HPV EPIDEMIOLOGY AND HPV VACCINATION IN EAST AFRICA

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Declaration

I, Kathryne Baisley, 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.

Abstract

Background

Cervical cancer is the leading cause of cancer-related mortality among women in sub-Saharan Africa (SSA), but almost entirely preventable through prophylactic human papillomavirus (HPV) vaccination. The introduction of HPV vaccines has been challenging in low and middle income countries (LMIC), with a major barrier being the costs and logistical challenges of delivering the vaccine as a multi-dose schedule. In addition, data on HPV epidemiology in SSA needed to inform HPV vaccination strategies have historically been limited.

Methods

This analytic commentary aims to synthesise and critically appraise the published results of two studies on HPV epidemiology and two studies on HPV vaccine in Tanzania. These studies included one of the first prospective studies of HPV epidemiology in East Africa, a cross-sectional study in adolescent girls around the time of sexual debut, the first trial of HPV vaccine in SSA (HPV-021), and the first randomised trial of single-dose HPV vaccination in girls in the target age range for vaccination (9-14 years), the DoRIS trial.

The main overarching objectives of these studies were to assess: the prevalence and incidence of HPV infection among adolescent girls and young women in Tanzania; the effect of malaria and helminth infections on HPV vaccine immune responses; and whether a single dose of HPV vaccine given to the target age for routine vaccination produces immune responses that are likely to be effective in preventing cervical cancer in SSA.

Results

Our cohort study found one of the highest HPV prevalences (55%) and incidence rates (51/100 person-years for high-risk (HR) HPV) described globally for young females in the general population. HPV prevalence among adolescent girls around the time of sexual debut was also very high (32%). However, in this age group, prevalence of most vaccine genotypes was low.

In the HPV-021 trial, there was no evidence that malaria or helminth infections decreased vaccine antibody responses following 3 doses of HPV vaccine. In the DoRIS trial, 99% of girls in the 1-dose arms were HPV16 antibody seropositive at month (M)24, and seropositivity was non-inferior to that in the 2 and 3 dose arms. Although the prespecified non-inferiority criteria were not met for HPV18, ≥98% girls in the 1-dose arms were HPV18 seropositive, and HPV 16/18 antibody avidity was non-inferior to that generated by 2 and 3 doses. HPV16/18 antibody geometric mean concentrations (GMCs) in the 1 dose arms were non-inferior to those in observational cohorts of women who received a single dose of HPV vaccine and in whom vaccine efficacy had been shown.

Conclusions

Data from our HPV epidemiology studies in Tanzania showed that, without HPV vaccination or screening programmes in place, it is likely that cervical cancer would continue to contribute significantly to morbidity and mortality among women in the region. The results from the DoRIS trial showed that one dose of HPV vaccine induces immune responses in young girls that are comparable with those seen in young women in whom efficacy has been shown, and are sustained for up to two years after vaccination. In 2022, the World Health Organization (WHO) endorsed a 1-dose regimen for individuals aged 9-20 years, based in part on evidence from the DoRIS trial.

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Table of Contents

List of abbreviations and acronyms

AIS	adenocarcinoma in situ
BV	bacterial vaginosis
CI	confidence interval
CIN	cervical intraepithelial neoplasia
CVT	Costa Rica Vaccine Trial
DNA	Deoxyribonucleic acid
DoRIS	Dose Reduction Immunobridging and Safety Study of two HPV vaccines in Tanzanian girls
EDCTP	European & Developing Countries Clinical Trials Partnership
ELISA	enzyme-linked immunosorbent assay
EPI	Essential Programme on Immunization
GAVI	Gavi, the Vaccine Alliance (formerly the Global Alliance for Vaccines and Immunization)
GM	geometric mean
GMC	geometric mean concentration
GSK	GlaxoSmithKline Biologicals
HIV	human immunodeficiency virus
HPV	human papillomavirus
HR	high risk (oncogenic) HPV genotypes
IARC	Agency for Research on Cancer
ITM	Institute of Tropical Medicine, Antwerp
IUSTI	International Union against Sexually Transmitted Infections
JCVI	UK Joint Committee on Vaccination and Immunisation
LMIC	Low and middle income country
LR	low risk (non-oncogenic) HPV genotypes
LSHTM	London School of Hygiene and Tropical Medicine
M( )	Month (visit month in studies)
MITU	Mwanza Intervention Trials Unit
NCI	United States National Cancer Institute
NIMR	National Institute of Medical Research, Tanzania

PCR	Polymerase chain reaction
PI	Principal Investigator
RHASA	Reproductive Health of Adolescents in sub-Saharan Africa study
SAGE	Strategic Advisory Group of Experts
SSA	sub-Saharan Africa
STI	sexually transmitted infection
USA	United States of America
VaIN	vaginal intraepithelial neoplasia
VE	vaccine efficacy
VIN	vulvar intraepithelial neoplasia
VLP	virus-like particles
WHO	World Health Organization

SECTION 1: ANALYTICAL COMMENTARY

Introduction

Human papillomavirus (HPV) infection, the primary cause of cervical cancer, is a major public health problem in sub-Saharan Africa (SSA). Cervical cancer is the leading cause of cancer-related morbidity and mortality among women in much of SSA, but almost entirely preventable through vaccination against HPV infection.[1] As part of its global strategy for cervical cancer elimination, the World Health Organization (WHO) has set a target of 90% of girls aged 15 years worldwide being vaccinated against HPV by 2030.[2] However, the introduction of HPV vaccines has been slow in low and middle income countries (LMIC), with a major barrier being the cost and logistical challenges of vaccine delivery. In 2021, only 37% of LMIC had HPV vaccination programmes in place and global vaccination coverage in girls aged 15 years was only 21%.[3]

This analytical commentary first describes my research in HPV epidemiology in Tanzania starting in 2006, at a time when there were limited data available on the prevalence and incidence of HPV infection in East Africa. It will then describe my HPV vaccine research, including the first trial of HPV vaccine in SSA and the first trial of a single-dose HPV vaccination schedule in girls in the target age range for vaccination.

