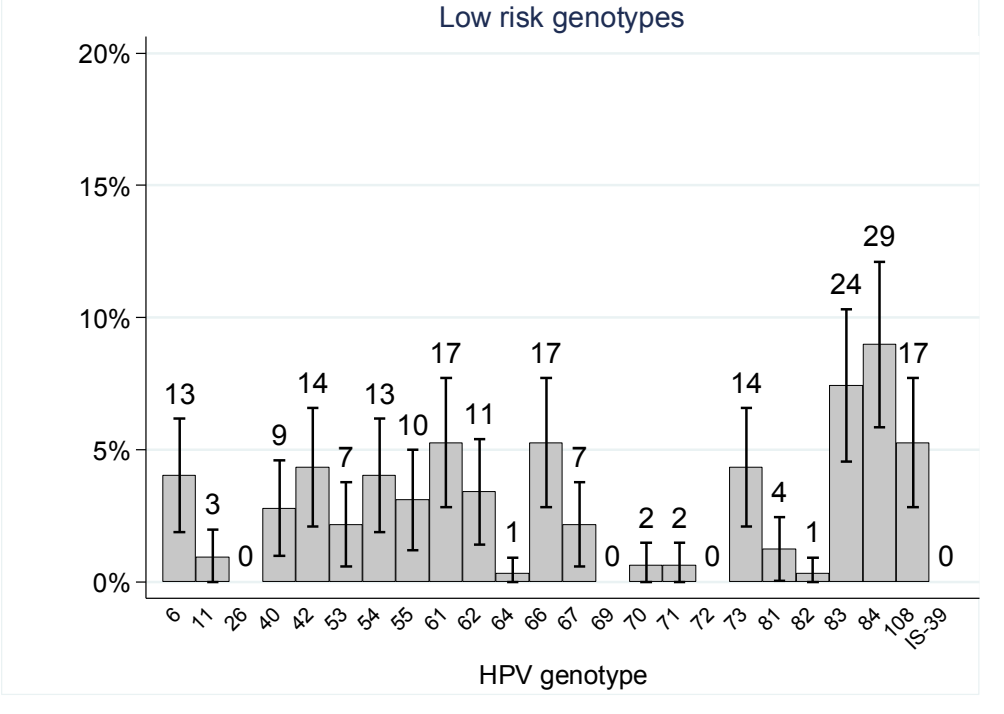


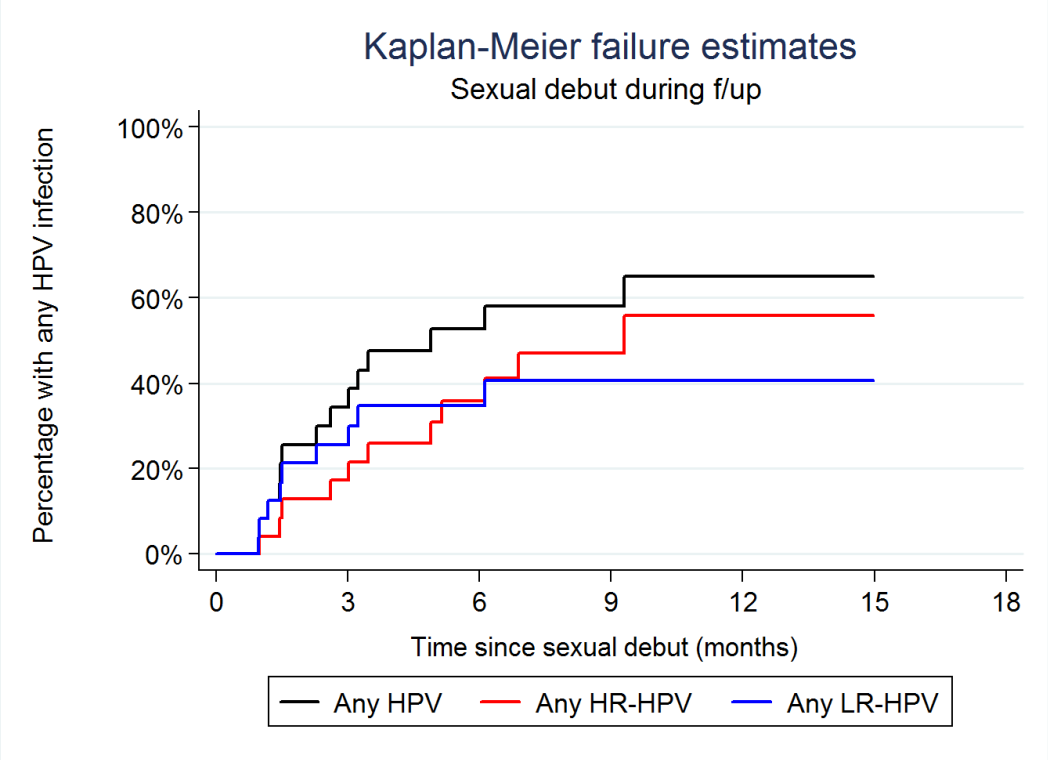
Figure 5:2. HPV genotype point prevalence (95% confidence interval), and number of infections at all visits after reported first sex in 105 adolescent girls





The HPV genotype-specific point prevalence was estimated as the number of visits where the genotype was detected, divided by the total number of visits after the reported date of sexual debut, including the enrolment visit. Visits with missing vaginal samples, or with samples that were β-globin negative, are excluded.

Figure 5:3. Time from sexual debut to first infection with any HPV, any HR HPV or any LR HPV, among 41 girls who reported sexual debut during follow-up and were HPV-naïve at time of reported sexual debut



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6 Manuscript 4: Comparison of Audio Computer-Assisted Self-Interview with face to face interview for disclosure of sexual behaviour in adolescent girls in Tanzania

6.1 Preamble

This manuscript describes the comparison of face-to-face interview with self-completed audio computer assisted interview (ACASI) for the disclosure of sexual behaviour and intra-vaginal practices to addresses the secondary objective of the research.

6.2 Cover sheet: manuscript 4

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I designed the data collection tools, coordinated the data collection, performed the statistical analysis, wrote the first draft of the manuscript and finalised it for publication

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6.3 Manuscript 4: Comparison of Audio Computer-Assisted Self-Interview with face to face interview for disclosure of sexual behaviour in adolescent girls in Tanzania

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Abstract:

Background

Valid identification of sexually transmitted infection risk factors, and evaluation of interventions to modify these, depend on accurate sexual behaviour data. Audio computer-assisted self-interviews (ACASI) have demonstrated increased reporting of sensitive behaviours, although studies from low-income countries are limited, and results somewhat inconsistent. We compared sexual behaviour reporting by ACASI and face-to-face (FtF) interviews in adolescent Tanzanian girls.

Methods

A cohort study examining the epidemiology of human papillomavirus (HPV) in Mwanza, Tanzania followed 503 girls 3-monthly for 18 months with FtF-interviews. At the 12-month visit, we randomly selected girls to participate in a cross-sectional comparison of ACASI (using a sub-set of FtF-interview sensitive behaviour questions) versus the standard FtF-interview. Participants completed both interviews, the order of which was randomly allocated. Agreement between methods was measured using kappa statistics. Proportions reporting each behaviour by interview method were compared using the Mainland-Gart test.

Results

Overall, 203 participants completed both interviews and correctly answered questions testing their understanding of the questions. Of these 203, 108 (53.2%) were aged 16 and 95 (46.8%) aged 17 years. Fourteen (7.4%) reported recent kissing during ACASI compared to 7 (3.4%) on FtF-interview (p=0.05, kappa=0.55). Conversely, ever having had vaginal sex was more frequently reported during FtF-interviews than ACASI (15 (7.4%) versus 6 (3.0%) respectively; p=0.02, kappa=0.45). There was no significant difference between methods for reporting of other behaviours, or order of interview. 84.2% of participants preferred ACASI to FtF-interviews, and 97.6% found ACASI easy to use.

Conclusion

In this study of adolescent Tanzanian girls, ACASI was feasible and acceptable, but compared to FtF-interviews did not result in higher reporting of vaginal sex or other sexual behaviours, with the exception of kissing. Reasons may include opportunities in

FtF-interviews to probe and explain sexual practices. Sexual behaviour interviews with ACASI should be evaluated in different settings before implementation.

Introduction

The HIV epidemic has disproportionately affected adolescent girls and young women[1]. In sub-Saharan Africa (SSA), 72% of 15-24 year olds with HIV are female[2]. Identified risk factors include younger age at first sex and sex with an older male partner[3–5]. Valid identification of sexual behaviour risk factors for HIV and other sexually transmitted infections (STI) in this population, and evaluation of interventions to modify these, are dependent on accurate collection of retrospective sexual behaviour data.

The disclosure of sexual behaviour is highly prone to social desirability bias, with interviewees responding as they believe the interviewer or wider society think is appropriate or acceptable[6]. This bias may be increased in studies of girls in SSA countries like Tanzania where sex outside marriage or whilst schooling may result in school expulsion, physical punishment or legal reprimand for the male partner[7,8]. The use of bio-markers of recent sex in this region have demonstrated that mis-reporting of previous sex is common[9,10].

Several methods to improve sexual behaviour data collection have been developed, including the use of audio computer-assisted self-interviews (ACASI). This method uses a desktop, laptop, tablet, or palm-top computer with headphones to play pre-recorded spoken questions, and the respondent selects answers using a mouse, keyboard or touch screen. It has been postulated that this method might reduce social desirability bias, intra-observer variability and embarrassment since the respondent has no direct human interaction while completing the questionnaire[11].

A systematic review of studies comparing ACASI with face-to-face (FtF) interviews in developing countries concluded that ACASI yielded higher overall reporting of sexual behaviours, and lower non-response rates[12]. A second review showed that increased reporting with ACASI is not consistently seen in studies in Africa[13]. The number of studies in both reviews was limited, and significant heterogeneity was seen between studies. Low and middle-income countries are diverse with respect to computer access, literacy, numeracy and social norms relating to adolescent sexual behaviour. It is therefore essential to explore the performance of ACASI in different settings in sub-Saharan Africa[12].

To evaluate the feasibility and acceptability of ACASI in adolescent girls in Tanzania and to compare rates of sexual behaviour disclosure between ACASI and FtF-interviews, we nested a cross-sectional comparison of ACASI and FtF-interview within a cohort study

examining acquisition of human papillomavirus (HPV) infection around the time of sexual debut.

Methods

HPV Epidemiology Study

The ACASI sub-study was carried out at the 12-month visit of the HPV Epidemiology Study, a cohort study investigating the epidemiology of HPV infection around the time of sexual debut in adolescent girls, which is described in detail elsewhere[14]. Briefly, between January and August 2012, 481 girls who reported no previous sex at enrolment, and 22 who reported sex (to reduce stigmatisation), who were aged 15-16 years, and who were attending or had attended 82 randomly selected primary schools, were enrolled. The schools were randomly selected from all primary schools in 3 districts in Mwanza Region in north-west Tanzania. Two of these districts were in Mwanza city and one was in a neighbouring rural district. Participants were seen every 3 months for a total of 18 months. At each visit, girls had a FtF-interview with a female study nurse using a structured pre-tested paper questionnaire, and provided one self-administered nurse-assisted vaginal swab. Questions were written in English, translated into Swahili and back-translated for verification, and included colloquial terms for sexual behaviours that had been collected during focus group discussions with similar-aged girls prior to commencement of the cohort study. The questionnaire covered demographic and socio-economic details, non-penetrative sexual behaviours including kissing, breast touching, oral sex, finger-genital contact with a partner, penetrative vaginal sex, anal sex and the numbers and characteristics of sex partners. At each follow-up visit, participants were asked about sexual behaviours since they were last seen by the study nurse. In order to check for pregnancy risk and potential passing of sexual debut between study visits, nurses had access to information on whether the participant had previously disclosed at an earlier visit ever having had sex, or breast touching or lip kissing (behaviours suggesting they were becoming increasingly sexually active). This information was coded on a form that was kept confidential. Nurses were trained to probe once if the participant offered an inconsistent response for a question that contradicted previously reported behaviours. Nurses did not disclose the answers from earlier visits and recorded the final answer given.

Sub-study population

Girls were randomly selected for this sub-study. To be eligible, the ACASI sub-study participants had to have attended the first follow-up visit (month 3), and to be resident in Mwanza city districts (Nyamangana and Ilemela) at that visit. The districts were selected for study logistics, and comprised both urban and rural administrative areas (known as wards). Further eligibility criteria were: attendance at the visit at which the sub-study was performed (month 12) and resident in Mwanza city districts at that visit, and being willing to participate in the sub-study.

Sub-study methods

Eligible girls who agreed to participate underwent both ACASI and FtF-interviews, the order of which (i.e. ACASI first or FtF-interview first) had been randomly allocated at the time they were selected for inclusion, in a 1:1 ratio. Both interviews had to be completed within 3 consecutive days. All interviews took place in private at the participant's home, school or a nearby health centre, depending on participant preference. The ACASI screen was only visible to the participant once she commenced the interview and FtF interviews were conducted where the interviewer and participant could not be overheard or seen.

Ethics

The London School of Hygiene and Tropical Medicine (LSHTM) Ethics Committee and the Ethics Committee of the Medical Research Coordinating Committee (MRCC) in Tanzania approved the main study protocol (LSHTM approval 6040, MRCC approval NIMR/HQ/R 8a./Vol IX/1249). Since participants were under 18 years old at the time of enrolment into the main HPV Epidemiology Study, written informed parental consent was required before participants were approached for their assent for the main study.

Both ethics committees approved the ACASI sub-study as an amendment to the original study protocol, and did not require alteration of the informed consent from the main study (LSHTM amendment approval A354, MRCC amendment approval NIMR/HQ/R 8a./Vol I/241). At the time of enrolment into the ACASI sub-study, it was explained to the selected participants and they were invited to participate. Separate written consent/assent was not requested for the sub-study. Participants were compensated for their time at each follow-up visit of the main study with a health-related item such as soap or a toothbrush. No additional compensation was offered for participating in the ACASI sub-study.

ACASI development

Development of the ACASI questionnaire and data management used QDS™ Version 2.6.1 software (NOVA Research Company, Bethesda, USA). The ACASI interviews were administered using a touch-screen tablet computer (ViewSonic® ViewPad 10Pro with Windows 7 Operating System). Structured questions, and a choice of answers or a number keypad, appeared on the screen at the same time as the audio-recorded female voice (in Swahili) was heard through headphones. The touch screen only became responsive once the audio track, including instructions on how to answer and the available answers, had finished. No pictures were shown on the screen. There were 8 initial questions designed to help the participant practice using the tablet and which were used as a final competence-check at analysis. These questions covered non-sensitive topics, including ever having made a trip to the market, the number of people living in the house and the person the respondent last spoke to before ACASI, and were completed by the participant with the assistance of a non-clinical research assistant. These 8 initial questions were then re-started and the participant was requested to complete the questions alone. The participant could then choose to repeat the 8 questions any number of times before commencing the interview. Participants who incorrectly answered 2 key competence-check questions of the 8 practice questions at the final attempt ('Are you in Tanzania now?' and 'Are you a boy or a girl?') were permitted to complete the full interview, but their data were excluded from the analysis. The questions in ACASI were a sub-set of questions from the FtF-interview and the same wording was used in both, including colloquial terminology to describe sexual behaviours such as oral sex. Participants were asked whether they had ever had vaginal sex and, for other sexual behaviours, whether they had experienced these since they were last seen in the study.

In the FtF questionnaire, nurses were not able to enter 'don't know' for the question on whether the participant had ever had sex. The limited response option for that question was intentionally included in the FtF-questionnaire from the start of the main HPV Epidemiology Study so that participants were probed to answer the question. It was felt that in ACASI the 'don't know' response should be included for this question because the format would then be consistent throughout the interview, and would prevent those who were confused from guessing a yes/no answer. The 'don't know' responses in ACASI are presented, but the analysis of the reporting of ever having had sex was restricted to participants who did not select 'don't know' responses in ACASI. Once participants had completed both interviews, the research assistant conducted a brief

face-to-face interview in Swahili to ascertain the participant's experience with, and opinions of, ACASI.

Data management and statistical analysis

FtF-interview questionnaires were double-entered into OpenClinica LLC (Akaza Research, Waltham, MA, USA). ACASI data were downloaded at the end of every day from the tablet computer. Data were merged and analysed using STATA V12.0 (StataCorp LP, College Station, Texas, USA) and are available on request from the Data Access Committee at the Mwanza Interventional Trials Unit, via the website http://www.mitu.or.tz. Participant characteristics were summarised using information collected during the FtF-interviews. Cross-tabulations of reported behaviours were performed, and agreement measured using kappa statistics. The Mainland-Gart test[15] for paired data was used to compare the proportion reporting each behaviour by (a) the order of interviews, and (b) whether the interviews were performed on the same or different days. Odds ratios (OR) and 95% confidence intervals (CIs) for the association of interview method with disclosure of each behaviour were calculated using exact conditional logistic regression. Interaction terms were not incorporated into the model since numbers reporting behaviours were small.

In secondary analyses, we compared reporting in FtF-interviews in those who had versus had not completed a prior ACASI, using Fisher's exact tests, in order to investigate the effect of previous ACASI on FtF-interview reporting. We further compared reporting in ACASI in those who had not completed a previous FtF-interview versus reporting in FtF-interview in those who had not completed a prior ACASI, to examine whether reporting differences between ACASI and FtF-interview in this sub-group were similar to the primary analyses.

Sample size calculations

Statistical power for the comparison of disclosure of sexual behaviours was estimated using methods for matched case-control studies, where the OR is calculated from the number of discordant pairs (those who answer differently between each method)[16]. A sample size of 200 participants was selected. For a behaviour with a disclosure prevalence of 10% in FtF-interview and 15% in ACASI (reported sex was expected to be this or higher in the study population[17,18]), this sample size gave 85% power to detect an OR of 6.0 or greater.

Results

Of 503 participants enrolled in the cohort study, 334 attended the 3 month visit and were resident in one of the two selected districts (Figure 6.1). Of those, 230 (69%) participants were randomly selected to participate in the ACASI sub-study. At the 12 month visit, 12 girls had moved outside the study districts, 7 had withdrawn from the cohort study, 4 were not found and 2 were deaf and not able to hear questions through the headphones. All 205 remaining participants were invited to complete an additional ACASI questionnaire and agreed to do so. Two were excluded from the analysis because they incorrectly answered the competence-check question "Are you in Tanzania now?".

Of the 203 included participants, 53% and 47% were aged 16 and 17 years, respectively (Table 6.1), the majority (72%, N=147) were attending secondary school, and one participant was married. Overall, 104 (51%) participants underwent ACASI first. Most participants (89%, N=181) underwent both interviews on the same day, 17 (8%) had the second interview on the following day and the remainder (2%, N=5) were interviewed two days later. The order of the interviews (ACASI first or FtF-interview first) was evenly distributed amongst those interviewed by both methods on the same day or different days (Fisher's exact test p>0.99). Half of the interviews (50%, N=101) took place in the participant's home, 71 (35%) at school, 25 (12%) at a clinic and 6 (3.0%) in other locations.

Twice as many participants reported that they had kissed a boy on the lips since they were last seen during ACASI compared to the FtF-interview (14 (7.4%) versus 7 (3.4%) respectively, p=0.05, kappa=0.55, Table 6.2). The OR of disclosure of having kissed on the lips with ACASI compared to FtF-interview was 8.0 (95% CI 1.1-355.0) (Table 6.2). Conversely, there were more than twice as many reports of ever having had vaginal sex during FtF-interview than by ACASI (15 (7.4%) versus 6 (3.0%) respectively, p=0.02, kappa=0.45). The OR of disclosure of ever having had sex with ACASI compared to FtF-interview was 0.10 (95% CI <0.01-0.70).

The reporting of other non-penetrative sexual behaviours was infrequent in both interviews, with no strong evidence of increased reporting by either interview method. Five girls reported recent kissing with tongues during FtF-interview, while 6 reported this in ACASI, and 6 reported breast touching in both interviews. Both the reporting of kissing with tongues and breast touching showed good agreement (kappa=0.91 and 0.66 respectively). In each of the ACASI and FtF-interviews, 3 participants reported that they had recently touched a boy's penis with their hand; 2 of the 3 reported this in both

interviews. In FtF-interview, 2 participants reported that a boy had touched their vagina with his hand, compared to 3 reporting this in ACASI (kappa=0.39). Only one participant reported this in both interviews. Oral-penile and oral-vaginal contact were both reported once in a FtF-interview and once in ACASI, by the same participant (kappa for both=1.00). Anal sex was reported by one participant during ACASI, and not reported during FtF-interview.

There was no evidence of an effect of interview order (ACASI first or FtF-interview first) (Table 6.2) or of whether the interview was carried out on the same day or different days (p=1.0 for all behaviours except hand-vaginal contact where p=0.69) on the reporting of sexual behaviours, although the power for these comparisons is low due to the low numbers reporting these behaviours. In the secondary analyses, comparing reporting in only FtF-interviews, there was no evidence of an effect on reporting of having previously completed an ACASI (Supplementary Table 6.4). Comparing reporting in ACASI with reporting in FtF-interview from the first interviews only (i.e. ACASI without a prior FtF-interview, and FtF-interview with a prior ACASI), we observed similar trends as for the primary analyses (Supplementary Table 6.5), but the power for the comparisons in these secondary analyses was low due to the low frequencies of reporting of behaviours. Combining any sexual behaviour into a single variable did not demonstrate any significant difference between interview methods.

The majority of participants felt that ACASI was more secret than FtF-interview (83%, N=168), and preferred ACASI to FtF-interview for questions about sex (84%, N=171) (Table 6.3). Two-thirds of participants (70%, N=141) found ACASI "easy" and 28% (N=57) found it "very easy". One participant (0.5%) found it "difficult".

Discussion

This study contributes important evidence to the debate around the feasibility, acceptability and validity of performing sensitive interviews using electronic self-completed questionnaires in adolescents in a sub-Saharan African setting. In Tanzanian girls aged 16-17 years, we have demonstrated significantly higher reporting of kissing with ACASI compared to FtF-interviews, but significantly lower reporting of ever having had penetrative vaginal sex.