In my portfolio for the PhD by prior publication, I have included 5 published papers arising from 3 studies, 2 on HPV epidemiology and 3 on HPV vaccination. In this commentary, I will discuss how these studies were designed to address a range of outstanding questions at the time that they were undertaken, and how the findings have contributed to the knowledge base on HPV epidemiology and HPV vaccination, as well as their impact on policy. I will also discuss the limitations of the studies and my research in the context of current discussions on HPV vaccination strategies for cervical cancer elimination.

The main overarching objectives of these studies were to assess:

1) the prevalence and incidence of HPV infection among adolescent girls and young women in Tanzania

2) the effect of malaria and helminth infection on HPV vaccine immune responses

3) whether a single dose of HPV vaccine produces immune responses that are likely to be effective in preventing cervical cancer in SSA

In the first chapter, I will present general background information on the HPV virus and associated disease, natural infection and immunity, and HPV vaccines. I will mainly focus on cervical infection, because my HPV research has been in women and because it is the main cause of cancer among women in SSA, where all my studies are based. In the second and third chapters, I will present the studies on HPV epidemiology and HPV vaccines, respectively, and describe the methods used, the main findings, and their strengths and limitations. In the fourth chapter, I will discuss how the findings from these studies have contributed to the knowledge base and informed subsequent research. In the final chapter, I will discuss remaining knowledge gaps and briefly describe some current studies that are addressing these. I will focus primarily on my work on HPV vaccines, since this has been (for the past 10 years) and continues to be my main area of research.

Chapter 1: Background

1.1 HPV infection and associated disease

HPV is a highly infectious DNA virus that is transmitted primarily through sexual intercourse. Based on the nucleotide sequence of the major capsid protein gene L1, over 200 genotypes have been identified of which 40 infect the genital mucosa.[4,5,6] The International Agency for Research on Cancer (IARC) has classified HPV genotypes based on their potential to cause cancer in humans.[7] Those with a strong association with cancer are referred to as oncogenic, or high-risk (HR), and comprise 13 genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68). Genotypes that are not generally associated with cancer are termed non-oncogenic or low-risk (LR). HR genotypes HPV16 and 18 are responsible for approximately 70% of cervical cancers, with the remaining 30% caused by other HR HPV types.[8,9] HPV16 and 18 are also responsible for 40–50% of invasive vulvar cancer and 70%

of vaginal cancer.[10] LR types include HPV6 and HPV11 which cause over 90% of cases of genital warts.[11,12]

Nearly all sexually active men and women will become infected with HPV at least once in their lifetime.[13,14] Most infections are asymptomatic and transient; however, persistent infection with HR HPV genotypes may result in disease. In women, persistent cervical HPV infection may lead to cellular abnormalities and lesions, termed cervical intraepithelial neoplasia (CIN), which, if left untreated, may progress to invasive cancer.[15] HPV infection is also associated with cancers of the head, neck, oropharynx and anogenital area in both men and women. Some studies indicate there could also be a link with colon cancer.[16,17] The proportion of cancers attributable to HPV varies by anatomic site, with nearly 100% of cervical, 88% of anal, and less than 50% of lower genital tract and oropharyngeal cancers attributable to HPV.[18,19] Overall, an estimated 8.6% of cancers worldwide in women and 0.8% in men are attributable to HPV.[18] Although HPV infects both men and women, the burden of HPV-related disease is much higher in women because of the biological susceptibility of the cervix to HPV infection and carcinogenesis.

Cervical cancer is the fourth most common cancer among women worldwide, and the second most common cancer in women aged 15-44 years.[1,20] Over the past decade, cervical cancer incidence rates have declined in high income countries, and around 90% of new cases and deaths in 2020 occurred in LMIC.[21] The burden is heaviest in low income countries, where the age-standardised incidence and mortality rates in 2020 were 23.8 and 17.4/100,000, respectively, compared with 8.4 and 2.53, respectively, in high income countries.[21] In SSA, cervical cancer is the most common cancer among women and East Africa has the world's highest age-standardised incidence and mortality rates, at 40.1 and 28.6/100,000, respectively.[21] The variation in cervical cancer rates is mainly a consequence of differences between countries in the prevalence of HPV infection and the availability of adequate screening, treatment of pre-cancerous lesions and HPV vaccination.

1.2 HPV virus life cycle

The HPV virus initially infects epithelial basal membrane cells, which are usually accessed through micro-abrasions in the superficial epithelial layer.[22,23] The undifferentiated basal

layer acts as a reservoir where the HPV genome is maintained at low copy number and only early viral genes are expressed. This is referred to as the 'non-productive' stage of infection. As the infected basal cells differentiate into epithelial cells, the 'productive' stage of viral infection is triggered, with amplification of viral DNA and expression of late viral genes, resulting in the production of new virions that are released from the differentiated epithelial cell surface. The HPV viral life cycle is exclusively intraepithelial and does not cause cell lysis, systemic viraemia or apparent inflammation, thus host innate immune responses are absent or reduced.[24]