To our knowledge, our findings are the first published evaluation of ACASI in Tanzania, and the results conflict with some previous studies from the region. Most studies of ACASI in sub-Saharan Africa, but not all, have shown higher reporting of sensitive behaviours compared to standard interview methods[12,13]. A large randomised trial, in 1495 individuals aged 15-23 years in Zimbabwe who had completed a baseline interview, allocated participants to one of four interview methods and found that the adjusted odds of reporting sex were doubled with ACASI compared to self-completed questionnaires (SCQ) or interviewer-administered questionnaires where the participant placed sensitive answers in a confidential voting box (12% reported sex with SCQ or using the confidential voting box compared to 16% with ACASI)[19]. Within a sub-set of 395 participants who completed both ACASI and SCQ, this trend held although was weaker. Other studies have nested a test-retest comparison (within the same participants) of methods within a longitudinal study, in a similar methodology to our study. A comparison of ACASI versus FtF-interview in male and female sex workers in Kenya aged 23-32 years was nested in the enrolment visit of a cohort study and demonstrated significantly higher reporting of sex partners in the past week and intra-venous drug use with ACASI, but no significant difference in the reporting of condom use, anal sex or forced sex[20]. A microbicides trial in Malawi compared ACASI used at a single visit to their standard FtF-interview which was used during quarterly follow-up (participants had completed a varying number of previous visits)[21]. In 585 women aged 18-53 years, reporting of anal intercourse and number of sex partners was higher using ACASI, and reported condom use was lower. Finally, a cohort study of adolescent sexual behaviour and schooling in Malawi, with participants aged 16-18 years, used ACASI administered at every annual round. This was compared with FtF-interview at the third round, and no significant difference was found in the reporting of sex[22]. These studies, all of which examined responses given by the same individual exposed to both ACASI and FtF-interviews during a longitudinal study, showed either higher reporting of sensitive sexual behaviours with ACASI, or no significant difference between the methods, in contrast to our finding of lower reporting of vaginal sex with ACASI.

An explanation for the significantly lower reporting of vaginal sex with ACASI observed in our study may be related to the longitudinal design and intensity of follow-up. Of the previous studies, one tested ACASI at the first interaction with a participant[20] and one nested a one-off FtF-interview when ACASI was used at every visit[22]. In our study, similar to the microbicides trial cohort in Malawi[21], participants had already seen a

study nurse at enrolment and at up to 3 follow-up visits, during which they had been asked sensitive questions on sexual behaviour and intravaginal practices. We propose 3 possible explanations for our results. Firstly the participants may have developed a more trusting and comfortable relationship with the nurses in our study, since our study team consisted of only 3 nurses who were very experienced in counselling and adolescent sexual behaviour research, and each nurse tried to see the same participant at each interview. Secondly our nurse-interviewers were encouraged to probe once, specifically if there were non-verbal cues in the adolescent suggesting commencement of sexual activity (e.g. changes in physical appearance, changes in body language during discussion of sexual practices), and thirdly our nurse-interviewers had access to previous data which would allow them to probe if there were inconsistencies in the participant's reporting of sexual behaviour over time. This last point is especially relevant since it has been clearly shown that, irrespective of interview method, individuals change their disclosure of sexual behaviours during longitudinal follow-up (e.g. reporting a lower number of sex partners over time)[23,24]. In the previous cohort studies comparing FtF-interviews with ACASI, it is not clear whether interviewers had access to previously-reported sexual behaviour data [21,22].

Explaining the meaning of medicalised terms for sexual practices is essential for accurate data collection, especially since what constitutes "sex" is not consistent within and between populations[25,26], and an interactive discussion of this may be helpful. In our study, sexual behaviours were described graphically (e.g vaginal sex was explained as "a penis going inside a vagina") and colloquial terms for non-penetrative sex behaviours such as oral-genital contact were also used (e.g. the Swahili term "kula muhogo", which directly translates as "eating cassava", is a colloquial term for fellatio used by some adolescents and adults in our study area). In a FtF-interview, the nurse could check the participant's understanding of each of these terms if the participant appeared confused, and the participant could ask questions. However, in ACASI, although the participants had heard these terms during previous FtF-interviews, the recorded question was followed by the audio list of colloquial terms. This may have been confusing, and could be one explanation for the lower rates of sex reported in ACASI. Previous studies have argued that use of images in sensitive interviews, for example in coital diaries, can improve the reporting of sensitive behaviours[27,28]. Such images were used in ACASI (but not the FtF-interview) in the Zimbabwe study which found higher reporting of sexual acts with ACASI[21]. However, we were advised that, because our study participants were considered legal minors, incorporation of explicit

images of sexual behaviours into ACASI was not appropriate.

Although we found lower reporting of sex with ACASI versus FtF-interview, there was significantly higher reporting of kissing on the lips but not kissing with tongues. This could also be due to a misunderstanding of what constitutes a sexual kiss on the lips and, with no nurse to clarify this, over-reporting could have been seen in ACASI.

Overall reporting of anal sex and non-penetrative sex behaviours was low, either because of under-reporting or because these behaviours are uncommon in girls of this age-group in the region[7]. Kissing and breast-touching were reported by girls who had, and those who had not, previously reported sex. However, oral sex and hand-genital contact were only reported by girls who also reported previous sex. The low frequency of reporting limited the power to detect a difference between reporting methods. This also limited the power to detect the effect of the order of the interviews or time between interviews, on reporting.

We were not able to validate reporting of sex with biological markers in this study. Although vaginal swabs were collected, and will be tested for HPV which is a common sexually transmitted virus, a longitudinal study conducted in the USA has shown that only 39% of girls acquired HPV during the 24 months after first sex, and therefore this infection has a limited role as a marker for sexual debut in our study[29]. In our region, antibodies to HSV2 have been shown to be a marker of sexual activity in females aged 15-29 years[30]. However, given the age of our study participants, as well as the selection criteria (which, for the majority, included reporting no previous sex), the number of times of previous sex in those reporting and not reporting previous sex may have been low. Since the transmission probability of HSV2 at first sex is not 100%, and it is not recommended as a marker of sexual debut, its use as a biomarker in our study may have been limited[31]. Alternative vaginal biomarkers of previous sex such as Y-chromosome DNA, semenogelin or prostate-specific antigen can only be utilised to validate self-reported sex if samples are taken within days of reported sex[32]. The use of in-depth interviews may have offered another source of data for validation of the interview methods, and may have provided insight into participants' understanding of questions during ACASI[10].

Further research should explore how well questions are understood with ACASI compared to other interview methods, and further attempts should be made to validate ACASI using in-depth interviews or biomarkers such as HSV2 if appropriate to the study population. Finally, our data demonstrate that the disclosure of sensitive behaviours with ACASI is population-specific and this method should be tested locally before implementation in studies collecting such data.

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Table 6:1. Socio-demographic characteristics of 203 girls in Tanzania in the ACASI versus FtF-interview sub-study

		Number (%)
Age (years)	16	108 (53.2)
	17	95 (46.8)
Currently married / living as married	No	202 (99.5)
	Yes	1 (0.5)
Area of residence	Urban	114 (56.2)
	Rural	89 (43.8)
Lives with	One or both parents[1]	142 (70.0)
	Other relative or friend	57 (28.1)
	Husband	1 (0.5)
	Employer	3 (1.5)
Occupation	Primary School	13 (6.4)
	Secondary School	147 (72.4)
	Working / vocational training	13 (6.4)
	At home & not working or schooling	30 (14.8)
Religion	Christian	179 (88.2)
	Muslim	21 (10.3)
	Other religion	2 (1.0)
	None	1 (0.5)
Passed menarche	No	12 (5.9)
	Yes	191 (94.1)

1. Living with one or both parents does not exclude other family members residing in the household

Table 6:2. Reporting of sexual behaviours in FtF-interview and ACASI in 203 girls in Tanzania aged 16 and 17 years

Sexual behaviour	Reported	FtF-interview N (%)	ACASI N (%)[2]	Responded yes in both N (%)[2]	Kappa	p for diff by order[3,4,5]	Odds Ratio[6] (95% confidence interval)	p-value[7]
Kissed (on lips) since last seen	Yes	7 (3.4)	14 (7.4)	6 (4.0)	0.55	0.44	8.0 (1.1-355.0)	0.04
	No	196 (96.6)	176 (92.6)					
	Don't know	0	13					
Kissed (using tongues) since last seen	Yes	5 (2.5)	6 (3.0)	5 (3.5)	0.91	1.0	- [8]	-
	No	198 (97.5)	191 (97.0)					
	Don't know	0	6					
Breast touching since last seen	Yes	6 (3.0)	6 (3.1)	4 (3.0)	0.66	1.0	1.0 (0.1-13.8)	1.00
	No	197 (97.0)	189(96.9)					
	Don't know	0	8					
Hand (F)- penis contact since last seen	Yes	3 (1.5)	3 (1.5)	2 (1.5)	0.66	1.0	1.0 (0.0-78.5)	1.00
	No	200 (98.5)	194 (98.5)					
	Don't know	0	6					
Hand (M)-vagina contact since last	Yes	2 (1.0)	3 (1.5)	1 (1.0)	0.39	1.0	2.0 (0.1-118.0)	1.00
	No	201 (99.0)	196 (98.5)					

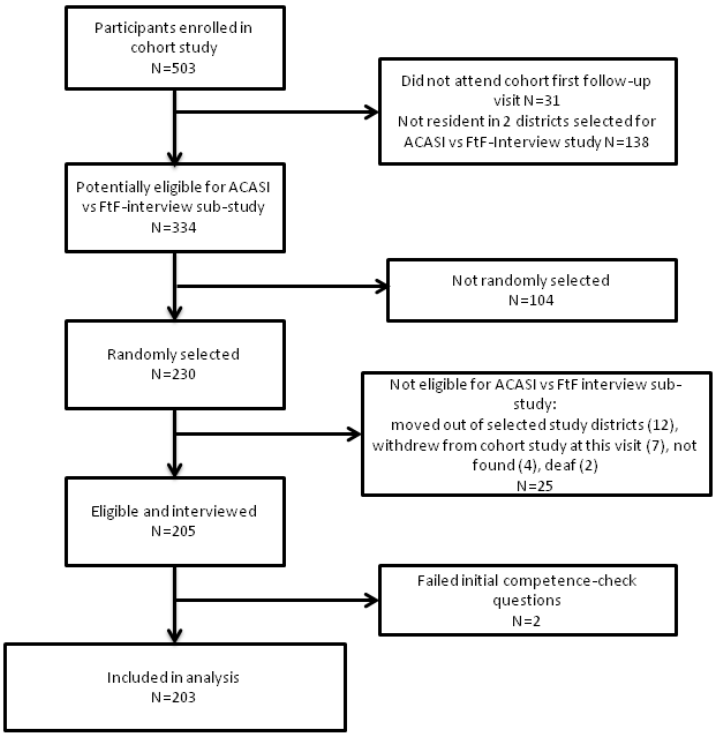
seen	Don't know	0	4					
Oral (F)-penis contact since last seen	Yes	1 (0.5)	1 (0.5)	1 (1.0)	1.0	1.0	- [8]	-
	No	202 (99.5)	197 (99.5)					
	Don't know	0	5					
Oral (M)-vagina contact since last seen	Yes	1 (0.5)	1 (0.5)	1 (1.0)	1.0	1.0	- [8]	-
	No	202 (99.5)	196 (99.5)					
	Don't know	0	6					
Anal sex since last seen	Yes	0	1 (0.5)	0	1.0	1.0	- [8]	-
	No	203 (100)	197 (99.5)					
	Don't know	0	5					
Vaginal sex, ever	Yes	15 (7.4)	6 (3.0)	5 (2.5)	0.45	1.0	0.10 (<0.01-0.70)	0.01
	No	188 (92.6)	192 (97.0)					
	Don't know	-[1]	5					

M is male, F is female. 1. For FtF-interviews, the "don't know" response was not available for questions on ever having had vaginal sex. 2. The denominator is those responding either yes or no, and does not include those who responded "don't know" in either interview. 3. From Mainland-Gart test; "don't know" responses were dropped from the analysis. The Mainland-Gart test compares the discordant responses by sequence (ACASI first or FtF first) so the p values are not based on the cell frequencies as displayed in this table. 4. Test for difference in response depending on which interview was given first (FtF-first or ACASI first). 5. p=1.0 indicates that the distribution is degenerate, due to one of the marginal totals for the Mainland-Gart test being zero. 6. Odds of reporting the behaviour in ACASI versus reporting the behaviour in FtF-interview from exact conditional logistic regression; "don't know" responses were dropped from the analysis. 7. Exact p-values based on the binomial distribution. 8. Odds ratios could not be estimated because there was only one or no discordant pairs.

Table 6:3 Perceptions of ACASI and FtF-interview in 203 Tanzanian girls aged 16 and 17 years

		Number (%)
Secrecy of ACASI compared to FtF-interview	ACASI more secret	168 (82.8)
	ACASI less secret	4 (2.0)
	Equal secrecy	26 (12.8)
	Don't know	5 (2.5)
Prefer ACASI or FtF-interview for questions on sex	ACASI	171 (84.2)
	Face-to-face	15 (7.4)
	No preference	6 (3.0)
	Don't know	11 (5.4)
ACASI difficult or easy to complete	Very easy	57 (28.1)
	Easy	141 (69.5)
	Neither easy nor difficult	4 (2.0)
	Difficult	1 (0.5)
	Very difficult	0
	Don't know	0

Figure 6:1. Flow diagram of inclusion in the ACASI versus FtF-interview sub-study.



Supplementary tables

Table S6:4 Reporting during FtF-interview in those who completed the FtF-interview first (without prior ACASI) and reporting during FtF-interview in those who completed the FtF-interview after a prior ACASI

Sexual behaviour	Reported	FtF-interview in those who had not completed a prior ACASI N (%)	FtF-interview in those who completed FtF-interview after ACASI N (%)	p[1]
Kissed (on lips) since last seen	Yes	2 (2.0)	5 (4.8)	0.44
	No	97 (98.0)	99 (95.2)	
	Don't know	0	0	
Kissed (using tongues) since last seen	Yes	1 (1.0)	4 (3.9)	0.37
	No	98 (99.0)	100 (96.2)	
	Don't know	0	0	
Breast touching since last seen	Yes	1 (1.0)	5 (4.8)	0.21
	No	98 (99.0)	99 (95.2)	
	Don't know	0	0	
Hand (F)- penis contact since last seen	Yes	0 (0.0)	3 (2.9)	0.25
	No	99 (100.0)	101 (97.1)	
	Don't know	0	0	
Hand (M)-vagina contact since last seen	Yes	0 (0.0)	2 (1.9)	0.50
	No	99 (100.0)	102 (98.1)	
	Don't know	0	0	
Oral (F)-penis contact since last seen	Yes	0 (0.0)	1 (1.0)	1.0
	No	99 (100.0)	103 (99.0)	
	Don't know	0	0	
Oral (M)-vagina contact since last seen	Yes	0 (0.0)	1 (1.0)	1.0
	No	99 (100.0)	103 (99.0)	
	Don't know	0	0	
Anal sex since last seen	Yes	0 (0.0)	0 (0.0)	1.0
	No	99 (100.0)	104 (100.0)	
	Don't know	0	0	

M is male, F is female. 1. Calculated using Fisher's exact test

Table S6:5. Reporting during ACASI in those who had not completed a prior F2F-interview and reporting in F2F-interview in those who had not completed a prior ACASI

Sexual behaviour	Reported	FtF-interview in those who had not completed a prior ACASI N (%)[1]	ACASI in those who had not completed a prior FtF-interview N (%)[1]	p[3]
Kissed (on lips) since last seen	Yes	2 (2.0)	7 (7.1)	0.17
	No	97 (98.0)	92 (92.9)	
	Don't know	0	5	
Kissed (using tongues) since last seen	Yes	1 (1.0)	4 (3.9)	0.37
	No	98 (99.0)	98 (96.1)	
	Don't know	0	2	
Breast touching since last seen	Yes	1 (1.0)	5 (5.0)	0.21
	No	98 (99.0)	96 (95.0)	
	Don't know	0	3	
Hand (F)- penis contact since last seen	Yes	0 (0.0)	2 (2.0)	0.50
	No	99 (100.0)	99 (98.0)	
	Don't know	0	3	
Hand (M)-vagina contact since last seen	Yes	0 (0.0)	2 (1.9)	0.50
	No	99 (100.0)	101 (98.1)	
	Don't know	0	1	
Oral (F)-penis contact since last seen	Yes	0 (0.0)	1 (1.0)	1.0
	No	99 (100.0)	101 (99.0)	
	Don't know	0	2	
Oral (M)-vagina contact since last seen	Yes	0 (0.0)	1 (1.0)	1.0
	No	99 (100.0)	101 (99.0)	
	Don't know	0	2	
Anal sex since last seen	Yes	0 (0.0)	0 (0.0)	1.0
	No	99 (100.0)	101 (100.0)	
	Don't know	0	3	
Reported any sexual behaviour[2], ever	Yes	8 (8.0)	10 (4.9)	0.81
	No	91 (92)	93 (90.3)	
	Don't know[4]	0	1	

M is male, F is female. 1. The denominator is those responding either yes or no, and does not include those who responded "don't know" in either interview. 2. This combined variable excludes vaginal sex. Vaginal sex is not included since the "don't know" option was not available in FtF-interviews. 3. Calculated using Fisher's exact test. 4. Includes only those who answered "don't know" to all sexual behaviours.

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7 Discussion

7.1 Summary of results

This chapter summarises the key results from each publication and how these link together. Since manuscripts 1 and 2 describe HPV prevalence and natural history in girls who did not report sex, they are summarised together.

7.1.1 Manuscript 1 and 2: Prevalence, incidence and clearance of HPV in adolescent girls before reported sexual debut

The first and second manuscripts in this thesis aimed to determine the prevalence and incidence of HPV in girls who reported no previous sex, and associated risk factors. The HPV vaccine is recommended for girls before first sex, since it has the highest efficacy in girls and women who have not been exposed to HPV genotypes in currently available vaccines. HPV prevalence and incidence have been described in girls before first sex in high-income countries, but not in SSA where the highest incidence of cervical cancer is seen and, therefore, where HPV vaccines may have the largest impact on cervical cancer incidence and mortality.

The prevalence of HPV DNA of any genotype in 474 girls who were enrolled into the cohort and who reported no previous sex was 8.4%, and of HR HPV DNA was 5.3%. HPV prevalence in these girls was associated with a more than doubled odds of ever having practiced intravaginal cleansing, with evidence of a dose response. It is possible that reported intravaginal cleansing at enrolment was a marker for unreported sex[1]. In a randomly selected subset of this group who did not report sex during 18 months of follow-up, and excluding one who spontaneously reported being HIV positive (119 girls), 51 new HPV infections were detected in 438 (11.6%) visits attended. The overall incidence rate of new HPV infections in these girls was 29.4 per 100 person-years (95%CI:15.9-54.2). Reporting having recently, or ever, cleansed inside the vagina during follow-up was not associated with a new incident HPV infection. The detection of a new HPV infection was associated with having recently (in the past 6 months) spent a night away from home and, therefore, may be due to unreported sex. This finding supports the delivery of the HPV vaccine to girls several years before the expected age of sexual debut in a population.

Although HPV was detected in 8.4% of 15 or 16 year old girls at enrolment who reported no previous sex, only 0.8% of these girls had infection with HPV16 or -18. During follow-up of these girls who reported no previous sex, HPV16 had the highest incidence, but was only detected in 2.1% of follow-up samples. HPV16 and -18 are associated with over 70% of cervical cancer cases, and are covered by both the bivalent and quadrivalent HPV vaccines which have a high efficacy at preventing infection and precancerous lesions with these genotypes[2,3]. Since

HPV16 and -18 were infrequently seen, the HPV vaccines are likely to have high efficacy against both infection with HPV16 and -18 and pre-cancerous lesions caused by these genotypes, even if given to girls who are 4-5 years older than the target age of 9-13 years.

7.1.2 Manuscript 3: HPV Incidence around the time of sexual debut in adolescent girls in Tanzania.

The third manuscript in this thesis describes the acquisition, duration and clearance of HPV in girls after reported sexual debut. Previous studies in middle and high-income countries have shown a very high cumulative incidence of HPV around the time of sexual debut (38.9% at 24 months)[4], and factors associated with HPV incidence, as well clearance have been identified in these girls[5–7]. There are few studies that describe HPV incidence and clearance in SSA[8–10], and none describing this around the time of sexual debut when incidence is likely to be highest.

Girls included in this analysis were those who reported that they had passed sexual debut in the year prior to the start of the study (n=29), and those who reported passing sexual debut during the study (n=77). In those who reported sexual debut during the study, and who were HIV negative if tested, the median time from the reported date of first sex to HPV detection was 4.9 months. Within 6 months from the reported date of first sex, 52.8% of girls had acquired HPV, and 35.8% had acquired a HR type. The incidence of a new HPV infection in all girls who reported sex before or during the study was 225 per 100 person-years (95%CI:166-305). The incidence of HPV girls in this study was one of the highest reported in the current literature in studies where incidence in person-years has been presented[10–15].

There was a more than a two-fold risk (aRR 2.48) of acquisition of an incident HPV infection in girls who reported having had sex once (compared to reporting no sex) in the past 3 months. This was in keeping with other studies of HPV incidence in predominantly middle-age women in North and South America, that report that recent sex is associated with HPV acquisition[16,17]. Knowing the most recent sex partner for at least 6 months, compared to knowing them for less than 1 month, was associated with a more than triple risk of new HPV infection (aRR 3.15). The reason for this is not clear, but the number of infections in those who had known their partner for under 6 months was small (26). Unlike other studies, we did not observe strong associations between factors associated with lower risk of HPV infection, such as most recent sex partner being circumcised[18] and condom use[7,19]. This may be because condom use was rare, and because girls were not able to identify whether their male partners were circumcised or not. This has been demonstrated in a study of female partners (age18-50 years) of men in Zambia and Swaziland: 11-15% of women mis-reported their male partner's circumcision (in both directions)[20].

The median duration of infection seen in our study was 6.1 months and was similar for HR and LR genotypes (6.0 and 6.1 months respectively). Studies in younger women in the USA have shown similar duration of infection: in women with a median age 15 years, the median duration of HR/LR HPV types were 7.5/5.6 months[21], and in women with a mean age of 24.2 years, were 9.8/4.3 months[16]. However, this is shorter than in many other reported studies. The median duration of HPV infection in women with a median age of 32.3 years in Columbia was 14.8 /11.1 months for HR/LR infections respectively[17]. Two studies in younger women in the US and USA showed a longer duration of infection that in our study. In the UK, women who had a median age of 18 years had a median duration of infection of 13.7 months[22], and the time to negative test in women with a mean age of 19 years in the USA was 9.4 months[23]. All of these studies included women who were already sexually active. It is likely that the short duration of infection seen in women in our study was due to both younger age and first exposure to HPV at first sex. Additionally, differences in host genetics, which influence immune response and therefore HPV clearance, and variance within HPV genotypes, particularly in HPV16, may explain some of the variation in the duration of infection[24–26].