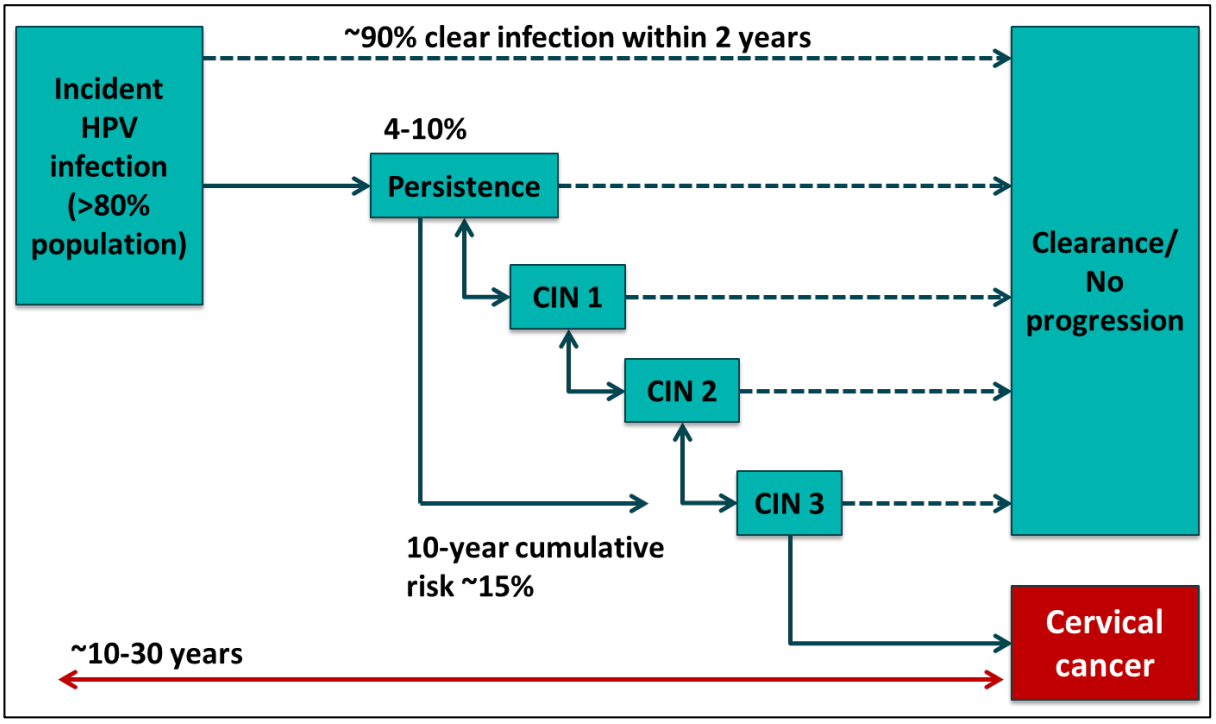
In healthy individuals, the immune system eventually clears the majority of HPV infections, with 90% being cleared within 2 years.[15,25,26] Estimates of duration of infection vary widely between studies, in part because of differences in sampling frequency, definitions of HPV clearance, and methods of HPV DNA detection. A 2013 meta-analysis of 86 studies found that the median duration of cervical infections ranged from 6–24 months, depending on geographic region and HPV type.[27]

A minority (<10%) of HPV infections are not cleared, and the risk of developing epithelial cell abnormalities and cancer is then increased. The major determinants of HPV persistence are HPV genotype and viral load.[26] HR HPV genotypes, on average, persist for longer than LR types and are less likely to be cleared. Older age has also been cited as a risk factor for persistence, although the evidence is inconsistent. A large population-based study in Costa Rica found women with persistent HPV infection were significantly older than those who cleared their infections (mean 56.4 years vs 35.8 years, respectively).[28] However, studies in Colombia and in Brazil found no association between clearance and age.[29,30] Smoking, oral contraceptives, co-infections with other sexually transmitted infections (STI) and high parity have been shown in some studies to be co-factors that increase the risk of cervical cancer in women with HPV infection.[31-35] However, it is unclear whether these co-factors increase the risk of HPV persistence.

The risk of both HPV infection and HPV-associated disease is also increased in individuals who are immunosuppressed. Women living with HIV have a higher prevalence of HPV infection, lower rates of clearance, and higher rates of HPV-related disease progression. Host genetic factors are also likely to play a role.

The progression of persistent cervical HPV infection leads to the development of premalignant lesions in the epithelial lining, ranging from low-grade (CIN1) to high-grade (CIN2 and 3) (Figure 1).[15,26] The stages before the development of cancer can regress spontaneously, although regression rates decrease with increasing severity of the lesions. Studies have shown that up to 90% of CIN1 and 15-40% of CIN3 lesions may spontaneously regress without treatment in immunocompetent women.[36-38] Progression from HPV infection to the development of high-grade CINs and invasive cervical cancer takes between 10–30 years, although cases of rapid progression do occur.[15,39] Persistence of specific HPV genotypes, particularly HPV16, is associated with a higher risk of developing CIN3. A long-term follow up of over 24,000 women in the UK found that, among women with normal cytology, 55% of HPV16 infections detected at study entry were still present after 1 year vs 39% of other HR HPV infections.[40] The 10-year risk of progression to CIN3, adenocarcinoma in situ or cervical cancer (CIN3+) among women with HPV16 infection at entry was 12.4% (95% confidence interval (CI)=9.3-16.5%) vs 6.9% for HPV18 and <5% for other HR genotypes.