7.1.3 Manuscript 4: Comparison of Audio Computer-Assisted Self-Interview with face-to-face interview for disclosure of sexual behaviour in adolescent girls in Tanzania

The fourth manuscript in this thesis describes the examination of alternative methods for the collection of sensitive sexual behaviour data in the study population. This study was conducted after we observed a higher than expected prevalence of HPV in girls who denied previous sexual activity at the enrolment visit of the cohort study, where there might have been potential under-reporting of penetrative sex or non-penetrative sexual behaviours. Higher rates of reporting of sensitive behaviours had been previously demonstrated with the use of ACASI compared to other interview methods in Kenya and Zimbabwe[27,28]. We elected to conduct a comparison of ACASI with FtF-interview at the 12 month visit of the study to examine reporting of sexual behaviours. This comparison could not be performed earlier in the cohort study, as a protocol amendment and resubmission for ethics committee approval were required. Overall, 203 of the 230 randomly selected girls met eligibility criteria and successfully completed an ACASI in addition to their standard FtF-interview, the order of which (ACASI first or FtF-interview first) was randomly allocated.

During ACASI, twice as many participants reported that they had kissed a boy on the lips since they were last seen compared to the reporting of this in the FtF-interview 17.4% vs. 3.4% respectively; p=0.05, kappa=0.55). In contrast, more than twice as many girls reported that they had had vaginal sex during FtF-interview than in ACASI (7.4 vs. 3.0%; p=0.02, kappa=0.45).

Other behaviours (kissing, breast touching, hand-genital contact, oral-genital contact and anal sex) were infrequently reported and were not reported at significantly different rates between interview methods.

These results reflect the challenge of obtaining consistent reports of sexual behaviours. Identifying which method is more valid is not possible, since there is no 'gold standard'; reviews of ACASI compared to other methods of interview demonstrate conflicting results[29–31]. The use of ACASI during the study was feasible, and 97.6% of participants found ACASI easy to use, and 84.2% preferred this interview method to FtF-interview. It is likely that the characteristics of the populations studied (e.g. age, sex, ethnicity, educational level), the setting (e.g. clinics, home), the study design (e.g. longitudinal, cross-sectional) and the technology employed (e.g. desk top computer, tablet, mobile phone) can influence the reporting of sexual behaviours in various studies[27,29,30,32]. In this study, it is likely that the nurse's ability to probe, especially given access to previous data, and the likelihood that nurses developed a trusting relationship with participants during previous FtF-interviews may have led to higher reporting of sex in FtF-interview. ACASI should be evaluated and compared to other interview methods in study settings that have not used this data collection modality before, prior to its use in studies that aim to collect accurate sexual behaviour data.

7.2 Methodological challenges

7.2.1 Strengths

Major strengths of this study include the representativeness of the sample and high retention rate; 416 of the 503 (82.7%) enrolled participants attended the final visit. Participants were enrolled from primary school registers and registration at a primary school is a legal requirement in Tanzania. Included schools were a random selection of all government primary schools in the district and enrolled participants included those living in both rural and urban areas.

The questionnaire included colloquial terms for sexual behaviours collected from girls of the same age and the interviewers employed were highly experienced in sexual behaviour research. Nurses in the study directly observed sample collection and requested repeat samples if necessary, leading to a low proportion of self-administered swabs being considered inadequate.

Another major strength of this study was the frequency of follow-up. HPV incidence and clearance as well as updated data on sexual and other behaviours were measured at 3 monthly visits, allowing more accurate description of the natural history of infection than is available from studies that record these every 4-6 months[4,11,12,14].

7.2.2 Limitations

The major limitations of this study include the potential for misclassification of sexual behaviour data. It is likely that sexual behaviours, including previous penetrative and non-penetrative sex (i.e. kissing, breast touching, hand-genital contact and oral sex) in our population of adolescent girls were under rather than over-reported. This reporting bias is likely to have reduced the strength of associations seen. Further important exposure data include the reporting of intravaginal cleansing. It is not clear whether participants were more or less likely to report this. Ever having performed intravaginal cleansing was reported by 34 of 106 (32.1%) who reported sex at enrolment or follow-up, and by 141 of 397 (35.5%) who did not report sex during follow-up (p difference=0.51). In the baseline data (Chapter 3) prevalent HPV was associated with the reporting of intravaginal cleansing with a clear dose response: increased frequency of cleansing was associated with higher odds of prevalent HPV. If performing intravaginal cleansing is a true risk factor for HPV acquisition, it is likely that a temporal association would be seen during follow-up (i.e. recent intravaginal cleansing would be associated with incident HPV), and no association was seen during follow-up in girls who did, and who did not report sex. However, this may be due to under-reporting of intravaginal cleansing during follow-up. We cannot rule out reporting bias if study nurses discouraged girls from practicing intravaginal cleansing because they were aware of potential adverse health consequences of this practice.

Reporting bias (inaccurate information disclosed by the interviewee) and interview bias (inaccurate information recorded by the interviewer) remain significant problems in studies collecting data on sensitive behaviours. Staff training and the exploration of alternative interview methods are essential techniques to address this. In addition to staff training during this study, ACASI was trialled during follow-up as an alternative interview method. This did not lead to higher reporting of penetrative vaginal sex, and was not continued during the study.

7.2.3 Sample size, study duration and missed visits

Another limitation of this study in terms of describing the incidence, clearance and duration of HPV in girls who reported sex and associated risk factors, was the sample size and the duration of follow-up.

We had aimed to enrol 500 girls who reported that they had not passed sexual debut, of whom 200 (40%) were expected to report sex during 18 months of follow-up based on a previous study in the region[33]. We further intended to include 26 girls who reported sexual debut before enrolment. At the end of the recruitment period, we had enrolled 481 girls who, at the time they were recruited, reported no previous sex (96.2% of the 500 expected), and 22 who

reported having passed sexual debut not more than one year prior to enrolment (84.6% of 26 expected). Of greatest concern was that, of the 481 who reported no previous sex at enrolment, 22.0% (N=106), rather than the 40% expected, reported first sex by the end of the study. This smaller sample size of girls who reported that they had ever had sex at the end of the study led to lower power to detect the same effect size. We were unable to increase the sample size because of logistic and budget constraints.

The proportion of girls expected to acquire HPV over 12 months after sexual debut was 28.5%, based on a study in the USA in girls reporting first sex during follow-up[34]. In our study the median (IQR) follow-up time after first sex was only 5.8 months (1.5-10.4). Therefore, both the number of girls included in the study of incident HPV after first sex, and the number of follow-up visits attended (due to the length of the study) were limited, reducing the period of exposure for analysis. However, due to the high incidence of HPV in the study population, after 6 months 52.8% of girls who had no HPV prior to first sex, and who passed sexual debut during follow-up, had acquired HPV, which mitigated the low power of the small study size. Exposures of interest (condom use, male circumcision, partner in a concurrent relationship, etc.) were reported by only a few girls, resulting in low power to detect associations between these and HPV prevalence, incidence and clearance. Further, because of the limited data, it was difficult to calculate an effect estimate adjusted for all potential confounders, and effect estimates may therefore be biased away from the null.

For budget reasons, in the analysis of girls who reported no sex prior to or during the study, only samples collected at enrolment and at months 6, 12 and 18 were tested for HPV. Samples collected at month 3, 9 and 15 were not tested. Sexual behaviour, intravaginal practice and socio-demographic data that had been collected during those visits (months 3, 9 and 15) were incorporated by generating variables which combined reported behaviour in the current visit and the visit 3 months earlier, if attended. Sensitivity analyses were performed to examine behaviours reported in the previous 3 months compared to behaviours reported over the previous 6 months, and no differences were seen in the associations between behaviour and HPV incidence. Since HPV infections can be transient (the median duration in our study of girls reporting sex was 6.1 months) it is likely that some infections, which were acquired and cleared between visits 6 months apart, were missed. Not capturing these transient infections may have diluted any associations between the infrequently reported non-penetrative sexual behaviours and HPV acquisition (i.e. resulted in a bias towards the null).

7.2.4 Study retention

The final study visit was attended by 416 of the 503 (82.7%) enrolled participants. Table 7.1 summarises sociodemographic factors and sexual behaviours reported at enrolment by participants who did and did not attend the final visit. There were no significant differences in reported sexual behaviours. However, those who did not attend the final visit were more likely to be of non-Christian religion, and were not attending school at enrolment. Importantly, those who did not attend the final visit were more likely to have HPV at enrolment (15 of 87 (17.4%) versus 32 of 415 (7.8%), p<0.001). Since girls did not know their HPV result from the enrolment visit, their attendance was not influenced by the outcome, and this may have only had the effect of reducing the sample size and reducing the power to detect associations between behaviours during follow-up and incident HPV. However, it is also possible that girls who were lost to follow-up were more likely to acquire HPV over follow-up (even if they did not differ in other baseline covariates) compared to those who remained in the study.

Table 7:1. Distribution of selected variables at baseline in girls among those who attended and those who did not attend the final study visit at 18 months

Baseline characteristics	Attended final visit n=416, n (%)	Did not attended final visit n=87, n (%)	p-value[1]
Age (years)			
15	201 (48.3)	37 (42.5)	
16	215 (51.7)	50 (57.5)	0.33
Residence			
Urban	198 (47.6)	47 (54.0)	
Rural	218 (52.4)	40 (46.0)	0.23
Composite household wealth			
Low	138 (33.2)	30 (34.5)	
Medium	139 (33.4)	29 (33.3)	
High	139 (33.4)	28 (32.2)	0.97
Occupation			
Schooling	292 (70.2)	50 (57.5)	
Working or vocational training	4 (1.0)	2 (2.3)	
Not working or schooling	120 (28.9)	35 (40.2)	**0.05**
Religion			
Christian	370 (89.0)	71 (81.6)	
Muslim	36 (8.7)	10 (11.5)	
Non-Muslim non-Christian	10 (2.4)	6 (6.9)	**0.06**
Kissed, ever			
Yes	18 (4.3)	3 (3.5)	
No	398 (95.7)	84 (96.6)	0.71
Hand-genital contact, ever			
Yes	4 (1.0)	1 (1.2)	
No	412 (99.0)	86 (98.9)	0.87
Breast touching, ever			
Yes	37 (8.9)	6 (6.9)	
No	379 (91.1)	81 (93.1)	0.54
Vaginal sex, ever			
Yes	25 (6.0)	3 (3.5)	
No	319 (94.0)	84 (95.6)	0.34
Cleaned inside the vagina, ever			
Yes	80 (19.2)	22 (25.3)	
No	336 (80.8)	65 (74.7)	0.20
HPV (any genotype)[2]			
Positive	32 (7.8)	15 (17.4)	

Negative	378 (92.2)	71 (82.6)	**0.006**
β-globin negative[3]	6	0	

[1]P value is from Chi squared test [2]One participant did not provide a sample at enrolment [3]Those β-globin negative were excluded from the comparison

7.2.5 Unmeasured variables

Due to budget constraints, we were unable to test for the presence of other STIs, with the exception of HIV, since voluntary counselling and testing for HIV was offered at the final visit. Girls who reported sex at any point during the study were verbally screened for symptoms of STIs at the visit they first reported sex, and every subsequent visit irrespective of further reports of sex. Diagnoses were syndromic and included vaginal discharge syndrome (VDS), genital ulcer disease (GUD) and pelvic inflammatory disease (PID). Only 5 of the 106 (4.7%) girls who reported ever having had sex were diagnosed with VDS, and this was not associated with incident HPV during the study. No diagnosis of GUD or PID were made.

Bacterial vaginosis (BV), a predominantly asymptomatic infection, has been associated with the detection of HPV in cross-sectional studies[35] and in longitudinal follow-up of women in the USA[36]. BV has been further associated with a reduced rate of clearance of HPV[36,37]. Infection with *Chlamydia trachomatis,* another reproductive tract infection which can cause asymptomatic infection, has been associated with HPV persistence[38]. It is possible that these unmeasured confounders affected our effect estimates of the association between sexual behaviours and the incidence and clearance of vaginal HPV in girls who reported sex. The detection of these, and other STIs such as Herpes simplex virus type 2 (HSV2) may have provided insights into the frequency unreported sex. However, a study in adolescent girls in the USA suggests that HPV acquisition may occur before bacterial STIs[39]. Although HSV2 has been shown to be a marker of previous sex in girls and women in Mwanza[40], measuring incident HSV2 would have required regular blood tests, potentially reducing cohort retention. Furthermore, the transmission of HSV2 per-sex act is not 100% suggesting that this may not be a reliable marker of sexual debut[40,41].

Host genetics (Human Leukocyte Antigen-type) influence both susceptibility to HPV infection, and the duration of HPV infection[42,43]. However, measurement of this was beyond the budget for this study and again would have required blood sampling.

HPV incidence and persistence are higher in HIV positive women compared to HIV negative women, and the rate of clearance of HPV is lower[12,44,45]. In this study, HIV testing was only offered to girls who reported sex, and only accepted by 49 of 91 (53.8%). One participant tested HIV positive and was excluded from the analysis of HIV incidence and clearance in girls who reported sex. Participants who reported no previous sex at enrolment or during the study were

not offered an HIV test, or asked about their HIV status. One participant in this group spontaneously reported being HIV positive (through presumed mother-to-child transmission), and was also removed from the analysis. Undiagnosed HIV-infected participants in both groups may have unknowingly been included in the analysis, and introduced confounding. However, any effect is likely to be minimal, since the HIV prevalence in Tanzania is reported as only 1.3% in 15-19 year olds[46].

7.2.6 Outcome misclassification

Self-administered vaginal swabs were used for specimen collection in this study. This was particularly challenging in this age-group since some girls had limited knowledge of their anatomy, and there was a risk that samples may reflect HPV genotypes from surrounding regions (e.g. external genital or peri-anal regions). Nurse-assisted self-administered swabs were collected for this reason. During the collection procedure, the study nurse would carefully observe placement of the swab and request repeat sample collection if they were not satisfied that the swab had entered the vagina. Further, β-globin (a marker for human cellular DNA) was considered an important marker of adequate vaginal sampling, and samples which tested negative for β-globin were treated as missing in all analyses. In girls who reported no previous sex, 20 of 438 (4.6%) samples tested were negative for β-globin, and in girls who reported sex, 30 of 353 (8.5%) were negative. Of these 50 β-globin negative samples, 5 samples contained HPV DNA. These possible infections were not recorded in prevalence or incidence estimates. It is possible that samples were contaminated with HPV DNA during testing, but positive and negative controls were run according to test protocols (per 12 samples) and batches of 12 were re-run if these controls were not valid.

Estimates of the duration of genotype specific infections included genotype-specific runs where a negative or missing sample was seen between two positive samples. This approach was based on evidence from studies that had examined intermittent detection of HPV genotypes[47,48]. In these studies, two samples with an intervening negative have been considered to be the same infection if those detected genotypes had very similar DNA sequences, allowing for intermittent non-detection over a short period of time. Genotypes are considered to be the same 'variant' (i.e. the same infection, not a new infection with the same genotype) if the DNA sequence which codes for the L1 protein varied less than 2%[49]. A study in the USA sequenced HPV16 genotypes that had been detected in students tested every 4 months who have an intervening negative sample between two HPV16 positive samples. The same HPV16 variant was detected at visits before and after the intervening negative, and this implied persistent infection rather than re-infection[48]. The same investigators repeated this with a further 10 HPV genotypes and had similar results, where genotypes could be intermittently absent between positive visits

during 4-monthly follow-up. Again these were classified as persistent and not newly acquired infections[47]. We therefore followed this approach and, in situations where there was one negative sample between two positive samples of the same genotype, the negative sample was considered a false negative and this constituted persistent infection with that genotype. This method may have led to the over-estimation of HPV duration. This would not have affected HPV incidence, since women were only considered to be at risk of HPV genotype-specific infection once. A further study to investigate the possibility of false negative samples is planned (section 7.3.2). It will remain challenging to differentiate persistent infection or reactivation of infection from reinfection if there has been no change in partner since theoretically the same HPV genotype variant may be re-acquired in the female partner.

After first sex, the incidence of new HPV infections was very high, and the duration of infection short. In intervals with a prolonged gap between samples (for example, if the girl didn't attend a visit or a valid sample was not provided), it is possible that a genotype may have been acquired and cleared. Any infection detected after a prolonged gap may not have been the first time the genotype was acquired. Therefore in the analysis of genotype-specific incidence among girls after reported first sex, if there were gaps of over 180 days between samples, the observation time was censored at the date of the last available sample before the gap. However, this approach led to the exclusion of incident infections that occurred after gaps, particularly for the more common genotypes. For example, all incident infections of HPV61 occurred after gaps in observation time. As a result, the rate of HPV61 was underestimated; in the sensitivity analysis where girls were allowed to become at risk again after gaps, the rate of HPV61 was 10.9 per 100 person-years. When the observation time after the gap was censored, the rate of HPV61 was 0 per 100 person-years. For other genotypes, there was no substantial change in incidence rate.

Gaps of over 180 days were also removed from the observation time in the analysis of any new HPV infection. However, since the acquisition and clearance of an HPV genotype during a gap would not substantially alter the risk of infection with other genotypes[50], in this analysis, girls were allowed to contribute at-risk time after the gap.

7.3 Future research

7.3.1 Fomite transmission

HPV was associated with intravaginal cleansing in girls who reported no previous sex at enrolment. The reporting of intravaginal cleansing was most likely a marker for under-reported sex. However, mucosal HPV types have been detected on fingernails of adolescent girls[51], on

the fingernails women and men with genital warts[52], and on toilet seats[53]. The ability of HPV to remain infectious to mouse cells after desiccation for up to 7 days has also been shown[54]. It is therefore possible that vaginal HPV infection could be acquired from shared cloths, washing basins, fingers or external genitals, since intravaginal cleansing involves the insertion of fingers or a cloth quite deep inside the vagina[55].

A fomite investigation study is therefore underway. Samples have been collected from bathroom surfaces of study participants using a Dacron swab, and from the fingertips of study participants using a cervical brush at the final visit. These will be tested using the Roche Linear Array to determine, in the first instance, whether HPV DNA can be detected on these potential fomites. If HPV DNA is detected, further studies investigating viral viability may be indicated.

In addition to the collection of samples from households and the fingertips of participants, we have collected oral rinse samples to determine the prevalence of oral HPV in the study population. HPV is associated with head and neck cancers, yet little data are available on oral HPV prevalence in sub-Saharan Africa[56].

7.3.2 Persistent infection with intermittent detection

In longitudinal follow up in this study, a negative sample between two samples positive for the same genotype was considered a false negative. The final positive sample in this situation could be due to re-infection with the same genotype (possibly from the same or a new sex partner) or the intermittent detection of a persistent infection. Only a few studies (and none from Africa) have sequenced the HPV genome of pairs of samples where there was an intervening negative sample, and considered this a false negative if the two samples on either side were considered the same 'variant', determined by similarities in the HPV DNA genome[47,57]. We have a unique opportunity to examine longitudinal sexual behaviour data and HPV DNA variance in genotype-specific sample pairs between which there was one negative sample. The available sexual behaviour data, detailing whether participants did or did report sex with the same, or a new partner will add valuable information and contribute to the understanding of intermittent detection of HPV genotypes in women. This study is currently under discussion with ICO.

7.3.3 Extending the age of vaccination in Tanzania

It has been clearly established that providing HPV vaccination to girls age 9 to 13 years is a cost-effective method of reducing cervical cancer[58]. Modelling studies in high-income countries have suggested that extending the age of vaccination to older girls may also be cost effective (up to age 22)[59]. Modelling studies in low-income countries are necessary in order to establish the potential impact of HPV catch-up campaigns in older girls in these countries. This study,

which describes genotype-specific rates of HPV acquisition, will provide data to model the cost-effectiveness of HPV catch-up campaigns for girls of different ages.

7.4 Conclusion

This study confirms the extremely high incidence of HPV in adolescent girls in Tanzania around the time of first sex. Previously, a very high incidence was seen in young, sexually experienced women in the same region of Mwanza, Tanzania at 74 per 100 person-years[10]. Similarly, a very high cumulative incidence was seen in young women in the 1-3 years after first sex at 39-44% in Brazil and the USA[4,11,60]. The rate of HPV acquisition seen in the study presented was higher than those previously reported; HPV incidence was 225 per 100 person-years, and cumulative incidence at 6 months was 53%. Further, the duration of HPV infection was shorter than that reported in most studies[22,23], and may reflect rapid clearance after first exposure to the virus at first sex.

The reporting of vaginal sex was lower than expected in the study population, and few non-penetrative sexual behaviours (kissing, breast touching, hand-genital contact) were reported. It is likely that previous sex was under-reported, and possible that non-penetrative sexual behaviours were infrequently practiced as well as under-reported. The study confirms the challenge of collecting accurate sexual behaviour data in adolescents and the importance of testing alternative methods of interview such as ACASI before implementing them.

Further research is underway to explore the possibility of fomite transmission of HPV in the context of a region with high rates of intravaginal cleansing, HPV and cervical cancer. Future studies also include further investigation of the possibility of intermittent detection of persistent HPV, and a study of oral HPV prevalence. Finally, modelling the most appropriate upper-age of vaccination will be performed using these data in order to inform HPV vaccination campaigns in this region.

7.5 References

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8 Annexes

Annex 1. Ethical approval from MRCC Tanzania

THE UNITED REPUBLIC OF
TANZANIA

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NIMR/HQ/R.8a/Vol. IX/1249

13th December 2011

Dr Deborah Watson-Jones
Mwanza Interventions Trials Unit
NIMR Mwanza Campus, Isamilo Road
P O Box 11936 MWANZA

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Epidemiology and natural history of human papilloma virus infection in a cohort of Tanzanian girls after sexual debut in Mwanza (Watson-Jones D et *al*), whose Local Investigator is John Changalucha, NIMR Mwanza, has been granted ethics clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health & Social Welfare and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine. NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Approval is for one year: 13th December 2011 to 12th December 2012.

Name: Dr Mwelecele N Malecela

Signature

CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr Deo M Mtasiwa

Signature

CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, SOCIAL WELFARE

CC: RMO
DMO

Annex 2. Ethical approval from LSHTM

London School of Hygiene & Tropical Medicine
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Observational / Interventions Research Ethics Committee

Dr Deborah Watson-Jones
Senior Clinical Lecturer
CRD/ITD
LSHTM

20 September 2012

Dear Dr Watson-Jones,

Study Title:	**Epidemiology and natural history of human papillomavirus infection in a cohort of Tanzanian girls after sexual debut**
LSHTM ethics ref:	**6040**
LSHTM amend no:	**A354**

Thank you for your application of 28 August 2012 for the amendment above to the existing ethically approved study and submitting revised documentation. The amendment application has been considered by the Observational Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above amendment to research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval for the amendment having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
LSHTM amendment application	n/a	13/08/2012
Study Protocol HPV Epi Study	V2	13/08/2012

After ethical review

Any further changes to the application must be submitted to the Committee via an E2 amendment form. The Principal Investigator is reminded that all studies are also required to notify the ethics committee of any serious adverse events which occur during the project via form E4. At the end of the study, please notify the committee via form E5.

Yours sincerely,

Professor Andrew J Hall
Chair
ethics@lshtm.ac.uk
http://intra.lshtm.ac.uk/management/committees/ethics/

Annex 3. Enrolment questionnaire

HPV Epidemiology Study Enrolment Questionnaire. **ID number [_P_|_V_|_G_|___|___|___|___]**

Mapping Study ID Number	HPV Epi Study ID	Lab sticker
[| | | | | | |]	PLACE STICKER HERE	PLACE STICKER HERE

Section 1 – Socio-demographics

	Question	Coding	SKIP /vaname
1	**Tarehe ya mahojiano** *Date of interview*	[__|__] [__|__|__] [_2_|_0_|__|__] DAY MONTH YEAR	dateenrol
2	**Alama ya anayehoji** *Code of interviewer*	[__|__|__]	staffcode
3	Name of school that pupil attended during at last mapping (check list) Code no. of school (do not enter "/")	_____ [__|__|__|__|__|__|__|__|__|__|__|__]	schname schcode
4	Code for district of current residence Code for ward of current residence	[__|__] [__|__]	distcode wardcode
5	**Una miaka mingapi?** *How old are you (years)?*	[__|__] ***Sijui =99*** don't know=99	age
6	**Ulizaliwa mwaka gani?** *What year were you born?*	[__|__|__|__] ***Sijui=9999*** don't know=9999	yrborn
7	**Je, ulizaliwa wapi?** *Where were you born?*	**Mkoa huu** *In this region* 1 **Mkoa mwingine katika Tanzania** *Other region of Tanzania* 2 **Nchi nyingine** ***(andika gani)*** *Another country*_____ 3 ***Sijui*** *Don't know* 4	regborn regbornoth
8	**Je, umeishi hapa <jina la kijiji au mji> kwa muda gani? (*Andika namba, zungushia wakati)*** *How long have you lived here <name of village/town>?* Enter duration and circle time unit.	**Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 [__|__|__] **Mwaka** *Year* 4 ***Sijui=999*** Don't know=999 **Sijui** Don't know 5	durlived durlivedx
9	**Je, muda wote unaishi na nani? *(Zungushia moja)*** *Who do you live with most of the time? (circle one only)*	**Mama tu** *Mother only* 1 **Baba tu** *Father only* 2 **Wazazi wangu wote** *Both parents* 3 **Ndugu Mwingine** *Another relative, no parents* 4 **(Ma)rafiki wa familia** *Family friend(s) no parents* 5 **Mwenza/mume** *Partner/husband* 6 ***Nyingine (fafanua)*** *Other*_____ 7	livew livewoth
10	**Bila kujihesabu mwenyewe unakaa na watu wangapi nyumbani/chumbani kwako? *(wakubwa na watoto)*** *Excluding yourself, how many people are living in the house you live in now? (adults+children)*	[__|__] ***Sijui =99*** 99=don't know	resnum

11	**Nitakusomea orodha ya vitu vifuatavyo tafadhali niambie kama mwenye miji anamiliki chochote kati ya hayo.** ***(Soma orodha zungushia yote yatakawotajwa)*** *I will read you a list of items and things. Please tell me if the head of your household owns any of these.* *(Read the list and circle all that apply)*	**Redio** *Radio*	1	radio
		SIMU ya mkononi *Cellphone*	2	phone
		Runinga/televisheni *Television*	3	tv
		Baisikeli *Bicycle*	4	bike
		Pikipiki *Motorcycle*	5	motorb
		Gari *Car*	6	car
		Mifugo *Live-stock*	7	livesto
		Shamba *Agricultural plot*	8	land
12	**Unafanya kazi gani kwa sasa?** ***(Zungushia moja)*** *What are you doing now? (i.e. "occupation" (circle one answer)*	**Ninahudhuria shule ya sekondari** Attending secondary school	1	
		Hana ujuzi wowote ***(mtunza usafi, mhudumu wa baa, mama lishe, msaidizi wa nyumbani)*** *unskilled manual (e.g. cleaner, bar worker, food handler, house girl, salon worker)*	2	**Skip to Q14**
		Kazi zenye ujuzi maalumu ***(makanika, fundi cherehani, fundi umeme)*** *(Skilled manual e.g. mechanic, tailor, electrician)*	3	
		Taaluma ***(mwalimu/nesi/mratibu/polisi)*** *Professional (eg. teacher/nurse/ accountant/police)*	4	
		Biashara *Business*	5	
		Mkulima *Agriculture/farming*	6	
		Mchimbaji *Mining*	7	
		Mvuvi *Fishing*	8	
		Hana kazi/anakaa nyumbani *No job/stay at home/unemployed*	9	occup
		Kazi nyingine _____ *other, (specify)*	10	occupoth
13	**Nitajie jina la sekondari na kidato ulichofikia?** *What is the name and form of the secondary school you attend?*	Name_____ Form [___]		secsch secform
14	**Sasa umeolewa au unakaa na mpenzi wako?** ***(Zungushia moja)*** *Are you currently married or living as married? Circle one answer*	**Nimeolewa** *Married*	1	marr
		Ninaishi na mwenzi *Living with partner as if married*	2	
		Mjane *Widow*	3	
		Nimeachika/tumetengana *Separated/divorced*	4	
		Bado sijaolewa Single (never married)	5	→ **skip to Q16**
15	**Ulikuwa na umri gani ulipoolewa na mume wa kwanza?** *How old were you when you first got married?*	[__|__] ***Sijui =99*** 99=don't know		agemarr
16	**Je, wewe ni kabila gani?** ***(Zungushia moja)*** *What is your tribe? (Circle one answer)*	**Msukuma**	1	
		Mjita	2	
		Mzinza	3	
		Nyiramba	4	tribe
		Mkara/Mkerewe	5	
		Mhaya	6	
		Mjaluo	7	
		Mkuria/Mshashi	8	
		Mchaga	9	
		Mwingine (Other Tanzanian)	10	
		Si Mtanzania (Non-Tanzanian)	11	
		Sijui Don't know	99	

17	**Dini yako, unaabudu wapi?** ***(Zungushia moja)*** *What is your religion? (Circle one answer)*	**Protestant** 1 **Catholic** 2 Other Christian ***(e.g. Lutheran, Baptist, Pentecostal, Jehovah's Witness, Methodist, Apostolic)*** 3 **Muslim** 4 **Other non-Christian, non-muslim religion** 5 **Sina dini** (No religion) 6	relig
18	**Umewahi kulala kwingine kukaa kwingine hata kwa siku moja tu katika miezi mitatu iliyopita?** *Have you slept a night away from home in the past 3 months? Probe if necessary*	**Ndiyo** *(yes)* 1 **Hapana** *(No)* 2 → **skip to Q20**	nightaway
19	**Kama ndiyo, je ni mara ngapi umekaa kwingine kwa zaidi ya wiki moja katika miezi mitatu iliyopita?** *If yes to above, how many times in past 3 months have you spent more than a week away from home?*	[__|__] ***Sijui =99***	totalaway
20	**Umewahi kunywa pombe?** *Have you ever drunk alcohol? Probe if necessary*	**Ndiyo** *(Yes)* 1 **Hapana** *(No)* 2 → **Skip to Q23**	alcany
21	**Ulikuwa na miaka mingapi ulipoanza kunywa pombe?** *What age were you when you first drank alcohol?*	[__|__] ***Sijui =99*** don't know=99	agealc
22	**Kwa kawaida, unakunywa pombe kiasi gani?** ***(Andika namba, zungushia wakati. Kandika hatumii pombe andika 00)*** *On average how many alcoholic "drinks" do you drink? (Enter number of drinks and circle time unit. Write 00 if she doesn't drink)*	1 drink=1 bottle of beer or 1 measure of Konyagi or 1 glass of wine or ½ litre of local brew **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 [__|__|__] ***Sijui =999*** **Mwaka** *Year* 4 don't know=999	alcnum alcnumx
23	**Je, umeshawahi kutumia** ***(zungushia yote yatakayotajwa)*** *Do you ever take (circle all that apply)*	**Bangi?** *Marijuana* 1 **Mirungi?** *Ghat* 2 **Unga?** *Cocaine* 3 **Valium?** *Valium* 4 **Heroin?** *Heroin* 5 **Gundi?** *Glue* 6 **Petroli?** *Petrol* 7 **Ugoro?** *Tobacco Powder?* 8 ***Nyingine (fafanua)*** *Other*_____ 9 **hakuna hata mmoja** *None of the above* 10 → **Skip to Q25**	maraj ghat coke val hero glue petrol tobaccop drugoth drugothsp nodrug
24	**Huwa unatumia mara ngapi?** ***Kama wakichagua zaidi ya moja chagua ile ambayo anatumia zaidi. Ingiza moja (1-8) kutoka swali 23. Ingiza ni kwa mana ngapi huwa anatumia halafu zungushia (siku/wiki/mwazi etc).*** *How often do you take this? If she circled several drugs, answer for the one most frequent. Enter the code number for the drug (between 1 and 7), the frequency and the time unit*	***Ingiza moja 1-8 kutoka swali 23*** [___] *enter the number from Q23* ***Ingiza ni kwa mana ngapi*** [__|__|__] enter the number of times **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi.***Month* 3 **Mwaka** *year* 4 **Sijui** Don't know 5	drugmost drugtime drugtimx
25	**Je, unavuta sigara?** *Do you smoke cigarettes?*	**Ndiyo** *(Yes)* 1 **Hapana** *(No)* 2 → **Skip to Q27**	smoke
26	**Kwa kawaida, unavuta sigara wangapi?** ***(Andika namba, zungushia wakati)*** *On average how many cigarettes do you smoke? (Enter the number of cigarettes and circle the time unit)*	***Andika numba*** [__|__|__] ***Sijui =999*** don't know=999 **Siku** *Day* **Wiki** *Week* **Mwezi** *Month* **Mwaka** *Year* **Sijui** Don't know	smoknum smoknumx

Section 2 - Sexual Behaviour

***Soma*: Sasa ningependa kukuuliza maswali kuhusu masuala ya kujamiiana ili kupata uelewa mzuri juu ya masuala yanayohusiana na afya ya uzazi. Nafahamu kwamba maswali haya ni nyeti, lakini ni matarajio yangu kwamba utayajibu kwa ufasaha ili kutusaidia kupata ufahamu zaidi juu ya afya na mambo ya uzazi.**

I am now going to ask you questions about sexual activity in order to gain a better understanding of issues related to reproductive health. I know these questions may be sensitive and difficult to answer, but I hope you will respond to help us to better understand some health and infection issues.

27	**Umeshawahi kumbusu mvulana mdomo? (fafanua)** *Have you ever kissed a boy on the lips? (explain)*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	lipkiss
28	**Umeshawahi kupeana denda na mpenzi/mume wako? (kula uroda, kula denda)** *Have you ever kissed a boy on (in) the mouth using tongues?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	tongkiss
29	**Mpenzi wako amewahi kuchezea matiti yako? (kujivinjari, kutomasatomasa, ku-survey)** *Has a boy ever played with / touched your breasts?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	breastrub
30	**Umeishawahi kufanya mapenzi ya kushikana na mwanaume/mvulana, ambapo unachezesha uume wake kwa mkono wako?** *Have you ever engaged in sexual touching with a man/boy where you touched his penis with your hand?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	penistouch
31	**Umeishawahi kufanya mapenzi ya kushikana na mwanaume/mvulana, ambapo anachezesha uke wako kwa mkono wake? (kupiga brush, kutomasatomasa)** *Have you ever engaged in sexual touching with a man/boy where he has touched your vagina with his hand?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	vagtouch
32	**Je, umewahi kunyonya uume wa mwanaume/mvulana kwenye mdomo wako? (kula muhogo, kula muwa, kula cone, kupiga mswaki, alamba)** *Have you ever had a man/boy put his penis in your mouth?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	penisoral
33	**Je, wanaume/mvulana amewahi kukunyonya sehemu zako za siri (kwenye uke)? (kufyonza embe, kuzama chumvini, kula pera)** *Has a man/boy ever put his mouth on your genitals?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	vagoral
34	**Je, umeishawahi kuchezesha chezesha uume kwenye mapaja miguu au matakoni au juu ya sehemu ya nje ya uke? *(kupiga puchu, kupiga katerelo)*** *Have you ever had a boy's penis rubbed between your thighs at the top of your legs or between your buttocks or over your outer genitals (without going inside your vagina)?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	penisrub
35	**Uume kuingia ndani ya njia yako ya hajakubwa? (*kufilana, kupakua kisamvu, kula tigo, kula mgongo*)** *Have you ever had anal sex? (penis entering your anus)*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 → **Skip to Q37**	analsex
36	**Kwa maisha yako, umefirwa mara ngapi?** *How many times in total in your life have you had anal sex? (penis entering your anus)*	[__|__|__] ***Sijui =999*** ***Tangu Kukaziwa 100=100*** don't know=999 *over 100=100*	analsexnum
37	**Je umeshawahi kujamiiana kwa kuingia ndani. Kwa hili namaanisha pale mwanaume anapoweka uume wake kwenye uke wako. *(Kuchakachuliwa, Kugongwa mshipa, Ku sex, Kutiana, Kuingia ngumu kutoka laini)*** *Have you ever had vaginal sexual intercourse? By that I mean where a man puts his penis in your vagina.*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 → **Skip to Vaginal Practices**	sa
	Remember, if she answered No to the above question, skip to section 3 - Vaginal practices		
38	**Je, ulikuwa na miaka mingapi ulipojamiana (tendo la ndoa) mara ya kwanza?** ***(prompt: Unakumbuka lini umejamiana kwa mara ya kwanza? Mpenzi wako alikuwa nani? Alikuona wapi mara ya kwanza? je unakumbuka ulikuwa na miaka mingapi?)*** *How old were you when you first had sex?*	[__|__] ***Sijui =99*** don't know=99	agesa
39	**Ulipojamiana mara ya kwanza ulipata maumivu?** *When you had sex for the first time did it hurt you?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	painsex1
40	**Ulipojamiana kwa mara ya kwanza je ulitokwa na damu?** *Did you bleed the first time you had sex?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	bleedsex1
41	**Ulitumia chochote mara ya kwanza ili upunguze maumivu kablaya kujamiiana? *(zungushia yote yatakayotajwa)*** *Did you use anything before sex the first time to reduce the pain? (Circle all that apply)*	**Kondomu** *Condom* 1 **Jelli** *Vaseline* 2 **Mate ya mpenzi** *His saliva* 3 **Mate yako mwenyewe** *My saliva* 4 **Maji** *Water* 5 **lotion** 6 **Sikutumia chochote** *Nothing* 7 **Nyingine** *Other*_____ 8	condsex1 vassex1 boysalsex1 girlsalsex1 watersex1 lotionsex1 nothsex1 othsex1 othsex1sp

42	**Je, katika maisha yako yote mpaka sasa hivi, umewahi kujamiiana (uume kwenye uke) na wanaume wangapi? *(uwahesabu na wale uliokutana nao mara moja tu na hata wale waliokulazimisha)*** *Throughout your whole life up to now, how many different men have you had sex (penis in vagina) with? (including current partners, past partners, spouse and all other kinds of partner; write the number)*	[__|__] ***Sijui =99*** *don't know=99*	totnumpart
43	**Je, kwa maisha yako, umejamiiana mara ngapi?** *How many sexual acts have you ever had? (this is the total number of times you have had sex even if it is with only one man)*	[__|__|__] → **If this number is 1 or 2, skip to 48** ***Sijui =999*** ***Tangu Kuzaliwa 100=100*** don't know=999 *over 100=100*	totsexnum
44	**Mara ngapi wapenzi/mume kabla ya kujamiiana wanachezea uke au sehemu ya nje ya uke? *(Zungushia moja)*** *How often does your lover play with your vagina / external genitals before having sex with you? (circle one answer)*	**Ndiyo mara zote** always 1 **Mara kwa mara** half of the time 2 **Mara nyingine** sometimes 3 **Mara chache** rarely 4 **Hamna kabisa** never 5	foreplay
45	**Mara ngapi wapenzi/mume wako, wamewahi kutumia mate yao katika uume wao au ndani ya uke wako? *(Zungushia moja)*** *How often does your lover put saliva on his penis or your vagina before sex? (circle one answer)*	**Ndiyo mara zote** always 1 **Mara kwa mara** half of the time 2 **Mara nyingine** sometimes 3 **Mara chache** rarely 4 **Hamna kabisa** never 5	salivasex
46	**Mara ngapi wapenzi/mume wako, wamewahi kutumia jeli ya Vaseline katika uume wao au ndani uke wako? *(Zungushia moja)*** *How often does your lover put vaseline on his penis or your vagina before sex? (circle one answer)*	**Ndiyo mara zote** always 1 **Mara kwa mara** half of the time 2 **Mara nyingine** sometimes 3 **Mara chache** rarely 4 **Hamna kabisa** never 5	vassex
47	**Mara ngapi wapenzi/mume wako, wamewahi kutumia kitu kingine chochote katika uume wao au ndani ya uke wako? *(ISIPOKUWA CONDOM. Zunguisha moja, andika kitu gani)*** *How often does your lover put something else on his penis or your vagina before sex? (EXCEPT CONDOMS, circle one or specify what)*	**Ndiyo mara zote** always 1 **Mara kwa mara** half of the time 2 **Mara nyingine** sometimes 3 **Mara chache** rarely 4 **Hamna kabisa** never 5 ***Andika kitu gani*** _____ Specify what 6	lubsex lubsexoth lubsexothsp
48	**Je umeshawahi kushawishiwa kufanya mapenzi ili upate usafiri, alama bora shuleni, hela za matumizi madogo madogo, au pesa ya kununua chakula nyumbani?** *Have you ever had sex in exchange for gifts or money, or any other kind of support for example food/lodging/transport/better grades/clothes?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 → **Skip to Q50**	giftsex
49	**Ni mara ngapi ulijamiiana kwa ajili ya zawadi/pesa?** *How many times have you had sex in exchange for gifts or money*	[__|__|__] ***Sijui =999*** ***Tangu Kuzaliwa 100=100*** don't know=999 *over 100=100*	numgiftsex
50	**Umewahi kujamiana ukiwa umelewa?** *Have you ever had sex whilst drunk?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	alcosex
51	**Je, uliwahi kulazimishwa na mwanaume kujamiiana naye bila hiari yako?** *Have you ever been forced by a man to have sex with him (against your will)?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	forcedsex
52	**Umewahi kujamiiana ukiwa katika siku zako za hedhi?** *Have you ever had sex whilst menstruating?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	menssex
53	***Umewahi kuwa mjamzito mara ngapi? Andika idadi*** *How many times have you been pregnant? Write number*	[__|__] ***Sijui =99*** ***kubwa kuliko=00*** → **If 00 skip to Q55** *Don't know=99* *Never=00*	numpreg

54	***Je nini matokeo ya mimba hizo? (zungushia yote yatakawotajwa)*** *What happened to those pregnancies? (circle all that apply)*	**Ilitoka yenyewe** *Miscarriage* 1 **Niliamua kuitoa** *Abortion* 2 **Nilijifungua mtoto** *Child* 3 **Ni mjamzito sasa** *Pregnant now* 4	miscar abort child preg
55	**Je unafanya kitu chochote au kutumia njia yoyote kuchele wesha au kuzuia kupata mimba kwa sasa?** *Are you doing something or used any method to delay or avoid pregnancy now?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 → **Skip to Q57**	contracep
56	**Je, unatumia nini ili kuzuia au kuchelewesha kupata mimba sasa? (*zungushia yote yatakawotajwa*)** *What are you doing/using to avoid or delay pregnancy now? Circle all that apply*	**Hakuna** *Nothing* 1 **Vidonge vya uzazi wa mpango** *Oral pill* 2 **Sindano** *Injection* 3 **Kondomu** *Condom* 4 **Kalenda wakati salama** *Calendar/safe period* 5 **Dawa za kienyeji** *Traditional medicine* 6 **Kutoa uume kabla ya kumwaga mbegu** *Withdrawal method* 7 **Mjamzito sasa** *Pregnant now* 8 **Kitanzi** *IUCD* 9 **Nyingine** Other_____ 10	methodcont1 methodcont2 methodcont3 methodcont4 methodcont5 methodcont6 methodcont7 methodcont8 methodcont9 methodcont10 methodothsp
57	**Je, umetahiriwa?** *Have you been circumcised?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	selfrepcirc

Ningependa kufahomu kuhusu wapenzi wako wa sasa na zamani. Anza na wa sasa hivi.
"I would like to know about your previous sexual partners. Let's start with your most recent lover"

		START HERE 1.	2.	3	(Complete for first ever lover if not previously described in 1 or 2 or 3) 4.
58	**Nitajie jina lake kwa kifupi? Tafadhali kama hukumbuki unaweza kunitajia jina lolote. Vile vile unaweza kunitajia jina lolote kama hutaki nifahamu majina yao.** *What were his initials? – please make some up if you can't remember. You can also make some up if you don't want me to know their initials.*	[__|__]	[__|__]	[__|__]	[__|__] partid
59	**Anaishi kata gani?** *Where does he live?*	_____	_____	_____	partres _____
60	**Kwa Kawaida huwa mnakutana sehemu gani kujaamiana?** *Where did you usually have sex with him? (write place or DK)*	_____	_____	_____	wheresex _____
61	**Alikuwa na umri gani?** *What age was this person? Guess if not sure.*	[__|__] ***Sijui* =99** → **If she knowns the age, skip to Q 63** *Don't know=99*	[__|__] ***Sijui* =99** → **If she knowns the age, skip to Q63** *Don't know=99*	[__|__] ***Sijui* =99** → **If she knowns the age, skip to Q63** *Don't know=99*	partage [__|__] ***Sijui* =99** → **If she knowns the age, skip to Q63** *Don't know=99*