Figure 1. Natural history of cervical HPV infection



Adapted from Pinto and Crum 2000 and Moscicki et al. 2012.[15,41]

1.3 Natural infection and immunity

After natural infection, around 70%–80% of women develop a measurable HPV genotype-specific serum antibody response, although antibody concentrations and binding strength are typically low.[42,43] The median time from infection to seroconversion is around 8–12 months.[44,45] In men, there is less response to natural HPV infection and few men seroconvert.[46,47] A longitudinal study of female university students in the United States found that 67% seroconverted after cervical HPV16 infection, with a median time to seroconversion of 8.3 months.[48] In contrast, a study of males at the same university found that only 7% seroconverted after genital HPV16 infection.[49]

Data on whether genotype-specific antibody responses to natural infection protect against reinfection with the same genotype are equivocal. HPV antibodies acquired through natural infection appear to provide modest protection against subsequent infection for women, but not for men. A 2016 meta-analysis based on data from 24,000 individuals found that women with detectable HPV16 or HPV18 antibodies induced through natural infection had some protection against subsequent infection with that genotype (pooled RR=0.65, 95%CI=0.50-0.80 for HPV16 and 0.70, 95%CI=0.43-0.98 for HPV18).[50] However, there was no evidence that naturally-acquired antibodies provided protection in men (pooled RR=1.22, 95%CI=0.67-1.77 for HPV16 and 1.50, 95% CI=0.46–2.55 for HPV18). One explanation for these findings may be that HPV infections in the mucosal epithelium of the cervix have greater exposure to the immune system than HPV infections on the keratinised epithelium of male genitals.[50] Similar findings for HPV16 infection were reported in a 2023 meta-analysis based on data from 63,000 individuals, although no protective effect was seen for naturally-acquired HPV18 antibodies in either women or men.[51]

The loss of HPV DNA detectability in genital samples may not always reflect true immune clearance. Instead, the immune system may control the infection below the limit of detection in a state of viral latency (i.e. the virus remains dormant in basal membrane cells without actively replicating).[52,53] Therefore, redetection of HPV DNA after a period of negative samples could be due to several reasons, including true reinfection from sexual exposure, intermittent detection of a latent infection, or simply transient deposition from a cross-infection at another epithelial site or from a recent sex act.

1.4 HPV epidemiology

The incidence of HPV infection rises quickly after sexual debut and HPV infection is most prevalent among young adults aged up to 30 years. In women, HPV prevalence generally declines after age 30 although a second peak in prevalence among women aged ≥45 years is observed in some countries.[26,54] The most comprehensive assessment of the prevalence of cervical HPV infection is from a 2010 meta-analysis of data from 194 studies comprising over 1 million women.[55] The authors estimated the worldwide prevalence of cervical HPV infection in women with normal cytology to be 11.7% (95%CI 11.6-11.7). The highest estimated prevalences were in sub-Saharan Africa (24.0%), Eastern Europe (21.4%), and Latin America (16.1%). There were large differences within each region, with country-specific cervical HPV infection prevalences ranging from 1.6% to 41.9%. Within geographical sub-regions, East Africa had one of the highest prevalences (31.7%, 95%CI 29.5-33.8). The most prevalent HPV genotypes among women worldwide were HPV16 (3.2%) and HPV18 (1.4%).

Of note, these estimates of prevalence refer to the period before HPV vaccination had been introduced in many countries. More recent estimates have shown a decrease in cervical HPV16/18 prevalence, the two genotypes that are targeted by all HPV vaccines, in countries where HPV vaccine has been introduced.[56-58]

1.5 HPV vaccines

Primary prevention of cervical cancer is through vaccination against HPV infection, with secondary prevention through screening and treatment for pre-cancerous lesions, which can be very effective if caught in the early stages. My analytical commentary will focus on prophylactic HPV vaccination rather than screening and treatment which have limited coverage in SSA and other LMICs.

HPV vaccines have been shown to be safe and highly effective in preventing HPV infection and related disease. There are currently six licensed prophylactic HPV vaccines, the first of which was licensed in 2006 (Table 1). All protect against the HR genotypes HPV16 and 18, and two also protect against HPV6 and 11. One vaccine protects against 9 genotypes (HPV6, 11, 16, 18, 31, 33, 45, 52 and 58). The vaccines also display some degree of cross-protection

against phylogenetically-related HPV types.[59] HPV vaccines are often referred to by their valency, i.e. the number of HPV genotypes included in the vaccine.

All of the current prophylactic HPV vaccines are subunit vaccines consisting of synthetically manufactured virus-like particles (VLP) made of the L1 capsid protein of the targeted HPV genotypes.[60] These VLPs closely resemble the outer surface of the HPV virion, but contain no viral DNA so are non-infectious. The high efficacy of HPV vaccines is thought to be due to the repetitive structure and spacing of the VLP epitopes, which stimulate an exceptionally strong immune response, similar to that induced by live vaccines.[61]

Table 1. Licensed HPV vaccines as of October 2024

Type	HPV genotypes targeted	Vaccine name	Manufacturer	Year first licensed
Bivalent	16, 18	Cervarix	GlaxoSmithKline	2007
		Cecolin	Innovax	2019
		Walrinvax	Yuxi Zerun	2022
Quadrivalent	6, 11, 16, 18	Gardasil	Merck	2006
		Cervavac	Serum Institute	2022
Nonavalent	16, 18, 6, 11, 31, 33, 45, 52, 58	Gardasil-9	Merck	2014

HPV vaccines are most effective in individuals who have not been previously infected with vaccine-related HPV genotypes. Therefore, the primary target age for vaccination is 9-14 years, generally before the start of sexual activity. Most HPV vaccine efficacy trials have been conducted in young women, generally aged 15-25 years, because the time that would be needed to accrue efficacy endpoints (HPV infection or cervical disease) in young girls makes trials of reasonable duration in this age group unfeasible. [62] In most trials, the primary efficacy endpoints are evaluated in women who are HPV vaccine-genotype DNA negative and seronegative at the time of vaccination.[63,64] This is felt to reflect efficacy in the current target age group for vaccination, who will largely be HPV naïve.

A 2018 Cochrane review of efficacy trials of three HPV vaccines (Cervarix, Gardasil, and a monovalent HVP16 vaccine that is no longer marketed), found that protection against HPV16/18-associated CIN2+ was consistently high among HPV16/18 DNA negative women aged 15-26 years who received at least 1 dose, with a pooled vaccine efficacy (VE) of 95%

(95%CI=90-97%).[62] In mid-adult women aged 24-45 years who were HPV16/18 DNA negative, efficacy was lower but still high (pooled VE=70%, 95%CI=19-89%). However, efficacy was much lower in the total vaccinated cohort, which included women who were HPV DNA positive at baseline. Among all women aged 15-26 years, the pooled VE against vaccine genotype-related CIN2+ was 54%. In mid-adult women, the pooled VE was only 26%.

Appendix 1 provides a summary of the key large-scale phase III efficacy trials of HPV vaccines that led to licensure. Several of the earlier trials have continued to follow participants for longer term outcomes after the original trial ended. A large trial of the bivalent vaccine, Cervarix, the Costa Rica Vaccine trial (CVT), invited participants in the HPV vaccine arm to enrol in a study extension, alongside a new unvaccinated control group.[65,66] Cumulative vaccine efficacy against HPV16/18 CIN2+ over 11 years among those who were HPV16/18 DNA negative at baseline was 97.4% (95%CI=88.0-99.6).[67] Women in the HPV vaccine arm of a large multi-country trial of the quadrivalent vaccine, Gardasil, (FUTURE II) in Nordic countries have been followed via cancer registries, where only one case of HPV16/18-related CIN2+ has been recorded over 12 years among those who were HPV16/18 DNA negative at baseline.[68] In Finland, women who were vaccinated in one of 3 early trials have been followed up to 17 years through the cancer registry and no HPV-associated cancer cases have been recorded.[69]

Although efficacy estimates from randomised trials are likely to be higher than effectiveness in real world settings, population-based studies in countries with high HPV vaccination coverage have shown considerable reduction in cervical HPV infection and cervical lesions. In Australia, one of the first countries to implement a HPV vaccination programme and with very high vaccine coverage, the rate of CIN2+ decreased by 47% in vaccinated women compared with unvaccinated women of the same age, within 5 years of introduction of the programme.[70] Within 9 years, the prevalence of cervical HPV infection with vaccine genotypes in women aged 18-35 years had decreased by 92%.[57] Similar decreases have been shown in Europe and North America. A 2019 meta-analysis of the population-level impact of HPV vaccination based on data from 65 studies in 14 high income countries found that the prevalence of cervical HPV16/18 reduced by 83% in girls aged 13-19 years, and by 66% in those aged 20-24 years, in the first 9 years after the introduction of vaccination.[71] In

Rwanda, the first country in Africa to introduce a national HPV vaccination programme, the prevalence of HPV vaccine genotypes (6/11/16/18) among women aged 17-29 years decreased by 53% over 7 years.[72]

HPV vaccines were originally licensed as a 3-dose regimen given over 6 months; however, in 2014, a 2-dose regimen was approved for girls aged 9-14 years, which I will discuss further in Chapter 3.[73] In 2022, the WHO Strategic Advisory Group of Experts (SAGE) on Immunization endorsed a 1-dose regimen for individuals aged 9-20 years, following growing evidence on the efficacy of a single dose.[74]

Chapter 2. HPV epidemiology studies

2.1 Knowledge gaps at time of studies included in commentary

The epidemiology studies included in this commentary were conducted between 2006-2013. Although cervical cancer is the most common cancer among women in SSA, and East Africa has the highest age-standardised incidence worldwide, there was limited information on HPV epidemiology in this region 15 years ago. Most of the data on HPV epidemiology and the natural history of HPV infection were from North America, Australia and Europe. The few studies that had been done in SSA at that time suggested that HPV infection was highly prevalent, and, as a cause of cervical cancer, would contribute significantly to morbidity and mortality. For example, the prevalence of cervical HPV DNA in a 2001 study of pregnant women in Tanzania was 34%.[75]

However, there were still large gaps in the epidemiological data from SSA. The prevalence of the different HPV genotypes, their distribution with age, and the HR genotypes that were responsible for cervical cancer in this region had not been well established. There were virtually no data on genotype-specific HPV incidence, persistence and clearance, and there were no data on HPV infection in school-age girls. HPV genotype-specific data are essential for estimating the impact of control strategies including screening and HPV vaccination programmes. Data from high income settings on the rate of acquisition of HPV after sexual debut had allowed robust mathematical modelling of the impact of vaccinating particular

age groups. However, studies characterising incidence, clearance and the diversity of HPV genotypes acquired over time by young women in SSA, where the majority of the disease burden occurs, were lacking.

In 2009, the Tanzanian Ministry of Health began to discuss the feasibility of introducing a national HPV vaccination programme. Given that the vaccines are most effective in individuals who have not yet acquired HPV, data on age of acquisition of HPV and on the genotypes that were most commonly acquired were essential for determining the appropriate target age for vaccination, and for estimating the proportion of cancers that might be averted by a vaccination programme. Although HPV16 was the most common HR genotype detected in the study of pregnant women in Tanzania (29% of infections), a study in Mozambique found that HPV35 was the dominant HR genotype (17%).[75,76] Therefore, the impact of the two available HPV vaccines at that time in preventing cervical cancer in some settings might be lower than expected.

Information was also needed on risk factors for HPV infection in SSA, which could help inform the design of HPV vaccination and cervical cancer prevention programmes. These data would also help modelling studies to provide a more accurate understanding of the HPV infection burden in different regions, and the development of effective intervention strategies.