62	**Alikuwa na umri gani, kadiria?** *Estimate the age of this person*	**Zaidi ya miaka kumi** More than 10 years older 1 **Kati ya miaka mitano hadi kumi** 5-10 years older 2 **Je unamzidi miaka mitano** less than 5 years older 3 **Je mnalingana** Same age 4 **Mdogo** Younger 5 **Sijui** Don't know 6	**Zaidi ya miaka kumi** More than 10 years older 1 **Kati ya miaka mitano hadi kumi** 5-10 years older 2 **Je unamzidi miaka mitano** less than 5 years older 3 **Je mnalingana** Same age 4 **Mdogo** Younger 5 **Sijui** Don't know 6	**Zaidi ya miaka kumi** More than 10 years older 1 **Kati ya miaka mitano hadi kumi** 5-10 years older 2 **Je unamzidi miaka mitano** less than 5 years older 3 **Je mnalingana** Same age 4 **Mdogo** Younger 5 **Sijui** Don't know 6	10old/5to10old/und5old/sameage/younger/dkage **Zaidi ya miaka kumi** More than 10 years older 1 **Kati ya miaka mitano hadi kumi** 5-10 years older 2 **Je unamzidi miaka mitano** less than 5 years older 3 **Je mnalingana** Same age 4 **Mdogo** Younger 5 **Sijui** Don't know 6
63	**Huyu mwanaume ndiye aliyekuwa mwenzi wako wa kwanza kabisa wa kimapenzi?** *Was this man/boy your first ever sexual partner?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 firstever
64	**Huyu mwanamume alikuwa nani wako? *(Zungushia moja)*** *What relationship was this boy/man to you? (Circle one)*				partrel partreloth
	Mume *husband*	1	1	1	1
	Mwenzangu ninayeishi naye *Cohabiting partner*	2	2	2	2
	Mpenzi wangu *Other regular partner or boyfriend*	3	3	3	3
	Mwanamume ambaye huwa unafanya naye tendo la kujamiiana lakini si mpenzi wako *Casual sex partner*	4	4	4	4
	Simfahamu (tulikutana mara moja) *Stranger (met once)*	5	5	5	5
	Mtu ambaye hataki kujamiiana na ye *Someone who I do not want to have sex with*	6	6	6	6
	nyingine *(otherspecify)*	7__________________	7__________________	7__________________	7__________________
65	**Anafanya kazi gani? *(Zungushia moja)*** *What is this person's job? (Circle one)*				partjob partjoboth
	Hana kazi/anakaa nyumbani *No job/stay at home*	1	1	1	1
	Kazi ya Madini *Mine employee*	2	2	2	2
	Mwafunzi wa shule ya msingi *At primary school*	3	3	3	3
	Mwafunzi wa shule ya sekondari *At secondary school*	4	4	4	4
	Mwanafunzi wa chuo/chuo kikuu *Student at colleage/university*	5	5	5	5
	Kazi ya kuajiriwa (eg. Muuguzi, polisi) *Skilled (eg. nurse/ accountant/police)*	6	6	6	6
	Mwalimu Teacher	7	7	7	7
	Mkulima *Farmer*	8	8	8	8
	Kazi ya kujiajiri ujuzi *Other manual (e.g. carpenter/tailor/fisherman)*	9	9	9	9
	Anaendesha gari kubwa/msaidizi wa gari kubwa *Truck driver/turnboy*	10	10	10	10
	Biashara *Business (e.g. duka owner; garage owner)*	11	11	11	11
	Kazi nyingine____ *(other specify)*	12__________________	12__________________	12__________________	12__________________
	Sijui don't know	13	13	13	13

66	**Ni kwa muda gani ulimfahamu huyu mwanaume kabla ya kufanya naye tendo la kujamiiana?** *How long you had you known this man before having sex with him the first time? (Enter number and circle time unit)*	[__|__|__]***Sijui =999*** *Don't know=999* **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 **Mwaka** *Year* 4	[__|__|__]***Sijui =999*** *Don't know=999* **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 **Mwaka** *Year* 4	[__|__|__]***Sijui =999*** *Don't know=999* **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 **Mwaka** *Year* 4	[__|__|__]***Sijui =999*** *Don't know=999* **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 **Mwaka** *Year* 4 timepresex/presexunit
67	**Ni siku/tarehe gani ulipojamiana nae kwa mara ya kwanza mwanamme/mvulana huyu?** *What date did you first have sex with this man/boy? (Write 99 or 999 if doesn't know)*	***Sijui =999 au 99*** *Don't know=999 or 99* [__|__] date [__|__|__]month [_2_|_0_|__|__]Year	***Sijui =999 au 99*** *Don't know=999 or 99* [__|__] date [__|__|__]month [_2_|_0_|__|__]Year	***Sijui =999 au 99*** *Don't know=999 or 99* [__|__] date [__|__|__]month [_2_|_0_|__|__]Year	***Sijui =999 au 99*** *Don't know=999 or 99* [__|__] date [__|__|__]month [_2_|_0_|__|__]Year datefirst
68	**Unaendelea kujamiiana na mwanaume huyu hadi sasa?** *Are you still having sex with this man/boy?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	currsex **Ndiyo** *(yes)* 1 **Hapana** *(no)* 2
69	**Je, huyu mwanamme /mvulana ulijamiiana naye tarehe ngapi/siku gani kwa mara ya mwisho?** *What was the date of the last sex with this man/boy (Write 99 or 999 if doesn't know)*	**(this is the last time she had sex)** [__|__] date [__|__|__]month [_2_|_0_|__|__]Year	[__|__] date [__|__|__]month [_2_|_0_|__|__]Year	[__|__] date [__|__|__]month [_2_|_0_|__|__]Year	datelast [__|__] date [__|__|__]month [_2_|_0_|__|__]Year
70		**Je, mlitumia kondomu mara ya mwisho ulipojamiiana?** *The last time you had sex, did you use a condom?* **Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	condomlasts		
71	**Huwa mnatumia kondom na mwanaume huyu?** ***(Zungushia moja)*** *Do you use condoms with this man/boy? (circle one)*				condfreq
	Situmii kabisa *Never use them*	1	1	1	1
	Mara chache *Few times/rarely*	2	2	2	2
	Mara nyingine *Sometimes*	3	3	3	3
	Mara nyingi *Often (frequently, most of the time)*	4	4	4	4
	Kila wakati *Always (everytime)*	5	5	5	5
	Nimejamiiana mara moja au mara mbili tu *I have only had sex once or twice*	6	6	6	6
72	**Umejamiiana na mwanamume huyu mara ngapi?** *How many times have you had sex with this man in total?*	[__|__|__] ***Sijui =999*** ***Tangu Kukaziwa 100=100*** don't know=999 *over 100=100*	[__|__|__] ***Sijui =999*** ***Tangu Kukaziwa 100=100*** don't know=999 *over 100=100*	[__|__|__] ***Sijui =999*** ***Tangu Kukaziwa 100=100*** don't know=999 *over 100=100*	numsex [__|__|__] ***Sijui =999*** ***Tangu Kukaziwa 100=100*** don't know=999 *over 100=100*
73	**Huyu mwanamume alitahiriwa? (alikatwa)** *Was this man/boy circumcised?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3 circum

74	**Wakati ulipokuwa na mahusiano ya kimapenzi na huyu mwanaume, alikuwa na wapenzi wengine pia?** *During the time you had been involved sexually with this man/boy, did he have another partner?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3 boyconc
75	**Wakati ulipokuwa na mahusiano ya kimapenzi na huyo mwanaume, ulikuwa na mpenzi/wapenzi wengine tofauti na yeye?** *During the time you had sexual encounters with this person, did <u>you</u> have another sexual partner?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sijui** *(DK)* 3 } **Skip the next Q**	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sijui** *(DK)* 3 } **Skip the next Q**	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sijui** *(DK)* 3 } **Skip the next Q**	girlconc **Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sijui** *(DK)* 3 } **Skip the next Q**
76	**Wakati huo ulikuwa na wapenzi wangapi tofauti?** *If you said yes, how many other sexual partners did <u>you</u> have at the time?*	[__|__] ***Sijui =99*** *99=don't know*	[__|__] ***Sijui =99*** *99=don't know*	[__|__] ***Sijui =99*** *99=don't know*	girlconcnum [__|__] ***Sijui =99*** *99=don't know?*

"Asante kwa kujibu maswali kuhusu mwanaume huyu. Ningependa sasa kukuuliza baadhi ya maswali kuhusu mwanaume wa mwisho uliyefanya naye tendo la kujamiiana" *Thank you for answering questions about this man/boy. I would now like to ask some questions about the last man you had sex with before this person.*
Go back to Q58, part 2

Asante kwa kujibu maswali kuhusu mwanamume huyu. Ningependa sasa kukuuliza baadhi ya maswali kuhusu mwanaume wa mwisho uliyefanya naye tendo la kujamiiana" *Thank you for answering questions about this man/boy. I would now like to ask some questions about the last man you had sex with before this person.*
Go to Q58, part 3 or 4

Thank the girl for her time. Go to the next section on vaginal practices

SECTION 3- Vaginal Practices

Sasa nitakuuliza maswali kuhusu maswala ya ngono na kujamiana ili nielewe zaidi juu ya afya na maambukizi. Najua maswali hayo yaweza kuwa magumu kwako lakini nakuomba ujibu hata hivyo ili utusaidie kuelewa mambo hayo muhimu kwa undani zaidi. Usiogope kuuliza ikiwa hulielewi vizuri swali linaloulizwa, nesi yuko tayari fafanua

<u>Vaginal practices</u> I am now going to ask you some questions about personal vaginal practices in order to gain a better understanding of issues related to health and infection. I know these questions may be sensitive and difficult to answer, but I hope you will respond to help us to better understand these important issues. Please do not be afraid to ask if you do not understand the question that is being asked, the nurse will be happy to explain.

77	**Unakuwa na umri gani ulipoona hedhi yako kwa mara ya kwanza?** *How old were you when you had your first period?*	[__|__] years **99= *sijui*** **00 = *si hedhi*** *99=don't know* *00= not yet menstruating*	agemens → **Skip to Q80**
78	**Unapokwa kwenye hedhi huwa unatumia nini kuzuia damu? *(Zungushia moja)*** *During menstruation, what do you use to catch the blood? (circle one)*	**Vitambaa/kanga** *Cloth* 1 **Chupi** *Pants* 2 **Karatasi ya choo** *Toilet paper* 3 **Pedi za madukani** *Sanitary napkins* 4 **Tampons** *Tampons* 5 **Nyingine** *Other*_____ 6	sanit sanitoth **Skip to Q80**
79	**Kama unatumia nguo, huwa unaiweka ndani ya uke au nje ya uke au zote pamoja? *(Zungushia moja)*** *You said that you use cloths to catch the blood, do you use these inside or outside the vagina or both? (circle one)*	**Ndani ya uke peke yake** *Only inside the vagina* 1 **Nje ya uke peke yake** *Only outside the vagina* 2 **Zote pamboja** *Both inside and outside* 3	cloth
80	**Baadhi ya wanawake husafisha uke kwa ndani. Inamaanisha kusafisha ndani ya uke kwa maji au kitu kingine?** *Some women cleanse inside the vagina. Cleansing is defined as cleaning inside the vagina with water or another substance. Have you ever cleansed inside the vagina?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 → **Skip to Q85**	everclean
81	**Kwa kwaida huwa unasafisha mara ngapi ndani ya uke? *Andika namba, zungushia wakati*** *In the past 3 months, about how often have you cleansed inside the vagina? Enter the number, circle the time.*	[__|__|__] ***Sijui =999*** don't know=999 **Siku** *Day* 1 **Wiki** *Week* 2 **Mwaka** *Year* 3 **Mwezi** *Month* 4	clean freqclean

82	**Ulisafisha na vitu vifuatavyo?** ***(zungushia yote yatakawotajwa)*** *Have you ever cleansed with any of the following? (circle all that apply)*	**Maji tu** *Plain water*	1	clnwat
		Sabuni au maji na sabuni *Soap/soap and water*	2	clnsoap
		Dawa ya kuzuia wadudu kwa mfano Dettol/Jik *Disinfectant e.g. Dettol/Jik*	3	clnjik
		Sabuni ya unga *Washing powder*	4	clnwashp
		Vinginevyo vinavyotumika_____ *(Other, write what)*	5	clnsuboth clnsubothsp
83	**Umenieleza kuhusu vitu vinavyovituma kusafisha, sasa niambie unasafishaje?** ***(usisome orodha, zungushia yote yatakawotajwa)*** *You have told me about what you use to clean, now tell me how exactly you do this? (Don't read out list, circle all that apply)*	**Vidole** *Fingers*	1	clnfing
		Nguo kamakitambaa *Cloth*	2	clncloth
		Pamba *Cotton wool*	3	clncotwl
		karatasi kama gazeti *Paper including Newspaper*	4	clnpaper
		Nyingine *other (specify)*_____	5	clnwoth clnwothsp
84	**Ni wakati gani hasa huwa unasafisha uke kwa ndani?** ***(Usisome orodha zungushia yote yatakawotajwa)*** *When do you usually clean inside the vagina? (Don't read out the list, circle all that apply)*	**Kila siku au ninapoenda chooni** *During daily toileting*	1	clntoilet
		Kila siku nina pooga *During bathing*	2	clnbath
		Wakati wa hedhi *During menses*	3	clnmense
		Kabla ya kujamiiana *To prepare for sex*	4	clnpresx
		Baada ya kujamiiana *Immediately after sex*	5	clnpostsx
		Unapokua na muwasho ukeni *When you have vaginal irritation or bad odor*	6	clnodor clnwhoth
		Viyingine *Other time (specify)*_____	7	clnwhothsp
85	**Je, umeishawahi kuweka vitu hivi ndani ya uke wako?** ***(Soma orodha zungushia yote yatakawotajwa)*** *Have you ever inserted any of the following substances into your vagina? (Read out list and circle all that apply)*	**limao au Ndimu** *Lemon or lime*	1	insertcitric
		Mitishamba *Traditional healer medicine*	2	insertmedm
		Dawa ya kuzuia wadudu kwa mfano Dettol/Jik *Disinfectant e.g. Dettol/Jik*	3	insertjik
		Sabuni ya unga *Washing powder*	4	insertwashp
		Ugoro *tobacco powder*	5	insertbac
		Nyingine *Other (specify)*_____	6	insertoth
		Sijawahi kuingiza chochote *never inserted*	7 → **Skip to Q88**	insertothsp neverinsert
86	**Ndani ya mwezi mmoja uliopita uliwahi kuigiza chochote ndani ya uke mara ngapi?** ***(Andika namba)*** *In the past month, about how often have you inserted any of these substances? (Enter number)*	[__|__|__] ***Sijui* =999** *don't know=999*		freqinsert
87	**Kwa nini umeviingiza hivi vitu ukeni, vina faida gani?** ***(usisome orodha zungushia yote yatakawotajwa)*** *Why did you insert these substances? (Don't read out the list, circle all that apply)*	**Kujisafisha baada ya haja ndogo** *To clean after toileting*	1	insertclean
		Kuwa msafi wakati wa hedh *During menses*	2	insertmens
		Kulainisha kabla ya kujamiiana *Before sex*	3	insertlub
		Baada ya kujamiiana *After sex*	4	insertpostsx
		Unapokuwa na muwasho ukeni *When you have vaginal irritation/odor*	5	insertodor
		Kutibu/zuia magonjwa ya zinaa au kuzuia vvu *Treat or prevent STI/HIV*	6	insertsti
		Kuzuia mimba *To prevent pregnancy*	7	insertcontrac
		Sababu Viyingine *Other reason (specify)*_____	8	insertwhoth insertwhothsp

88. Date of next follow up appointment [__|__] [__|__|__] [_2_|_0_|__|__] apptdate
DAY MONTH YEAR

89. Place of next appointment apptloc/apptlocsp

Sekou Toure	**1**
Home	**2**
Other_____	**3**

90. Tick here when the swab has been taken ☐ swabconf

Thank the girl for her time

For Data Management use only

	ENTRY 1	ENTRY 2
Initials		
Date		

Annex 4. Follow-up questionnaire

HPV Epidemiology Study **Follow-up Questionnaire.**

HPV Epi Study ID	Lab sticker
PLACE STICKER HERE	PLACE STICKER HERE

Section 1. Socio-demographics

	Question	Coding	SKIP /vaname
1	**Tarehe ya mahojiano** *Date of interview*	[__|__] [__|__|__] [_2_|_0_|__|__] DAY MONTH YEAR	dateenrol
2	**Alama ya anayehoji** *Code of interviewer*	[__|__|__]	staffcode
3	**Hudhurio la ngapi?** *Which visit is this?*	3 month 1 6 month 2 9 month 3 12 month 4 15 month 5 18 month 6	visitno
4	Code for district of current residence Code for ward of current residence	See list of districts in Tanzania [__|__|__] If district is not 1 or 2 or 3 write "00" [__|__]	distcode wardcode
5	**Umehama pale ulipkuwa unaishi mara ya mwisho tulipokuona?** *Have you moved from where you were living since we last saw you?*	**Ndiyo** 1 **Hapana** 2 → **Skip to Q8**	movedhome
6	**Kama ndio, umehamia wapi?** *If yes, where have you moved to?*	**Nimehamia nyumba nyingine katika kijiji/mji ule ule** 1 *Moved to another house in same village/town* **Nimehamia kwingine mkoani Mwanza** 2 *Moved to another town/town in Mwanza region* **Nimehamia nje ya mkoa wa Mwanza** 3 *Moved out of Mwanza region*	movedto
7	**Kwa nini ulihama?** *Why did you move?*	**Kwa ajili ya shule** 1 *For school* **Kufanya kazi** 2 *For work* **Familia imehama** 3 *Family were moving* **Ameolewa** 4 *For marriage* **Nyingine (fafanua)**_____ 5 *other (specify)*	reasonmv reasonmvesp
8	**Unafanya kazi gani kwa sasa?** ***(Zungushia moja)*** *What are you doing now? i.e. "occupation" (circle one answer)*	**Anasoma shule ya sekondari** 1 Attending secondary school Kazi za kawaida ***(mfanya usafi, mhudumu wa baa, mama lishe, mfanyakazi wa nyumbani)*** 2 *Unskilled manual (e.g. cleaner, bar worker, food handler, house girl, salon worker)* Kazi zenye ujuzi maalumu ***(makanika, fundi cherehani, fundi umeme)*** 3 *(Skilled manual e.g. mechanic, tailor, electrician)* Taaluma ***(mwalimu/nesi/mratibu/polisi)*** 4 *Professional (eg. teacher/nurse/ accountant/police)* **Biashara** 5 *Business* **Mkulima** 6 *Agriculture/farming* **Mchimbaji wa madini** 7 *Mining* **Mvuvi** 8 *Fishing* **Hana kazi/anakaa nyumbani** 9 *No job/stay at home/unemployed* **Kazi nyingine** _____ 10 *other, (specify)* **Anarudia shule ya msingi pale pale aliposomea awali** 11 *Repeating at the same primary school* **Anarudia shule ya msingi sehemu tofauti. Taja jina la schule**_____ 12 *Repeating at a different primary school. Specify the name of the school*	occup occupoth pschoth **Skip to Q10**
9	**Nitajie jina la sekondari na kidato ulichofikia?** *What is the name and form of the secondary school you attend? Code the name of the secondary school.*	Code [__|__|__|__|__|__|__|__|__|__|__|__|__] Form [___]	secsch secform
10	**Tangia tulipoonana na wewe mara ya mwisho.....?** ***(Zungushia moja)*** *Since we last saw you, did you get....?* *(circle one)*	**Umeolewa** 1 Married **Umeachika** 2 Divorced **Wametengana** 3 Separated **Mjane** 4 Widowed **Hakuna hata moja** 5 → **skip to 12** None of these	mstatus

11	**Tarehe ngapi ilipotokea?** *What date did this happen?*	**Sijui = "99" or "999"** [__|__] [__|__|__] [_2_|_0_|__|__] DAY MONTH YEAR	mdate
12	**Umewahi kulala kwingine kukaa kwingine hata kwa siku moja tu katika miezi mitatu iliyopita?** *Have you slept a night away from home in the past 3months?*	**Ndiyo** 1 **Hapana** 2 → **Skip to Q15**	nightaway
13	**Kama ndiyo, kwa nini ulilala nje ya nyumbani?** ***(zungushia yote yatakawotajwa)*** *If yes, why did you spend a night away from home? (circle all that apply)*	**Shule ya bweni** *Boarding school* 1 **Kuishi na ndugu kwa ajili ya masomo** *Staying with relatives to go to school* 2 **Kutembelea rafiki au ndugu (likizo, sherehe, mazishi)** *Visit friends or relatives (including for holiday/celebration/funeral)* 3 **Biashara au kazi** *Business or work* 4 **Hospitali au Kliniki** *Hospital or clinic visit* 5 **Nyingine (fafanua)**_____ *Other (specify)* 6	awayboardsc awaysc awayvfr awaywork awayhosp awayoth awayothsp
14	**Kama ndiyo, je ni mara ngapi umekaa kwingine kwa zaidi ya wiki moja katika miezi mitatu iliyopita?** *If yes to above, how many times in past 3 months have you spent more than a week away from home?*	[__|__] ***Sijui =99 Hakuna=00***	totalaway
15	**Tangia tulipoonana na wewe mara ya mwisho ume kunywa pombe? Kama ndiyo, mara ngapi kwa kawaida?** ***(Andika namba, zungushia wakati. Kama hatumii pombe andika 000)*** *Since we last saw you, on average how many alcoholic "drinks" did you drink? (Enter number of drinks and circle time unit. Write 00 if she doesn't drink)*	1 drink=1 bottle of beer or 1 measure of Konyagi or 1 glass of wine or ½ litre of local brew **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 [__|__|__] ***Sijui =999 Hakuna=000*** **Mwaka** *Year* 4 Don't know=999 None=000	alcnum alcnumx
16	**Umeshawahi kutumia vitu vifuatavyo?** ***(zungushia yote yatakawotajwa)*** *Since we last saw you have you taken (circle all that apply)*	**Bangi?** *Marijuana* 1 **Mirungi?** *Ghat* 2 **Unga?** *Cocaine* 3 **Valium?** *Valium* 4 **Heroin?** *Heroin* 5 **Gundi?** *Glue* 6 **Petroli?** *Petrol* 7 **Ugoro?** *Tobacco Powder?* 8 **Nyingine (fafanua)** *Other*_____ 9 **Hakuna hata mmoja** *None of the above* 10 → **Skip to Q18**	maraj/ghat coke/ val hero/glue petrol/ tobaccop drugoth/ drugothsp/ nodrug
17	**Wewe hutumia vitu hivi mara ngapi?** ***Kama wakichagua zaidi ya moja chagua ile ambayo anatumia zaidi. Ingiza muja (1-9) kutoka swali 16. Ingiza ni kwa mara ngapi huwa anatumia halafu zungushia (siku/wiki/mwazi etc).*** *How often do you take this? If she circled several drugs, answer for the one most frequent. Enter the code number for the drug (between 1 and 9), the frequency and the time unit*	**Siku** *Day* 1 **Wiki** *Week* 2 ***Ingiza muja 1-9 kutoka swali 16*** [___] **Mwezi** *Month* 3 *Enter the number from Q16* ***Ingiza ni kwa mara ngapi*** [__|__|__] **Mwaka** *Year* 4 *Enter the number of times* **Sijui** Don't know 5	drugmost drugtime drugtimx
18	**Je, unavuta sigara?** *Do you smoke cigarettes?*	**Ndiyo** 1 **Hapana** 2 → **Skip to Q20**	smoke
19	**Kwa kawaida, wewe huvuta sigara ngapi?** ***(Andika namba, zungushia wakati)*** *On average how many cigarettes do you smoke? (Enter the number of cigarettes and circle the time unit)*	**Siku** *Day* **Wiki** *Week* ***Andika numba*** [__|__|__] **Mwezi** *Month* ***Sijui =999*** **Mwaka** *Year* don't know=999 **Sijui** Don't know	smoknum smoknumx

Section 2. Sexual Behaviour

Mara ya mwisho nimekuuliza maswali kuhusiana na ngono na kuhusu mambo unayoyafanya ukiwa na mvulana. Nitakuuliza tena, ni muhimu kujua kilichotokea ili tugundue mambo yasababishayo kuambukizwa na HPV Yote utakayoniambia huwa siri yetu. Last time I asked you some questions about your sexual behaviour and things you have done with boys. I am going to ask you the same questions again, it is important to find out what has happened last time because we want to see which behaviours lead to HPV infection. Everything you tell me is kept completely secret.

20	**Tangia tumeonana na wewe mara ya mwisho, mara ngapi umeshambusu mwanaume kwenye midomo yake? (fafanua)** *Since we last saw you how many times have you kissed a boy on the lips? (explain and offer answers: "once, twice, ten times!")*	[__|__|__] ***Sijui =999 Hakuna=000***	lipkiss
21	**Tangia tumeonana na wewe mara ya mwisho, mara ngapi umeshawahi kupeana denda na mpenzi/mume wako? (kula uroda, kula denda)** *Since we last saw you, how many times have you kissed a boy on the mouth using tongues?*	[__|__|__] ***Sijui =999 Hakuna=000***	tongkiss
22	**Tangia tumeonana na wewe mara ya mwisho, mara ngapi mpenzi wako amewahi kuchezea matiti yako? (kujivinjari, kutomasatomasa, ku-survey)** *Since we last saw you how many times has a boy played with / touched your breasts?*	[__|__|__] ***Sijui =999 Hakuna=000***	breastrub

23	**Tangia tumeonana na wewe mara ya mwisho, mara ngapi umeshawahi kufanya mapenzi ya kushikana na mwanaume/mvulana, ambapo umeshika kwa mkono wako?** *Since we last saw you, how many times have you touched a man/boy's penis with your hand?*	[__|__|__] ***Sijui =999 Hakuna=000***	penistouch
24	**Tangia tumeonana na wewe mara ya mwisho, mara ngapi umeishawahi kufanya mapenzi ya kushikana na mwanaume/mvulana, ambapo anachezesha uke wako kwa mkono wake? (kupiga brush, kutomasatomasa)** *Since we last saw you how many times have you engaged in sexual touching with a man/boy where he has touched your vagina with his hand?*	[__|__|__] ***Sijui =999 Hakuna=000***	vagtouch
25	**Tangia tumeonana na wewe mara ya mwisho, mara ngapi umewahi kuwekewa uume wa mwanaume/mvulana kwenye mdomo? (kula muhogo, kula muwa, kula cone, kupiga mswaki, alamba)** *Since we last saw you, how many times has a man/boy put his penis in your mouth?*	[__|__|__] ***Sijui =999 Hakuna=000***	penisoral
26	**Tangia tumeonana na wewe mara ya mwisho, mara ngapi wanaume/mvulana amewahi kukunyonya sehemu zako za siri (kwenye uke)? (kufyonza embe, kuzama chumvini, kula pera)** *Since we last saw you, how many times has a man/boy put his mouth on your genitals?*	[__|__|__] ***Sijui =999 Hakuna=000***	vagoral
27	**Tangia tulipoonana na wewe mara ya mwisho, mara ngapi uume wa mwanaume umeshakugusa kwenye mapaja, katikati ya matako au sehemu zako za siri (bila kuingia ndani ya uke wako)? *(kupiga puchu, kupiga katerelo)*** *Since we last say you how many times have you had a boy's penis rubbed between your thighs at the top of your legs or between your buttocks or over your outer genitals (without going inside your vagina)?*	[__|__|__] ***Sijui =999 Hakuna=000***	penisrub
28	**Tangia tulipoonana na wewe mara ya mwisho, mara ngapi umewahi kujamiiana kwa njia ya haja kubwa? (*kufilana, kupakua kisamvu, kula tigo, kula mgongo*)** *Since we last saw you have how many times have you had anal sex? (penis entering your anus)*	[__|__|__] ***Sijui =999 Hakuna=000***	analsex
	Check Annex 14 follow-up list, was she SA at the last visit?	**Ndiyo** *(yes)* 1 → **skip to Q33** **Hapana** *(no)* 2	
29	**Je, tangia tulipoonana mara ya mwisho, mara ngapi umeshawahi kujamiiana kwa njia ya uke. Kwa hili namaanisha pale mwanaume anapoweka uume wake kwenye uke wako?** *Since we last saw you how many times have you had vaginal sexual intercourse? By that I mean where a man puts his penis in your vagina.*	[__|__|__] Hakuna=000	**If 000, skip to vaginal practice BUT answer Q 48-50** sa
30	**Ulipojamiana mara ya kwanza ulipata maumivu?** *When you had sex for the first time did it hurt you?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	painsex1
31	**Ulipojamiana kwa mara ya kwanza je ulitokwa na damu?** *Did you bleed the first time you had sex?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	bleedsex1
32	**Ulitumia chochote mara ya kwanza ili upunguze maumivu kablaya kujamiiana? *(zungushia yote yatakawotajwa)*** *Did you use anything before sex the first time to reduce the pain? (Circle all that apply)*	**Kondomu** *Condom* 1 **Jelli** *Vaseline* 2 **Mate ya mpenzi** *His saliva* 3 **Mate yako mwenyewe** *My saliva* 4 **Maji** *Water* 5 **lotion** 6 **Sikutumia chochote** *Nothing* 7 **Nyingine** *Other*_____ 8	condsex1 vassex1 boysalsex1 girlsalsex1 watersex1 lotionsex1 nothsex1 othsex1 othsex1sp
33	**Je, katika maisha yako yote mpaka sasa hivi, umewahi kujamiiana (uume kwenye uke) na wanaume wangapi? (uwahesabu na wale uliokutana nao mara moja tu na hata wale waliokulazimisha)** *Throughout your whole life up to now, how many different men have you had sex with? (inc all kinds of partner)*	[__|__] ***Sijui =99*** *DK=99*	totnumpart
34	**Je, kwa maisha yako, umejamiiana mara ngapi?** *How many sexual acts have you ever had? (this is the total number of times you have had sex even if it is with only one man)*	[__|__|__] → ***Sijui =999 Tangu Kukaziwa 100=100*** don't know=999 *over 100=100*	totsexnum **If this is 1 or 2, skip to Q 39**
35	**Mara ngapi wapenzi/mume kabla ya kujamiana anachezea uke au sehemu ya nje ya uke? *(Zungushia moja)*** *How often does your lover play with your vagina / external genitals before having sex with you? (circle one answer)*	**Mara zote** always 1 **Mara kwa mara** half of the time 2 **Mara nyingine** sometimes 3 **Mara chache** rarely 4 **Hamna kabisa** never 5	foreplay
36	**Mara ngapi wapenzi/mume wako, wamewahi kutumia mate yao katika uume wao au ndani ya uke wako? *(Zungushia moja)*** *How often does your lover put saliva on his penis or your vagina before sex? (circle one answer)*	**Mara zote** always 1 **Mara kwa mara** half of the time 2 **Mara nyingine** sometimes 3 **Mara chache** rarely 4 **Hamna kabisa** never 5 **Sikumbuki** don't know 6	salivasex

37	**Mara ngapi wapenzi/mume wako, wamewahi kutumia jeli ya Vaseline (Mafuta) katika uume wao au ndani uke wako? *(Zungushia moja)*** *How often does your lover put vaseline on his penis or your vagina before sex? (circle one answer)*	**Ndiyo mara zote** always 1 **Mara kwa mara** half of the time 2 **Mara nyingine** sometimes 3 **Mara chache** rarely 4 **Hamna kabisa** never 5 **Sikumbuki** don't know 6	vassex
38 a	**Mara ngapi wapenzi/mume wako, wamewahi kutumia kitu kingine chochote katika uume wao au ndani uke wako? *(ISIPOKUWA CONDOM. Zunguisha moja)*** *How often does your lover put something else on his penis or your vagina before sex? (EXCEPT CONDOMS, circle one)*	**Ndiyo mara zote** always 1 **Mara kwa mara** half of the time 2 **Mara nyingine** sometimes 3 **Mara chache** rarely 4 **Hamna kabisa** never 5 → **Skip to Q39** **Sikumbuki** don't know 6	lubsex lubsexoth lubsexothsp
38 b	**Kitu anachotumia** *specify what*	***Andika kitu gani***_____Specify what 1 **Sikumbuki** don't know 2	
39	**Tangia tulipoonana na wewe mara ya mwisho, mara ngapi ulijamiiana kwa ajili ya zawadi/pesa?** *Since we last saw you, how many times have you had sex in exchange for gifts or money*	[__|__|__] ***Sijui =999*** → If 000 skip to Q 46	numgiftsex
40	**Ilikuwa zawadi gani?** ***(zungushia yote yatakawotajwa)*** *What was the gift? (Circle all that apply)*	**Pesa** Money 1 **Chakula** Food 2 **Nguo** Clothes 3 **Matokeo ya shule/ Kufaulu mtihani** School grades/pass exams 4 **Pombe** Alcohol 5 **Kusafiri** Lift 6 **Ningine** _____ 7	money food clothes grades alcohol transp othergift othergiftsp
41	**Tangia tulipoonana na wewe mara ya mwisho, mara ngapi umeshawahi kujamiana ukiwa umelewa?** *Since we last saw you, how many times have you had sex whilst drunk?*	[__|__|__] ***Sijui =999***	alcosex
42	**Tangia tulipoonana na wewe mara ya mwisho, mara ngapi uliwahi kulazimishwa na mwanaume kujamiiana naye bila hiari yako?** *Since we last saw you, how many times have you been forced by a man to have sex with him (against your will)?*	[__|__|__] ***Sijui =999***	forcedsex
43	**Tangia tulipoonana na wewe mara ya mwisho tulipokuona uliwahi kujamiiana ukiwa kwenye hedhi?** *Since we last saw you how many times have you had sex whilst menstruating?*	[__|__|__] ***Sijui =999***	menssex
44	**Tangia tulipoonana na wewe, umeshabeba ujauzito mara ngapi? *Andika idadi*** *Since we last saw you, how many times have you been pregnant? Write number*	[__|__] ***Sijui =99*** ***kubwa kuliko=00*** *Don't know=99, Never=00* → If 00 skip to Q 46	numpreg
45	***Je nini matokeo ya mimba hizo? (zungushia yote yatakawotajwa)*** *What happened to those pregnancies? (circle all that apply)*	**Ilitoka yenyewe** *Miscarriage* 1 **Niliamua kuitoa** *Abortion* 2 **Nilijifungua mtoto** *Child* 3 **Ni mjamzito sasa** *Pregnant now* 4	miscar abort child preg
46	**Je unafanya kitu chochote au kutumia njia yoyote kuchele wesha au kuzuia kupata mimba kwa sasa?** *Are you doing something or used any method to delay or avoid pregnancy now?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 → **Skip to Q48**	contracep
47	**Je, unatumia nini ili kuzuia au kuchelewesha kupata mimba sasa? (*zungushia yote yatakawotajwa*)** *What are you doing/using to avoid or delay pregnancy now? Circle all that apply*	**Hakuna** *Nothing* 1 **Vidonge vya uzazi wa mpango** *Oral pill* 2 **Sindano** *Injection* 3 **Kondomu** *Condom* 4 **Kalenda wakati salama** *Calendar/safe period* 5 **Dawa za kienyeji** *Traditional medicine* 6 **Kutoa uume kabla ya kumwaga mbegu** *Withdrawal method* 7 **Mjamzito sasa** *Pregnant now* 8 **Kitanzi** *IUCD* 9 **Nyingine** *Other*_____ 10	methodcont1 methodcont2 methodcont3 methodcont4 methodcont5 methodcont6 methodcont7 methodcont8 methodcont9 methodcont10 methodothsp

48	**Umeshawahi kutumia mtandao (kutuma barua pepe, kutafuta muziki, mitandao ya kijamii nk)** *Have you ever used the internet (e.g. sending email, downloading music, social networking)*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	usewww
49	**Umeshawahi kutazama video/filamu ya ngono?** *Have you ever seen a pornographic video/movie?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	pmovie 2, 3 → **Skip to Q51**
50	**Ikiwa ndiyo umezitazama wapi?** *If yes, where did you see this?*	**Umeonyeshwa na rafiki** *Friend showed you* 1 **Umeonyeshwa na mpenzi** *Boyfriend/lover showed you* 2 **Umeonyeshwa na ndugu** *Family member showed you* 3 **Nyingine** *Other* ________________ 4	pmoviewhere pmoviesp

We only want to know about people she has had sex with since she was last seen. Check Q 29. Ask about most recent first (1.)

		START HERE 1.	2.	3	(Complete for first ever lover if not previously described) 4.
51	**Nitajie jina lake kwa kifupi? Tafadhali kama hukumbuki unaweza kunitajia jina lolote. Vile vile unaweza kunitajia jina lolote kama hutaki nifahamu majina yao.** *What were his initials*	[__|__]	[__|__]	[__|__]	partid [__|__]
52	**Je ulimtaja mara ya mwisho tulipoonana?** *Have you told us about this boy before?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **If Yes skip to Q62**	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **If Yes skip to Q62**	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **If Yes skip to Q62**	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **If Yes skip to Q62**
53	**Anaishi kata gani?** *Where does he live?*	____________	____________	____________	partres ____________
54	**Kwa kawaida huwa mnakutana sehemu gani kujaamiana?** *Where did you usually have sex with him?*	____________	____________	____________	wheresex ____________
55	**Alikuwa na umri gani?** *What age was this person? Guess if not sure.*	[__|__] *Sijui =99* *Don't know=99* → **If she does know the age, skip to Q 57**	[__|__] *Sijui =99* *Don't know=99* → **If she does know the age, skip to Q 57**	[__|__] *Sijui =99* *Don't know=99* → **If she does know the age, skip to Q 57**	partage [__|__] *Sijui =99* *Don't know=99* → **If she does know the age, skip to Q 57**
56	**Unadhani alikuwa na umri gani?** *Estimate the age of this person* 10old/5to10old/und5old/sameage/younger/dkage	**Zaidi ya miaka kumi** More than 10 years older 1 **Kati ya miaka mitano hadi kumi** 5-10 years older 2 **Ananizidi siyo zaidi ya miaka mitano** less than 5 years older 3 **Je mnalingana** Same age 4 **Mdogo** Younger 5 **Sijui** Don't know 6	**Zaidi ya miaka kumi** More than 10 years older 1 **Kati ya miaka mitano hadi kumi** 5-10 years older 2 **Ananizidi siyo zaidi ya miaka mitano** less than 5 years older 3 **Je mnalingana** Same age 4 **Mdogo** Younger 5 **Sijui** Don't know 6	**Zaidi ya miaka kumi** More than 10 years older 1 **Kati ya miaka mitano hadi kumi** 5-10 years older 2 **Ananizidi siyo zaidi ya miaka mitano** less than 5 years older 3 **Je mnalingana** Same age 4 **Mdogo** Younger 5 **Sijui** Don't know 6	**Zaidi ya miaka kumi** More than 10 years older 1 **Kati ya miaka mitano hadi kumi** 5-10 years older 2 **Ananizidi siyo zaidi ya miaka mitano** less than 5 years older 3 **Je mnalingana** Same age 4 **Mdogo** Younger 5 **Sijui** Don't know 6

57	**Huyu mwanamume ndiye aliyekuwa mwenzi wako wa kwanza kabisa wa kimapenzi?** *Was this man/boy your first ever sexual partner?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 firstever
58	**Huyu mwanamume alikuwa nani wako?** ***(Zungushia moja)*** *What relationship was this boy/man to you? (Circle one)*				partrel partreloth
	Mume. *husband*	1	1	1	1
	Mwenzangu ninayeishi naye *Cohabiting partner*	2	2	2	2
	Mpenzi wangu *Other regular partner or boyfriend*	3	3	3	3
	Mwanamume ambaye huwa unafanya naye tendo la kujamiana lakini si mpenzi wako *Casual sex partner*	4	4	4	4
	Simfahamu (tulikutana mara moja) *Stranger (met once)*	5	5	5	5
	Mtu ambaye sitaki kujamiiana naye *Someone who I do not want to have sex with*	6	6	6	6
	nyingine *(other, specify*	7_____	7_____	7_____	7_____
59	**Anafanya kazi gani?** ***(Zungushia moja)*** *What is this person's job? (Circle one)*				partjob partjoboth
	Hana kazi/anakaa nyumbani *No job/stay at home*	1	1	1	1
	Kazi ya Madini *Mine employee*	2	2	2	2
	Mwanafunzi wa shule ya msingi *At primary school*	3	3	3	3
	Mwafunzi wa shule ya sekondari *At secondary school*	4	4	4	4
	Mwanafunzi wa chuo/chuo kikuu *Student at colleage/university*	5	5	5	5
	Kazi ya kuajiriwa (eg. Muuguzi, polisi) *Skilled (eg. nurse/ accountant/police)*	6	6	6	6
	Mwalimu Teacher	7	7	7	7
	Mkulima *Farmer*	8	8	8	8
	Kazi ya kujiajiri ujuzi *Other manual (e.g. carpenter/tailor)*	9	9	9	9
	Anaendesha gari kubwa/msaidizi wa gari kubwa *Truck driver/turnboy*	10	10	10	10
	Biashara *Business (e.g. duka owner; garage owner)*	11	11	11	11
	Kazi nyingine____*(other specify)*	12_____	12_____	12_____	12_____
	Sijui don't know	13	13	13	13
60	**Ni kwa muda gani ulimfahamu huyu mwanaume kabla ya kufanya naye tendo la kujamiiana?** *How long you had you known this man before having sex with him the first time? (Enter number and circle time unit)*	[__|__|__]***Sijui =999*** *Don't know=999* **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 **Mwaka** *Year* 4	[__|__|__]***Sijui =999*** *Don't know=999* **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 **Mwaka** *Year* 4	[__|__|__]***Sijui =999*** *Don't know=999* **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 **Mwaka** *Year* 4	timepresex/presexunit [__|__|__]***Sijui =999*** *Don't know=999* **Siku** *Day* 1 **Wiki** *Week* 2 **Mwezi** *Month* 3 **Mwaka** *Year* 4

61	**Ni siku/tarehe gani ulipojamiana naye kwa mara ya kwanza mwanamume/mvulana huyu?** *What date did you first have sex with this man/boy? (Write 99 or 999 if doesn't know)*	***Sijui =999 au 99*** *Don't know=999 or 99* [__|__] date [__|__|__]month [_2_|_0_|__|__]Year	***Sijui =999 au 99*** *Don't know=999 or 99* [__|__] date [__|__|__]month [_2_|_0_|__|__]Year	***Sijui =999 au 99*** *Don't know=999 or 99* [__|__] date [__|__|__]month [_2_|_0_|__|__]Year	***Sijui =999 au 99*** *Don't know=999 or 99* [__|__] date [__|__|__]month [_2_|_0_|__|__]Year datefirst
62	**Unaendelea kujamiiana na mwanaume huyu hadi sasa?** *Are you still having sex with this man/boy?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	currsex **Ndiyo** *(yes)* 1 **Hapana** *(no)* 2
63	**Je, huyu mwanamme /mvulana ulijaamiana naye tarehe ngapi/siku gani kwa mara ya mwisho?** *What was the date of the last sex with this man/boy (Write 99 or 999 if doesn't know)*	**(this is the last time she had sex)** [__|__] date [__|__|__]month [_2_|_0_|__|__]Year	[__|__] date [__|__|__]month [_2_|_0_|__|__]Year	[__|__] date [__|__|__]month [_2_|_0_|__|__]Year	datelast [__|__] date [__|__|__]month [_2_|_0_|__|__]Year
64		**Je, mlitumia kondomu mara ya mwisho mlipojamiiana?** *The last time you had sex, did you use a condom?* **Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3			condomlasts
65	**Huwa mnatumia kondom na mwanaume huyu?*(Zungushia moja)*** *Do you use condoms with this man/boy? (circle one)*				condfreq
	Situmii kabisa *Never use them*	1	1	1	1
	Mara chache *Few times/rarely*	2	2	2	2
	Mara nyingine *Sometimes*	3	3	3	3
	Mara nyingi *Often (frequently, most of the time)*	4	4	4	4
	Kila wakati *Always (everytime)*	5	5	5	5
66	**Umejamiiana na mwanamume huyu mara ngapi?** *How many times have you had sex with this man in total?*	[__|__|__] ***Sijui =999*** ***Tangu Kuzaliwa 100=100*** don't know=999, *over 100=100*	[__|__|__] ***Sijui =999*** ***Tangu Kuzaliwa 100=100*** don't know=999, *over 100=100*	[__|__|__] ***Sijui =999*** ***Tangu Kuzaliwa 100=100*** don't know=999, *over 100=100*	numsex [__|__|__] ***Sijui =999*** ***Tangu Kuzaliwa 100=100*** don't know=999, *over 100=100*
67	**Huyu mwanamume alitahiriwa? (alikatwa)** *Was this man/boy circumcised?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	circum **Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3
68	**Wakati ulipokuwa na mahusiano ya kimapenzi na huyu mwanaume, alikuwa na wapenzi wengine pia?** *During the time you had been involved sexually with this man/boy, did he have another partner?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2 **Sikumbuki** *(DK)* 3 boyconc
		"Asante kwa kujibu maswali kuhusu mwanaume huyu. Ningependa sasa kukuuliza baadhi ya maswali kuhusu mwanaume wa mwisho uliyefanya naye tendo la kujimiiana" ***Go back to Q51, part 2***	**Asante kwa kujibu maswali kuhusu mwanamume huyu. Ningependa sasa kukuuliza baadhi ya maswali kuhusu mwanaume wa mwisho uliyefanya naye tendo la kujimiiana"** ***Go to Q51, part 3 or 4***		*Thank the girl for her time. Go to the next section on vaginal practices*

Section 3. Vaginal Practices

Mara ya mwisho nimekuuliza pia kuhusu taratibu zako za kujitunza. Nitakuuliza tena maswali hayo, usiogope kuuliza ikiwa hunielewe vizuri Nitafurahi kukuelewehsa vizuri.