2.2 Research questions

In light of this background information, we designed several studies to answer the following questions about HPV epidemiology in Tanzania:

1. What is the prevalence and incidence of genotype-specific HPV infection in young women, and the rates of persistence and clearance?

2. What is the prevalence of genotype-specific HPV infection in the early period after sexual debut?

3. What are the behavioural and demographic factors associated with the prevalence and incidence of HPV infection?

In the following sections, I will describe two of these studies: the methods used and the methodological considerations during their design, the findings (with full details given in the accompanying publications), and their strengths and limitations.

2.3 HPV-021 trial supplementary epidemiology study (paper 1)

The first trial of any HPV vaccine in Africa (HPV-021, which evaluated immunogenicity and safety of the bivalent vaccine Cervarix) was conducted in Tanzania and Senegal in 2007.[77] The trial was sponsored by GlaxoSmithKline (GSK) Biologicals, and the Mwanza Intervention Trials Unit (MITU) in Mwanza was selected as the Tanzanian site, with Deborah Watson-Jones as the site Principal Investigator (PI). I will describe this trial in more detail in Chapter 3, when I introduce my HPV vaccine research. This trial also provided an ideal opportunity for us to include a supplementary study of HPV epidemiology. This epidemiological study was conducted at the Tanzanian site only and I was a co-investigator.

2.3.1 Methods

The study was conducted from October 2007 to July 2010. Trial participants were recruited from schools, colleges and family planning clinics in Mwanza. Females were eligible for enrolment if they were aged 10–25 years, healthy, HIV-negative, not pregnant, and had no more than 6 lifetime sexual partners. Participants were randomly assigned (2:1) to receive either 3 doses of HPV vaccine or placebo and were followed for 12 months.

At enrolment and Month 12, participants were interviewed about sexual activity. A genital examination was performed on all participants who reported current or prior sexual activity, and cervical swabs were collected for HPV DNA testing. Swabs were sent to the Catalan Institute of Oncology in Barcelona where they were tested and genotyped by the Roche Linear array assay (Roche, Branchburg, USA), which detects 37 different HPV genotypes.

The primary objectives of the study were to measure the prevalence of HPV genotype-specific infections at enrolment, and incidence over 12 months. For each genotype, the number of new infections (genotype not present at enrolment), persistent infections (same genotype at enrolment and 12 months) and cleared infections (positive for the genotype at enrolment but negative for that genotype at 12 months) was tabulated by treatment arm

and overall. The incidence of any HPV genotype, and any HR genotype, was calculated among women who were negative for all genotypes, or negative for all HR genotypes, respectively, at enrolment. Person-years at risk were calculated from date of enrolment until date of HPV acquisition, assumed to occur midway between the last negative and first positive results. Logistic regression was used to obtain ORs and 95%CI for factors associated with prevalent HPV infection at enrolment.

The sample size was fixed based on the main trial design. The trial aimed to enrol 330 participants in Tanzania. Assuming that 50% were sexually active, this sample size would allow us to estimate the prevalence of HPV infection with a precision of ±3.3% for genotypes with prevalence of 5%, or a precision of ±4.7% for prevalences of 10%.

2.3.2 Results

The study enrolled 334 participants in Tanzania (221 in the HPV vaccine arm and 113 in placebo), of whom 142 (42.5%) reported being sexually active at enrolment. Among those, 117 (82.4%) provided cervical samples. At M12, 136/308 (44.3%) participants reported that they were sexually active, of whom 122 (89.7%) provided cervical samples (85/122 [69.7%] in the vaccine arm). The median age of those providing samples at either visit was 20 years (IQR=18-22).

The prevalence of HPV infection among sexually active participants at enrolment was 73.5%, and that of HR HPV was 54.7%. The most common HR genotypes were HPV45 (16.2%), HPV16 (12.8%) and HPV58 (12.8%). Prevalence of any HPV infection rose rapidly with age, from 36.4% in those aged <16 years, to 85.7% in those aged 19-20 years, then dropping to 64.3% in those aged ≥23 years.

Among the 97 participants who had HPV results at both time points, 65 (67.0%) were infected with a new HPV genotype at the 12 month visit. Of 187 genotype-specific infections detected at enrolment, 51 (27.2%) were present at 12 months. Persistence was similar between HR and LR genotypes (27.9% and 26.7%, respectively). Cumulative incidence of HR genotypes ranged from 1% to 12%, and was highest for HPV 51 (12.9%), HPV 39 (12.1%) and HPV 35 (8.7%) in the vaccine arm, and HPV 51 (9.1%), HPV 16 (4.8%) and HPV 58 (4.8%) in

the control arm. The overall incidence of HR HPV infection was 51/100 person-years (95%CI=46-126).

In the unadjusted analysis, age, condom use and hormonal contraception were associated with prevalent HPV infection. However, after adjusting for age, only condom use had some evidence of an association with HPV infection. There was no evidence of an association with the number of sexual partners, age at first sex or any reproductive tract infection (gonorrhoea, chlamydia, syphilis, bacterial vaginosis (BV) or trichomoniasis) in either the unadjusted or adjusted analysis, although the prevalence of these infections was very low.