Last time I also asked you some questions about personal vaginal practices. I am going to ask you the same questions again, please do not be afraid to ask if you do not understand the question that is being asked, I am happy to explain.

69	**Tangu tulipoonana mpaka sasa umetumia nini kuzuia damu wataki wa hedhi? *(Zungushia moja)*** *In the last three months, what have you been using during menstruation to catch the blood? (circle one)*	**Vitambaa/kanga** *Cloth* 1 **Chupi** *Pants* 2 **Karatasi ya choo** *Toilet paper* 3 **Pedi za madukani** *Sanitary napkins* 4 **Tampons** *Tampons* 5 **Nyingine** *Other*_____ 6 **Sina hedhi** *Not menstruating* 7	**Skip to Q 71**	sanit sanitoth
70	**Kama unatumia nguo, huwa unaiweka ndani ya uke au nje ya uke au zote pamoja? *(Zungushia moja)*** *You said that you use cloths, do you use these inside or outside the vagina or both? (circle one)*	**Ndani ya uke peke yake** *Only inside the vagina* 1 **Nje ya uke peke yake** *Only outside the vagina* 2 **Zote pamoja** *Both inside and outside* 3		cloth
71	**Tangu tulipoonana umesafisha uke kwa ndani. Inamaanisha kusafisha ndani ya uke kwa maji au kitu kingine?** *In the past three months have you cleansed inside the vagina?*	**Ndiyo** *(yes)* 1 **Hapana** *(no)* 2	**Skip to Q 76**	everclean
72	**Kwa kwaida huwa unasafisha mara ngapi ndani ya uke? *Andika namba, zungushia wakati*** *In the past 3 months, about how often have you cleansed inside the vagina? Enter the number, circle the time.*	[__|__|__] ***Sijui =999*** don't know=999 **Siku** *Day* 1 **Wiki** *Week* 2 **Mwaka** *Year* 3 **Mwezi** *Month* 4		clean freqclean
73	**Tangu tulipoonana ulisafisha na vitu vifuatavyo? *(zungushia yote yatakawotajwa)*** *In the past three months have you cleansed with any of the following? (circle all that apply)*	**Maji tu** *Plain water* 1 **Sabuni au maji na sabuni** *Soap/soap and water* 2 **Dawa ya kuzuia wadudu kwa mfano Dettol/Jik** *Disinfectant e.g. Dettol/Jik* 3 **Sabuni ya unga** *Washing powder* 4 **Vinginevyo vinavyotumika**_____ *(Other, write what)* 5		clnwat clnsoap clnjik clnwashp clnsuboth clnsubothsp
74	**Umenieleza kuhusu vitu unavyovitumia kusafisha, sasa niambie unasafishaje? *(usisome orodha, zungushia yote yatakawotajwa)*** *You have told me about what you use to clean, now tell me how exactly you do this? (Don't read out list, circle all that apply)*	**Vidole** *Fingers* 1 **Nguo kama kitambaa** *Cloth* 2 **Pamba** *Cotton wool* 3 **Karatasi kama gazeti** *Paper including Newspaper* 4 **Nyingine** *other (specify)*_____ 5		clnfing clncloth clncotwl clnpaper clnwoth clnwothsp
75	**Ni wakati gani hasa huwa unasafisha uke kwa ndani? *(Usisome orodha zungushia yote yatakawotajwa)*** *When do you usually clean inside the vagina? (Don't read out the list, circle all that apply)*	**Kila siku au ninapoenda chooni** *During daily toileting* 1 **Kila siku nina pooga** *During bathing* 2 **Wakati wa hedhi** *During menses* 3 **Kabla ya kujamiiana** *To prepare for sex* 4 **Baada ya kujamiiana** *Immediately after sex* 5 **Unapokua na muwasho ukeni** *When you have vaginal irritation or bad odor* 6 **Viyingine** *Other time (specify)*_____ 7		clntoilet clnbath clnmense clnpresx clnpostsx clnodor clnwhoth clnwhothsp
76	**Umeishawahi kuweka vitu hivi ndani ya uke wako? (*Soma orodha zungushia yote yatakawotajwa)*** *In the last three months have you inserted any of the following substances into your vagina? (Read out list and circle all that apply)*	**limao au Ndimu** *Lemon or lime* 1 **Mitishamba** *Traditional healer medicine* 2 **Dawa ya kuzuia wadudu kwa mfano Dettol/Jik** *Disinfectant e.g. Dettol/Jik* 3 **Sabuni ya unga** *Washing powder* 4 **Ugoro** *tobacco powder* 5 **Nyingine** *Other (specify)*_____ 6 **Sijawahi kuingiza chochote** *never inserted* 7	**Skip to Q 79**	insertcitric inserttradm insertjik insertwashp inserttobac insertoth insertothsp neverinsert
77	**Ndani ya mwezi mmoja uliopita uliwahi kuigiza chochote ndani ya uke mara ngapi? *(Andika namba)*** *In the past month, about how often have you inserted any of these substance? (Enter number)*	[__|__|__] ***Sijui =999***		freqinsert

78	**Kwa nini umeviingiza hivi vitu ukeni, vina faida gani? (*usisome orodha zungushia yote yatakawotajwa)*** *Why did you insert these substances? (Don't read out the list, circle all that apply)*	**Kujisafisha baada ya haja ndogo** *To clean after tolleting*	1	insertclean
		Kuwa msafi wakati wa hedh *During menses*	2	insertmens
		Kulainisha kabla ya kujamiiana *Before sex*	3	insertlub
		Baada ya kujamiiana *After sex*	4	insertpostsx
		Unapokuwa na muwasho ukeni *When you have vaginal irritation/odor*	5	insertodor
		Kutibu/zuia magonjwa ya zinaa au kuzuia vvu *Treat or prevent STI/ HIV*	6	insertsti
		Kuzuia mimba *To prevent pregnancy*	7	insertcontrac
		Sababu Viyingine *Other reason (specify)*_____	8	insertwhoth insertwhothsp

79. Date of next follow up appointment [__|__] [__|__|__] [_2_|_0_|__|__] apptdate

DAY MONTH YEAR

80. Place of next appointment

Sekou Toure	**1**	
Other Health Facility/dispensary/School_____ **(write name)**	**2**	
Home	**3**	

apptplace

Thank the girl for her time. Give her the insentive, remind her that there is an insentive at every visit.

Perform <u>STI screen</u> if she has ever had sex even if no sex since last visit.

Document in your diary whether she will be collected from <u>school</u> or <u>home</u> if the appointment is at a clinic or dispensary. Document which school.

Ask for a contact telephone number (s).

Annex 5. Informed consent (parent)

Annex 1

HPV Epidemiology Study

CONFIDENTIAL

INFORMED CONSENT FORM FOR HPV NATURAL HISTORY STUDY FOR SUBJECT BELOW LEGAL AGE OF CONSENT

Study Identification: MITU-002

Study Title: Epidemiology and natural history of human papillomavirus infection in a cohort of Tanzanian girls after sexual debut

Abbreviated Title: HPV Epidemiology Study

Version Number: [1]

Date: 11th Aug 2011

Subject Identification: _____

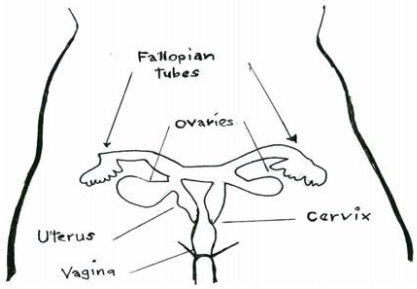
Please present this document to the subject in full and explain the contents to the subject.

HPV Epidemiology Study
CONFIDENTIAL

Introduction

You may have heard about infections that can be passed from person to person when two people have sex. These infections are called sexually transmitted diseases. One of the most common of these is a virus called human papillomavirus that is known as HPV. This virus is very important as it can cause cancer of the cervix in women many years after they have caught HPV. This is a serious disease and cancer of the cervix the most common cancer in women in Africa.

The cervix is in the lower part of the uterus or womb that separates the uterus from the vagina. The location of the cervix is shown in the picture of a woman's sexual organs (see below). When a girl catches HPV this virus enters cells in the cervix. In many girls the virus is removed from her body by the girl's immune system. In other girls the virus stays inside the cervix for many years. In a small number of those girls who have long term infection with HPV, the virus starts changing the cells in the cervix and if left untreated, this can lead to cancer 10 or more years later. There are many types of HPV virus but only some of these types cause cervical cancer.



There are many different types of HPV but only some types of HPV cause cervical cancer. Fortunately there are now vaccines that can prevent people from being infected with some of the HPV types that cause cervical cancer. The vaccines have been licensed in Tanzania but are not yet available through government health services. The Ministry of Health and Social Welfare is planning to introduce the vaccine in the next few years.

We do not know how quickly girls in Africa start catching HPV once they start having sex with boys or men. In order to plan for vaccine programmes we need more information about when girls catch HPV, how quickly the HPV virus is cleared by the body, and which types of HPV are common in Tanzania. This information will help us to decide which age groups of girls should be given the vaccine. The vaccine works best in girls who have not yet caught the types of HPV that cause cancer of the cervix and so we are asking girls who have not yet started sex or who have only recently started having sex to participate in a study to help us find out more about HPV in Tanzanian girls.

HPV Epidemiology Study

CONFIDENTIAL

Your daughter is being asked to participate in a small study to see what types of HPV are being passed to girls from their sexual partners once they start having sex and how quickly the girls can clear the HPV viruses.

If your daughter participates in the study, she will need to be seen by the study team every three months for 18 months. This is a total of seven visits. At these visits we will ask her some questions about whether she has had sex, whether she used a condom and some other questions about her sexual behaviour. At the same visit we will be asking her to take a swab from her vagina. A nurse will teach her how to do this and may be present whilst she takes the swab. This is painless and safe and will not harm her in any way. We will be asking around 400 girls to be involved in the study. If they are aged less than 18 years, we will be asking their parent's permission.

I understand that

If I accept to participate in this study and my daughter agrees, the following will happen:

I will have to sign (or thumbprint) this form and my daughter will have to sign another form.

My daughter will then be asked to have an interview with one of the female nursing staff from NIMR. She will be asked to answer questions about her age, schooling, family, friends, health and sexual history and whether she is using any contraception.

She will be asked to take a swab from her vagina at every visit. Taking the swab is painless and safe and will not harm her in any way. A nurse may be watching whilst she takes the swab.

I understand that my daughter and I can refuse to participate in the study without giving any reason. This will have no consequences for our future health care.

My daughter and I will benefit from the interview because we will be able to ask questions about HPV infection and cervical cancer and how to prevent it.

My daughter will benefit from the HPV testing because if she is found to have been infected with the same type of HPV for a long time, she will be referred for a check-up. She will also receive free treatment for sexually transmitted diseases if the nurse thinks she needs this. She will be offered an HIV test at the end of the study if she has had sex. Having this HIV test is completely voluntary and is for her own health.

My daughter and I will not receive any direct payment for participation in this study. I will be reimbursed for any transport costs incurred in bringing my daughter to study visits at Sekou Toure Regional Hospital.

If I decide that my daughter can take part in the study and my daughter agrees, a study identification card will be given to her with her subject identification number, her name, her photograph, her address, her date of birth, the date of the visits she will have to go to, and the name and phone number of the senior study team members.

My daughter will be asked to have seven study visits where she will either be visited by a team member or asked to attend a clinic at Sekou Toure Regional Hospital. These visits will be at Months 0 (her first visit), 3, 6, 9, 12, 15 and 18 and the dates of these visits will be written on her study identification card. My daughter will be advised by the study team of the date of her next visit. In total, the study will last approximately 18 months.

I understand that if I have any questions on this study, or if there are things that I do not understand, I will contact the following people:

- **Dr Catherine Houlihan,** Mwanza Interventions Trials Unit, National Institute for Medical Research, Isamilo, Mwanza. Tel: 0769396532

- **Dr Deborah Watson-Jones,** Mwanza Interventions Trials Unit, National Institute for Medical Research, Isamilo, Mwanza. Tel: 0754 056066

I understand that if I have any questions about my daughter's rights as a research study participant, I can contact the following people:

- Dr Mwele N Malecela-Lazaro, Chair, National Health Research Ethics Review Sub-Committee, National Medical Research Coordinating Committee, National Institute for Medical Research, P.O. Box 6953, Dar es Salaam. Tel: c/o Joyce Ikingura - 0713 438263

- Professor Andrew Hall, Chair, Ethics Committee, London School of Hygiene & Tropical Medicine, England. Tel +44 207 636 8636

I understand that information about my participation and my daughter's participation in this study will remain confidential. The information collected may be seen by people supervising the study or people who work for or with the London School of Hygiene & Tropical Medicine. All will be asked to keep the information confidential. All forms will be kept safe and locked in the study office in Mwanza.

Confirmation

I confirm that I have read the written information (or have had the information read to me) for this interview study.

I have had time to discuss the Informed Consent Form, and the study procedures have been explained to me by the study team.

I confirm that I have had the opportunity to ask questions about this study and I am happy with the answers and explanations that have been provided.

I have been given enough time and opportunity to decide whether I want my daughter to take part in this study.

I agree that the authorized persons described in the information sheet can have access to the data.

I understand that my daughter can leave the study at any time and that this will not affect her or me accessing other healthcare services in Mwanza in any way

I agree that my daughter may be visited at home or at school by the study team.

I agree that my daughter can take part in this study

Subject's name		Thumbprint of subject unable to sign
Subject's signature	Date (DD/MM/YY)	
Name of person conducting the consent		
Signature of the person conducting the consent	Date (DD/MM/YY)	
Name of impartial witness		
Signature of impartial witness	Date (DD/MM/YY)	

Annex 6. Informed assent (participant)

Annex 2

HPV Epidemiology Study

CONFIDENTIAL

INFORMED ASSENT FORM FOR HPV NATURAL HISTORY STUDY FOR SUBJECT BELOW LEGAL AGE OF CONSENT

Study Identification: MITU-002Study Title: Epidemiology and natural history of human papillomavirus infection in a cohort of Tanzanian girls after sexual debut

Abbreviated Title: HPV Epidemiology Study

Version Number: [1]

Date: 11th August 2011

Subject Identification: _____

Please present this document to the subject in full and explain the contents to the subject.

Introduction

You may have heard about infections that can be passed from person to person when two people have sex. These infections are called sexually transmitted diseases. One of the most common of these is a virus called human papillomavirus that is known as HPV. This virus is very important as it can cause cancer of the cervix in women many years after they have caught HPV. This is a serious disease and cancer of the cervix the most common cancer in women in Africa.

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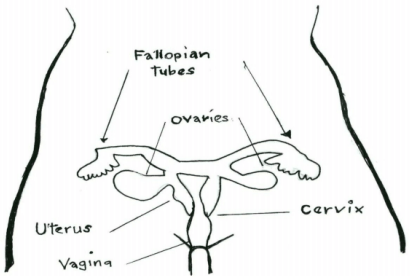

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We do not know very much about how quickly girls in Africa start catching HPV once they start having sex with boys or men. In order to plan for vaccine programmes we need more information about when girls catch HPV, how quickly the HPV virus is cleared by the body, and which types of HPV are common in Tanzania. This information will help us to decide which age groups of girls should be given the vaccine. The vaccine works best in girls who have not yet caught the types of HPV that cause cancer of the cervix and so we are asking girls who have not yet started sex or who have recently started having sex to participate in a study to help us find out more about HPV in Tanzanian girls.

You are being asked to participate in a small study to see what types of HPV are being passed to girls from their sexual partners once they start having sex and how quickly the girls can clear the HPV viruses.

If you participate in the study, you will need to be seen by the study team every three months for 18 months. This is a total of seven visits. At these visits we will ask you some questions about whether you have had sex, whether you used a condom and some other questions about your sexual behaviour. At the same visit we will be asking you to take a swab from your vagina. A nurse will teach you how to do this and may be present whilst you take the swab. This is painless and safe and will not harm you in any way. We will be asking around 400 girls to be involved in the study. If the girls are aged less than 18 years we will be asking their parent's permission.

I understand that

If I accept to participate in this study and my parents/legal guardian agree, the following will happen:

I will have to sign this form and, if I am aged less than 18 years, my parent/legal guardian will sign another form.

I will be asked to have an interview with one of the female nursing staff from NIMR. I will be asked to answer questions about my age, schooling, family, friends, health and sexual history and whether I am using any contraception.

I will be asked to take a swab from my vagina at every visit. Taking the swab is painless and safe and will not harm me in any way. A nurse may be watching whilst I take the swab.

I understand that I can refuse to participate in the study without giving any reason. This will have no consequences for future health care.

I will benefit from the interview because I will be able to ask questions about HPV infection and cervical cancer and how to prevent it.

I will benefit from the HPV testing because if I am found to have been infected with the same type of HPV for a long time, I will be referred for a check-up. I will also receive free treatment for sexually transmitted diseases if the nurse thinks I will need this. I will be offered an HIV test at the end of the study if I have had sex. Having this HIV test is completely voluntary and is for my own health.

I will not receive any direct payment for my participation in this study. If I decide to take part in the study and my parents/legal guardian agree, a study identification card will be given to me with my subject identification number, my name, my photograph, my address, my date of birth, the date of the visits I will have to go to, and the name and phone number of the senior study team members.

There will be seven study visits where I will either be visited by a team member at home or at school or asked to attend a clinic at Sekou Toure Regional Hospital. These visits will be at Months 0 (my first visit), 3, 6, 9, 12, 15 and 18 and the dates of these visits will be written on my study identification card. My parents/legal guardian and I will be advised by the study team of the date of my next visit). In total, the study will last approximately 18 months.

I understand that if I have any questions on this study, or if there are things that I do not understand, I will contact the following people:

- **Dr Catherine Houlihan,** Mwanza Interventions Trials Unit, National Institute for Medical Research, Isamilo, Mwanza. Tel: 0769396532

- **Dr Deborah Watson-Jones,** Mwanza Interventions Trials Unit, National Institute for Medical Research, Isamilo, Mwanza. Tel: 0754056066

I understand that if I have any questions about my rights as a research study participant, I can contact the following people:

- Dr Mwele N Malecela-Lazaro, Chair, National Health Research Ethics Review Sub-Committee, National Medical Research Coordinating Committee, National Institute for Medical Research, P.O. Box 6953, Dar es Salaam. Tel: c/o Joyce Ikingura - 0713 438263

- Professor Andrew Hall, Chair, Ethics Committee, London School of Hygiene & Tropical Medicine, England. Tel +44 207 636 8636

I understand that information about my participation in this study will remain confidential. The information collected may be seen by people supervising the study or people who work for or with the London School of Hygiene & Tropical Medicine. All will be asked to keep the information confidential. All forms will be kept safe and locked in the study office in Mwanza.

Confirmation

- I confirm that I have read the written information (or have had the information read to me) for this study.

- I have had time to discuss the Informed Assent Form, at school or at home, and the study procedures have been explained to me by the study team.

- I confirm that I have had the opportunity to ask questions about this study and I am happy with the answers and explanations that have been provided.

- I have been given enough time and opportunity to decide whether I want to take part in this study.
- I agree that the authorized persons described in the information sheet can have access to the data.
- I understand that I can leave the study at any time and that this will not affect me accessing other healthcare services in Mwanza in any way.
- We do not know very much about how quickly girls in Africa start catching HPV once they start having sex with boys or men.

I agree to take part in this study

Subject's name		Thumbprint of subject unable to sign
Subject's signature	Date (DD/MM/YY)	

Name of person conducting the consent

Signature of the person conducting the consent	Date (DD/MM/YY)

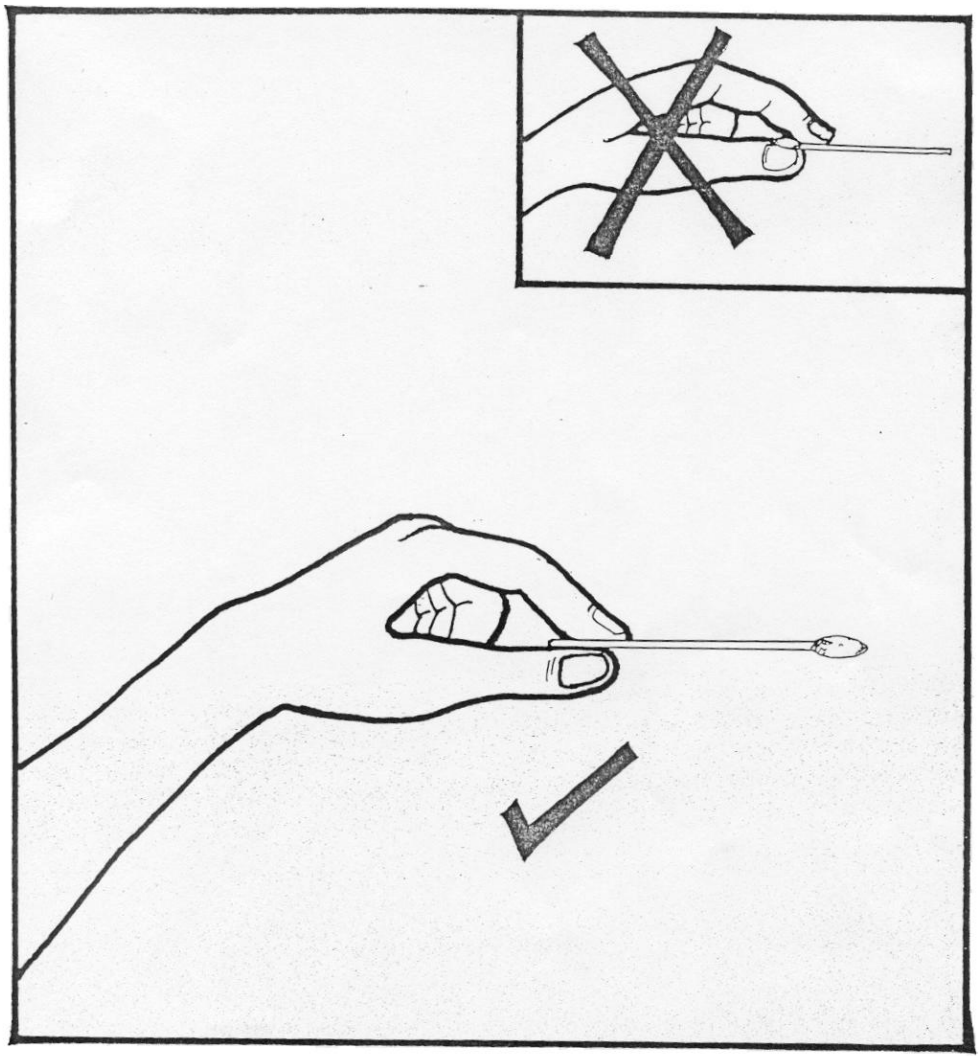
Name of impartial witness

Signature of impartial witness	Date (DD/MM/YY)

Annex 7. Nurse-assisted self-administered vaginal swab pictures

Annex 10. Self-Administered Vaginal Swab Pictures
Picture 1

Picture 2



Picture 3

Annex 8. Nurse-assisted self-administered vaginal swab instructions

Annex 9a HPV Epi Study. Self-Administered Vaginal Swab Explanation Sheet (English)

The nurse should say the following to each girl:

"One way in which we can test for HPV in your private parts is to ask you to insert the cotton part of a swab like this (***Hold up the demonstration swab***) a little way inside the entrance to your "private parts". Later, we shall test the fluids that are collected on the swab for HPV. I am now going to explain how to use the swab, and I will then ask you whether you are willing to do it."