2.3.3 Strengths and limitations

A strength of this study is that it was one of the first prospective studies of HPV epidemiology in East Africa, and one of the first studies which included adolescent females. It was also one of the first studies in this region to recruit participants from a more general population (e.g. schools), rather than from potentially higher risk populations such as antenatal or reproductive health clinics. We tested for a large number (n=37) of HPV genotypes, using a well-validated assay with a high sensitivity and specificity. We also tested for reproductive tract infections, which have been associated with HPV persistence in some studies.[34,35,78]

Limitations of the study were that we only tested for HPV in participants who reported being sexually active, and that sampling was not frequent enough to distinguish between new infections vs. transient detection, or to measure clearance or persistence. With the 12-month interval between samples, we could not accurately determine the timing of new infections first detected at month 12, and this would have missed any new infections that were acquired and cleared in that period. Therefore, our estimates of HPV incidence in this study are likely to be conservative. With only 24 participants in the placebo arm providing samples at both timepoints, we were unable to obtain a reliable estimate of the incidence of HPV16/18 (or other HPV genotypes for which the vaccine provides cross-protection) in unvaccinated participants.

2.4 Reproductive Health of Adolescents in sub-Saharan Africa (RHASA) (paper 2)

The RHASA study was conducted to explore the acceptability of studies of reproductive and sexual health in adolescent girls around the time of sexual debut. The overall aim was to inform future trials of interventions to improve the reproductive health in this population, including vaginal microbicides and vaccines. The study was funded by EDCTP and the PI was Anne Buve (Institute of Tropical Medicine, Antwerp); I was a co-applicant on the proposal and a study co-investigator.

RHASA included a qualitative component and a cross-sectional study. The primary objective of the cross-sectional study was to characterise the vaginal microbiome of adolescent girls, and to measure the prevalence of reproductive tract infections, including HPV.

2.4.1 Methods

We enrolled girls aged 17-18 years from schools in Mwanza from November 2013 to June 2014. Girls and their parents were invited to attend a study information meeting, and then approached individually for informed consent (or informed assent with consent from parents if aged 17 years) before being enrolled and interviewed at the study clinic. Since it was expected that around half of the girls would report never having had sex, we decided not to perform a speculum examination. Instead, we asked all participants to collect nurse-assisted, self-administered vaginal swabs. This procedure had been found to be acceptable in previous studies in Mwanza, and had been shown to have similar sensitivity for HPV detection as clinician-collected specimens.[79,80] Therefore, we were able to obtain a more accurate estimate of HPV prevalence among adolescent girls than in the HPV-021 study, where our results were restricted to participants who reported having had sex. HPV detection and genotyping was done using the Roche Linear Array assay at the National Institute for Medical Research (NIMR) laboratory in Mwanza.

The study aimed to enrol 400 girls, an estimated 50% of whom were expected to report no previous sexual activity and 50% who would report having passed their sexual debut. This sample size was based on feasibility given the time and budget constraints of the project, rather than on any formal sample size calculations.

2.4.2 Results

The study enrolled 401 girls aged 17-18 years, of whom 385 (96.0%) had HPV results; of those, 222 (57.7%) reported not having passed their sexual debut. The overall prevalence of any HPV infection was 32.5% (125/385), and of any HR HPV was 18.2% (70/385). The most prevalent HR genotypes were HPV16 (3.9%), HPV39 and HPV52 (both 3.1%), and HPV58 (2.9%). HPV18 was detected in 3 girls (0.8%).

HPV prevalence varied by reported sexual activity, with HPV infection detected in 84/163 (51.5%) sexually active girls and in 41/222 (18.5%) girls who reported no previous penetrative sex. In addition to penetrative sex, HPV infection was associated with lower socioeconomic status, vaginal cleansing, menstrual hygiene and some reproductive tract infections (gonorrhoea, BV and *Mycoplasma genitalium*). There was a strong inverse association between HPV and the presence of *Lactobacillus* species, including *L. crispatus* and *L. jensenii*, key constituents of the healthy vaginal microbiota.

2.4.3 Strengths and limitations

A strength of this study was its population of healthy adolescent girls, providing information on HPV prevalence around the time of sexual debut. The collection of vaginal swabs, although self-administered, was observed by nurses who assisted when necessary; 97% of participants provided a swab for HPV testing and all but 2 specimens contained β-globin (a marker for human cellular DNA), indicating successful sampling and specimen processing. We used the same well-validated assay as the HPV-021 epidemiology study for HPV detection and genotyping. We also tested for other STIs and for vaginal microbiota, allowing us to examine the association of prevalent HPV infection with these factors.

Limitations included the cross-sectional design, so we were unable to estimate incidence, persistence or clearance. Face-to-face interviews may have increased social desirability bias in responses. We enrolled girls who were still in school; many girls in this age range in Tanzania are no longer in school. Furthermore, only 55% of parents and girls who were invited attended the study information meeting, which suggests possible selection bias.

We subsequently designed another study (the HPV natural history study) to address some of the limitations of the HPV-021 epidemiology and RHASA studies. I will discuss that study in more detail in Chapter 4.

Chapter 3. HPV vaccine studies

3.1 Knowledge gaps at the time of the studies included in commentary (2007-2015)

As described in Chapter 2, the first trial of an HPV vaccine in Africa was conducted in Tanzania and Senegal in 2007-2010. This was a phase III trial of Cervarix, which was a new HPV vaccine that was only licensed in Australia at the time the trial was planned. The trial was sponsored by GSK and provided the first data on the immunogenicity and safety of HPV vaccines in SSA.

At that time, HPV vaccines were still very recent and it was not known whether their efficacy in SSA would be similar to that in other regions. In Sept 2007, the WHO Global Advisory Committee on Vaccine Safety recommended research on safety and efficacy of the vaccines in Africa as a priority.[81] There are a number of factors that could have potentially affected the immune response in SSA, such as malnutrition, HIV or other intercurrent illnesses or conditions. Parasitic infections are common in SSA and it was not known whether these infections would influence the vaccine response. Malaria and helminths, in particular, have been shown to negatively impact immune responses to a number of vaccines such as BCG, typhoid, tetanus and polio.[82-86] The GSK trial provided an ideal opportunity for us to nest supplementary studies within the trial to answer some of these questions.