"First, I want to check that you know what I mean by "private parts". The "private parts" is the hole between your legs here (***point in the general direction of your own vagina***) where the blood comes out when you menstruate each month. Do you know which hole I am talking about when I talk about the "private parts"? Can you find it easily?

"If you agree to do this, I would like you to go behind the screen over there and raise your skirt and lower your underpants. I will hand you a swab like this (***Hold up the demonstration swab***). Put one foot on a chair or stool and open your legs ***(Show Picture 1)***. Use the fingers of one hand to open the labia, like the woman in this picture ***(Show Picture 3)***. Then take the swab out of its packet (***Demonstrate***). You should hold the swab at this end, like this ***(Demonstrate & also show Picture 2)***. Please be very careful not to touch the cotton part of the swab with your fingers at any time."

"You should put this end (***Point to the end with the cotton wool on it***) with the cotton on it only about one and a half inches (***Demonstrate about 1.5 inches using your finger and thumb)*** inside your private parts. If you feel resistance then stop. Do not press the swab in hard, just until you feel that the cotton part has gone inside. Then turn the swab around three or four times, like this (***Demonstrate, using a slow stirring motion***). The correct movement is a bit like if you slowly and gently stir soup that you are cooking. Then, remove the swab, and hand it back to me, holding the swab like this (***Demonstrate***). You should not let the cotton part of the swab touch anything except the inside of your private parts while you are doing this. I will observe you taking the swab so that you do it correctly."

"please tell me if you accidently drop the swab or touch the cotton part and I will give you a fresh one"

"Taking the swab should not cause you any pain, at all. Also, it is completely safe, even if you have never had sex, are pregnant or are menstruating."

"Do you have any questions about what I am asking you to do?"

"Are you willing to do this?"

If yes; say:

"I'd just like to be sure you know what you have to do, so please hold this demonstration swab and tell me what you will do.

Repeatedly stress that taking the swab should not cause any pain at all, and that it is completely safe.

Recheck she is willing to continue and has understood the explanation by asking:

"Is that okay?"

"Do you have any questions?"

Don't forget to help her wash her hands with soap both before and after taking the swabs, and to give her the hand towel to take home if she wants to keep it.

NOTE: if she touches the cotton part of the swab or drops the swab, replace with a fresh one.

Annex 9. Colloquial terms for sexual behaviours

Term in English/Swahili	Swahili slang	Direct translation	Notes
Sex	Ku-do	sex	
	Kuchakachuliwa	sex	
	Kugongwa mshipa	sex	
	Ku sex	sex	
	Kutiana	sexual intercourse	?more formal terminology
	Kuingia ngumu kutoka laini	hard to enter, soft to remove	
	Kuingia kavu kutoka mbichi	dry entering, wet while removed	
	Malavidavi		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Kiss with tongues / busu-la kunyomyana	Kula denda	Saliva sucking	
	Kula uroda		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Boy touching girls breasts / Kushika matiti	Ku-survey		
	Kutomasatomasa		
	kujivinjari		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Anal sex / Kujamiiana kwa njia ya hajakumbwa	Kula mgongo	eat back	
	Kula tigo	eat tigo	
	Kupakua kisamvu	eat cassava leaves	
	Kufilana	anal sex	

Term in English/Swahili	Swahili slang	Direct translation	Notes
Boys penis rubbed between thighs, buttocks or outer genitals / Kuchezesha uume kwenye mapaja, matakoni, nje ya uke	Kupiga katerelo		
	Kupiga puchu		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Oral sex, penis in mouth / Kuweka uume mdomoni	Kula cone	eat cone (ice-cream cone)	
	Kula muwa	eat sugar cane	
	kula ndizi	eat banana	
	Kula muhogo	eat cassava	
	kula tango	eat cucumber	
	Kula pipi ya kijiti	eat sweet roll	
	Kupiga mswaki	tooth brushing	
	Alamba		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Oral sex, mouth on vagina / Kulamba uke	Kuzama chumvini	drown in salt	
	kula pera	eat pear	
	kula apple	eat apple	
	Kufyonza embe	sucking mango	
Term in English/Swahili	Swahili slang	Direct translation	Notes
Boy touching girls vagina / Kushika uke kwa mkono	Kupiga brush	brushing	
	Ku survey	surveying	
	Kutomasatomasa		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Girls word for boyfriend	Kipapatio		
	Basha langu		
	boy-friend		
	Ki manzi	boy-friend	
	Kichuana		
	Mshikaji	boyfriend	
	Jamaa yangu		
	Buzi		
	Shalobalo		
	Wa ukeweli		
	HB	handsome boy	
	My Carpet		
	Partiner		
	Asali ya moyo	honey of my heart	
	Baby boy		
	Sweetie		
	Special boy		
	Honey		
	My Chocolate		
	My Lover		
	Maabuba		
	La Azizi		
	Mshua		
	Pedeshee		These terms apply to male partner over 35yrs old
	Bakurutu		
	Sabantele		
	Fataki		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Boys word for girlfriend	Kifunishi	parcel	
	Ki-portable		

	Wa kuzugia		
	Candy		
	Demu	girl-friend	
	Toto langu	my baby	
	Fisi maji	? in the water	
	Ngau		
	Kichenchede		These terms apply to girls if the boy has multiple partners.
	Kicheche		
	La Wote		
	Paka mapepe		
	Jamvi la wageni		
	girl-friend		
	Kisura		
	Mpondo	big buttocks	
	Mrembo	beauty	
	Mrupo		
	Habipt		
	Kipimajoto	temperature measure	
	Blanket		
	Ubavu-wangu	Partner	
	Nyongo mkaliaini		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Vagina / Uke	Kitumba		
	Bakuli	bowl	
	Kisima	well	
	Buyu la asali	bowl of honey	
	Chunga	pot	
	Andazi		
	Mgodi	mine	
	Bulibul		
	Mbuda		
	Kinu	local motor	

	Kidondandugu	chronic wound	
	Mtaro wa maji machafu		
	pochi	wallet	
	Kichongeo	sharpener	
	Kibumbu		

Term in English/Swahili	Swahili slang	Direct translation	Notes
Penis / Uume	Gogo	big penis	
	Kiboroso	small penis	
	Pencil		
	Muhogo	cassava	
	Dudu		
	Alikafimbo		Penis which cannot become erect
	Mshipa	muscles	
	Mwanafalsafa		
	Mchafuzi wa mazingira		
	Kidumu		
	Mkoromboko		
	Mchi	big penis	
	Mashine	Machine	

Annex 10. Clinical outcomes

Table 2.2 Criteria for referral for clinical follow up of persistent HPV at study completion

Participant (number)	Follow-up samples which were tested for HPV	Criteria for clinical referral at study completion
Reported sex either before or during study (106). **Group 1**.	All samples; 3, 6, 9, 12, 15, 18 months	Three consecutive positive samples for the same HR HPV genotype where the final sample was from the final visit attended
Did not report sex either before or during the study but randomly selected (120). **Group 2.**	6 month samples; 6, 12, 18 months	Two consecutive positive samples for the same HR HPV genotype where the final sample was from the final visit attended
Did not report sex either before or during the study and **not** randomly selected (277). **Group 3.**	If the enrolment sample was positive for a HR HPV genotype, the final (attended) visit sample was tested	Sample at final visit was positive for the same HR HPV genotype as enrolment

Summary of clinical follow-up

Group 1

Of the 106 girls who reported sex, 11 (10.4%) met criteria for clinical follow-up at study completion. One participant refused follow-up, and 10 girls were seen at Bugando Medical Centre (BMC) for visual inspection with ascetic acid (VIA). None had abnormal findings.

Group 2

Of the 120 girls who reported not having had sex before or during the study, and who were randomly selected for the incidence study, 5 met clinical criteria for follow-up at study completion. One was not found, and one refused to be seen at BMC. Three underwent VIA, and none had abnormal findings.

Group 3

Of the 277 girls who did not report sex before or during the study, and who were not randomly selected for the incidence study, 9 had HR HPV at enrolment into the study. Of those, 5 had the same HR HPV genotype detected at the last visit they attended and therefore met criteria for clinical follow-up. Of those, 1 was not found, and 2 refused to be seen at BMC. Two underwent VIA at BMC and none had abnormal findings.

Annex 11. ACASI questions

Test Questions:

	Question	Possible responses	Verbal instructions – for ACASI on laptop	Skip
1	Wewe ni msichana au mvulana? *Are you a boy or a girl*	Mvulana Msichana	Chagua moja kati ya mvulana au msichana *Choose one*	
2	Una umri wa miaka mingapi? *What is your age?*	Enter number	Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia *Enter number. If you don't know, choose I don't know on the right*	
	Unaishi na nani katika kaya(nyumba) unayoishi? *Who lives in your house with you?*	Baba Mama Kaka Dada Shangazi Mjomba Mama mdogo Mama mkubwa Baba mdogo Baba mkubwa Babu Bibi Rafiki Ndugu wengine Watu wengine	*choose all that apply*	
3	Uko Tanzania kwa sasa? *Are you currently in Tanzania?*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia *Choose yes or no.If you don't know choose I don't know on the right*	
4	Ulishawahi kwenda sokoni? *Have you ever been to the market*	Ndiyo Hapana *yes/no*	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia *Choose yes or no.If you don't know choose I don't know on the right*	Skip next q if answer "no"
5	Katika miezi mitatu iliyopita ulienda sokoni mara ngapi? *In the past three months how many times have you been to the market?*	Mara moja Mara mbili Mara tatu Mara nne Mara tano Mara sita Zaidi ya mara sita	Changua jibu sahihi. Au unaweza ukabahatisha jibu sahihi.	
6	Ni nani uliyeongea naye kwa mara ya mwisho kabla haujaja katika haya majadiliano? anaweza kuwa mototo au mtu yeyote. Pia inawezekana ikawa jana kama ulikuwa haujaongea na mtu yeyote *Who was the last*	Mama Baba Dada Kaka Bibi Babu Shangazi Mjomba Rafiki Mtoto Nesi Au mtu yeyote ambaye hayupo kafika orodha hii	Changua jibu sahihi kati ya haya	

	person you spoke to before this interview? Not this could be anyone including a small baby and it could be yesterday if you didn't speak to anyone today...			
7	Huyo mtu uliyeongea naye ana umri wa miaka mingapi? *What age was that person*	Zaidi ya miaka kumi Kati ya miaka mitano hadi kumi Ananizidi siyo zaidi ya miaka mitano Tunalingana Mdogo *More than 10yrs* *More than 5y older less than 10yrs older* *older but less than 5yrs* *Same age* *younger*	Changua jibu sahihi. Au unaweza ukabahatisha jibu sahihi.	

Demographic, sexual behaviors and IVP questions for ACASI
In Swahili…"are you ready to do the main interview? If you need help, ask the assistant, if you want to do the practice again choose option 1. If you are ready to do the main interview, choose 2. The assistant will sit where you can see her, but will only come to help if you ask her. Choose 1 to do the practice again, or 2 to proceed to the interview"

	Question	Possible responses	Verbal instructions – for ACASI on laptop	Skip
1	Unafanya nini kwa sasa? *What work are you doing now*	Nina fanya kazi Ninakaa nyumbani / sina kazi Ninarudia shule ya msingi Ninasoma shule ya sekondari Nina soma mafunzo ya ufundi Ninafanya kazi nyingine	Kuchagua jibu moja wapo,kama hujui chagua sijui kutoka orodha upande wa kulia	
2	Je, umeshawahi kutumia moja wapo kati ya madawa ya kulevya yafuatayo *Have you ever taken any of the following...*	Pombe Sigara Bangi Mirungi Unga Valium Heroin Gundi Petroli Ugoro Nyingine Hakuna hata mmoja *Alcohol, cigarette, marijuana, mirungi, cocaine, valium, heroin, gundim petrol, glue, other, not one of these…*	Kuchagua madawa yote amabayo umewahi kutumia	
3	Sasa umeolewa au unakaa na mpenzi wako? *Are you married or living as married*	Nimeolewa Ninaishi na mpenzi Sijaolewa	Kuchagua moja kati ya hayo	
4	Umeshawahi kumbusu mwanaume/mvulana midomoni? *Have you ever kissed a boy on the lips*	Ndiyo Hapana		

4a	Tangia tumeonana na wewe mara ya mwisho, mara ngapi umeshambusu mwanaume kwenye midomo yake? *Since we last saw you how many times have you kissed a boy on the lips*		Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
5	Umeshawahi kupeana denda na mpenzi au mume wako? (kula uroda, kula denda) *Have you ever kissed a boy in the mouth using tongues (known as,,,colloquial terms)*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
5a	Tangia tumeonana na wewe mara ya mwisho, mara ngapi umeshawahi kupeana denda na mpenzi/mume wako? (kula uroda, kula denda) *Since we last saw you, how many times..... (as above)*		Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
6	Mpenzi au mume wako amewahi kuchezea matiti yako? (kujivinjari, kutomasatomasa, ku-survey) *Have you ever let a boy play with/touch your breasts*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
6a	Tangia tumeonana na wewe mara ya mwisho, mara ngapi mpenzi wako amewahi kuchezea matiti yako? (kujivinjari, kutomasatomasa, ku-survey) *Since we last saw you, how many times..... (as above)*		Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
7	Umeshawahi kufanya mapenzi ya kushikana na mwanaume/mvulana, ambapo unachezesha uume wake kwa mkono wako? *Have you ever touched a boys penis with your hand?*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	

7a	Tangia tumeonana na wewe mara ya mwisho, mara ngapi umeshawahi kufanya mapenzi ya kushikana na mwanaume/mvulana, ambapo umeshika kwa mkono wako? *Since we last saw you, how many times..... (as above)*	Wangapi = XX	Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
8	Umeshawahi kufanya mapenzi ya kushikana na mwanaume/mvulana, ambapo anachezesha uke wako kwa mkono wake? (kupiga brush, kutomasatomasa) *Have you ever let a boy touch your vaginal with his hand?*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
8a	Tangia tumeonana na wewe mara ya mwisho, mara ngapi umeishawahi kufanya mapenzi ya kushikana na mwanaume/mvulana, ambapo anachezesha uke wako kwa mkono wake? (kupiga brush, kutomasatomasa) *Since we last saw you, how many times..... (as above)*	Wangapi = XX	Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
9	Je, umewahi kunyonya uume wa mwanaume/mvulana kwenye mdomo wako? (kula muhogo, kula muwa, kula cone, kupiga mswaki, alamba) *Have you ever had a boys penis in your mouth*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
9a	Tangia tumeonana na wewe mara ya mwisho, mara ngapi umewahi kuwekewa uume wa mwanaume/mvulana kwenye mdomo? (kula muhogo, kula muwa, kula cone, kupiga mswaki, alamba) *Since we last saw you, how many times..... (as above)*	Wangapi = XX	Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
10	Je, wanaume/mvulana	Ndiyo	Chagua "Ndiyo" au "Hapana"	

	amewahi kukunyonya sehemu zako za siri (kwenye uke)? (kufyonza embe, kuzama chumvini, kula pera) *Have you ever had a boy put his mouth on your vvagina?*	Hapana	Kama hujui chagua sijui kutoka orodha upande wa kulia	
10 a	Tangia tumeonana na wewe mara ya mwisho, mara ngapi wanaume/mvulana amewahi kukunyonya sehemu zako za siri (kwenye uke)? (kufyonza embe, kuzama chumvini, kula pera) *Since we last saw you, how many times..... (as above)*	Wangapi = XX	Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
11	Umewahi kujamiana kwa njia ya haja kubwa? *(Kufilana, kupakua, kisamvu, kula tigo, kula mgongo)* *Have you ever had anal sex?*			
11 a	Tangia tulipoonana na wewe mara ya mwisho, mara ngapi umewahi kujamiiana kwa njia ya haja kubwa? (kufilana, kupakua kisamvu, kula tigo, kula mgongo) *Since we last saw you, how many times..... (as above)?*			
12	Je umeshawahi kujamiiana kwa kuingia ndani. Kwa hili namaanisha pale mwanaume anapoweka uume wake kwenye uke wako. *(Kuchakachuliwa, Kugongwa mshipa, Ku sex, Kutiana, Kuingia ngumu kutoka laini*) *Have you ever had vaginal sex? (explanation, and colloquial terms…)*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
	ONLY ASK THIS SECTION IF Q11 ANSWER IS YES			
12 a	Je, katika maisha yako yote mpaka sasa hivi, umewahi kujamiiana	Wangapi = XX	Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande	

	na wapenzi wangapi? *In your whole life to now, how many men/boys have you had sex with?*		wa kulia	
12 b	Je, tangia tulipoonana mara ya mwisho, mara ngapi umeshawahi kujamiiana kwa njia ya uke. Kwa hili namaanisha pale mwanaume anapoweka uume wake kwenye uke wako? *Since we last saw you, how many times have you had sex? This is the number of sex acts.*	Wangapi = XX	Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
13	Mwanamume uliyejamiiana naye mara ya kwanza alikuwa nani wako? *Think about the fitst person you had sex with – who was this?*	Mume Mpenzi Simfahamu (tulikutana mara moja) Mtu ambaye sitaki kujamiiana naye Nyingine	Chagua Moja kutoka kwenye Orodha au " Sijui" "	
14	Mwanamume uliyejamaiiana naye mara ya kwanza, unadhani alikuwa na umri gani? *How old was your first lover?*	Zaidi ya miaka kumi Kati ya miaka mitano hadi kumi Ananizidi siyo zaidi ya miaka mitano Tunalingana Mdogo	Chagua Moja kutoka kwenye Orodha au " Sijui"	
15	Mwanamume uliyejamiiana naye mara ya nwisho alikuwa nani wako *Who was your first lover*	Mume Mpenzi wangu Simfahamu (tulikutana mara moja) Mtu ambaye sitaki kujamiiana naye Nyingine	Chagua Moja kutoka kwenye Orodha au " Sijui"	This question is asked if Q11a is >1
16	Mwanamume uliyejamiiana naye mara ya mwisho unadhani alikuwa na umri gani? *How old was your first lover?*	Zaidi ya miaka kumi Kati ya miaka mitano hadi kumi Ananizidi siyo zaidi ya miaka mitano Tunalingana Mdogo	Chagua toka kwenye orodha au sijui	This question is asked if Q11a is >1
17	Je, mlitumia kondomu mara ya mwisho mlipojamiiana *Did you use a condom the last time you had sex?*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	

18	Je umeshawahi kushawishiwa kufanya mapenzi ili upate usafiri, alama bora shuleni, hela za matumizi madogo madogo, au pesa ya kununua chakula nyumbani? *Have you ever had sex in exchange for gift/money*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
18 a	Katika miezi mitatu iliyopita mara ngapi ulijamiiana kwa ajili ya zawadi/pesa *How many times in the past three moths...as above*	Wangapi = XX	Ingiza namba au sijui	
19	Je, uliwahi kulazimishwa na mwanaume kujamiiana naye bila hiari yako? *Have you ever been forced to have sex against your will?*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
19 a	Katika miezi mitatu iliyopita mara ngapi uliwahi kulazimishwa na mwanaume kujamiiana naye bila hiari yako? *How many times in the past three moths...as above*		Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
20	Umewahi kujamiiana ukiwa katika siku zako za hedhi? *Have you ever had sex whilst menstruating*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
20 a	Katika miezi mitatu iliyopita mara ngapi tulipokuona uliwahi kujamiiana ukiwa kwenye hedhi? *How many times in the past three moths...as above*		Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
21	Umewahi kuwa mjamzito *Have you ever been pregnant?*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
21 a	Umeshabeba ujauzito mara ngapi *How many time have you been pregnant?*	.	Ingiza idadi, Kama huna uhakika chagua sina uhakika kutoka orodha upande wa kulia	
	THESE QUESTIONS ARE FOR EVERYONE AGAIN			
22	Unapokwa kwenye hedhi huwa unatumia nini kuzuia damu *What do you use to catch the blood during menstruation*	Vitambaa/kanga Chupi Karatasi ya choo Pedi za madukani Tampons	Chagua moja toka kwenye orodha	

		Nyingine Si hedhi		
22 a	Kama unatumia nguo, huwa unaiweka ndani ya uke, nje ya uke au ndani na nje ya uke *Do you use this inside/outside the vagina or both*	Ndani ya uke peke yake Nje ya uke peke yake Ndani na nje ya uke	Chagua moja toka kwenye orodha	
23	Umewahi kusafisha ndani ya uke wako? *Have you ever cleansed inside your vagina?*	Ndiyo Hapana	Chagua "Ndiyo" au "Hapana" Kama hujui chagua sijui kutoka orodha upande wa kulia	
24	Je, umeishawahi kuweka vitu hivi ndani ya uke wako? *Have you ever inserted anything inside your vagina?*	limao au Ndimu Mitishamba Dawa ya kuzuia wadudu kwa mfano Dettol/Jik Sabuni ya unga Ugoro Nyingine Au sijawahi kuingiza chochote	Chagua yote ambayo yahanahusika	

End of questionnaire. You can go back through the questionnaire to check or change your answers by pressing the "last question" button.
Thank you for giving us this information. It is completely anonymous, and safe.
Please call the assistant if you are finished.