In 2010, HPV vaccines were still being delivered in a 3-dose schedule. Although SSA had the highest age-standardised cervical cancer incidence and mortality rates in the world, there were no national HPV vaccination programmes in SSA at this time because of the high cost of the vaccines (GAVI had not yet approved a HPV vaccination programme) and logistical complexity of delivering a multi-dose schedule to young adolescent girls.[87-89] This age group is not usually targeted for routine immunisation or for other health interventions, so

few countries in SSA had existing systems in place to implement an HPV vaccination programme.

Early trials of HPV vaccines had shown that the 3-dose schedule in young girls aged 9-14 years induced antibody geometric mean concentrations (GMC) that were around double those in young women aged 15-25 years, the age group in which efficacy had been evaluated.[90,91] The observed strong antibody responses, coupled with the costs and challenges of delivering 3 doses to young adolescents, prompted discussions about a reduced dose schedule in this population. Subsequent immunological bridging ('immunobridging') studies in high and upper-middle income countries showed that antibody GMCs after 2 doses in young girls were non-inferior to those after 3 doses in young women, which led to the approval of a 2-dose schedule in girls aged <15 years, first by the European Medicines Agency in 2013.[92-94] Although there is no known immunological correlate of protection for HPV vaccines, antibody concentrations are the recommended endpoint for immunobridging because the vaccines' principal mechanism of protection is considered to be through neutralising antibodies.[95]

Following a review of the evidence, and recognising the cost-saving and programmatic advantages, WHO released a position paper in 2014, recommending the 2-dose schedule for girls aged <15 years.[96] However, they noted the need for further research on the efficacy and durability of immune responses with the 2-dose schedule, and in young girls in LMIC, and in malaria endemic areas. At that point there was only one observational study providing data on immune responses with fewer than 3 doses in SSA, and the numbers were small.[97] It was conceivable that the efficacy of the 2-dose schedule might be different in SSA, because of intercurrent infections or other factors.

Around this time, there was also emerging observational evidence of the efficacy of a single dose of HPV vaccine. A post-hoc analysis of results of the CVT showed that women who received only 1 dose had equivalent protection against persistent HPV16/18 infection as those who received 3 doses, and antibody levels were stable over 4 years.[98] In addition, those receiving 1 dose had similar antibody avidity, a measure of the overall antibody binding strength to antigen, to those receiving 3 doses. However, because women were not

randomised to receive fewer than 3 doses, there may have been differences between the dose groups in HPV exposure or other factors that could bias the results.

A key driver for research on the single-dose schedule was that cost continued to be a barrier to HPV vaccine introduction in LMIC.[88,99,100] However, there were questions related to the suitability of different HPV vaccines for a single-dose schedule. HPV vaccine types use different adjuvants which may differ in their immunogenicity. Head-to-head comparisons of the first two licensed vaccines had shown that HPV16 antibody GMCs were up to 5 fold higher with 3 doses of Cervarix than with Gardasil, and HPV18 GMCs up to 12 fold higher, at 4 years after vaccination.[101] The clinical relevance of these findings was unclear given the high efficacy of both vaccines, the high antibody GMCs induced by both vaccines compared with natural infection, and the lack of an immunological correlate of protection. In 2014, the WHO Strategic Advisory Group of Experts (SAGE) on Immunisation highlighted the need for head-to-head comparisons of reduced dose schedules of different HPV vaccines as a research priority.[102]

Furthermore, there was considerable debate about the biological plausibility of the efficacy of the single-dose schedule. The finding from the CVT that a single dose was protective against persistent HPV16/18 infection was unexpected. HPV vaccines, like other subunit vaccines (so called because they are composed of only parts of the pathogen) were thought to require a 'prime-boost' dose schedule to provide long-term protection.[103,104] The originally recommended HPV vaccination schedule was to give the first 2 doses a few months apart to prime the immune system to recognise the antigen. The priming elicits both antigen-specific plasma cells, which produce neutralising antibodies, and memory B cells, which do not produce antibodies but exist in a resting state and are activated by re-exposure to the same antigen to differentiate into antibody-secreting plasma cells.[103,105] The antibodies produced in response to the initial dose have a range of binding strengths (affinities) for the vaccine antigen. A process of 'affinity maturation' takes place over 4-6 months after priming, during which memory B cells are selected for their ability to generate increasingly high affinity antibodies. The third booster dose is given after a sufficient period has elapsed after priming (6 months or longer), which reactivates the pool of memory B cells. After 3 doses, the immune response has generated a large population of highly antigen-specific memory B cells and plasma cells, some of which survive for life.[103,106] The

long-lived plasma cells continuously produce IgG antibodies and are responsible for long term antibody persistence. With only a single priming dose of HPV vaccine, some researchers expressed concerns about waning antibody concentrations over a few years and weaker memory B cell responses, arguing that these would not be adequate to confer long-term protection against HPV infection and disease.

3.2 Research questions

In light of this background information, we designed several studies to answer the following questions:

1) Did malaria or helminth infections adversely impact the immune response to HPV vaccine?

2) Did the 1- and 2-dose schedules produce immune responses that were comparable to the 3-dose schedule in young girls in SSA?

3) Did 1- and 2-dose schedules produce immune responses that were likely to be protective against HPV vaccine-genotype related persistent infection and associated disease in SSA?

4) Were there important differences between HPV vaccines in immune responses after 1 or 2 doses?

5) How durable were immune responses after 1 and 2 doses in SSA?

In the following sections, I will describe the methods used in these studies, including the methodological considerations during their design, summarise the findings, and discuss their strengths and limitations.

3.3 HPV-021 trial supplementary studies (paper 3)

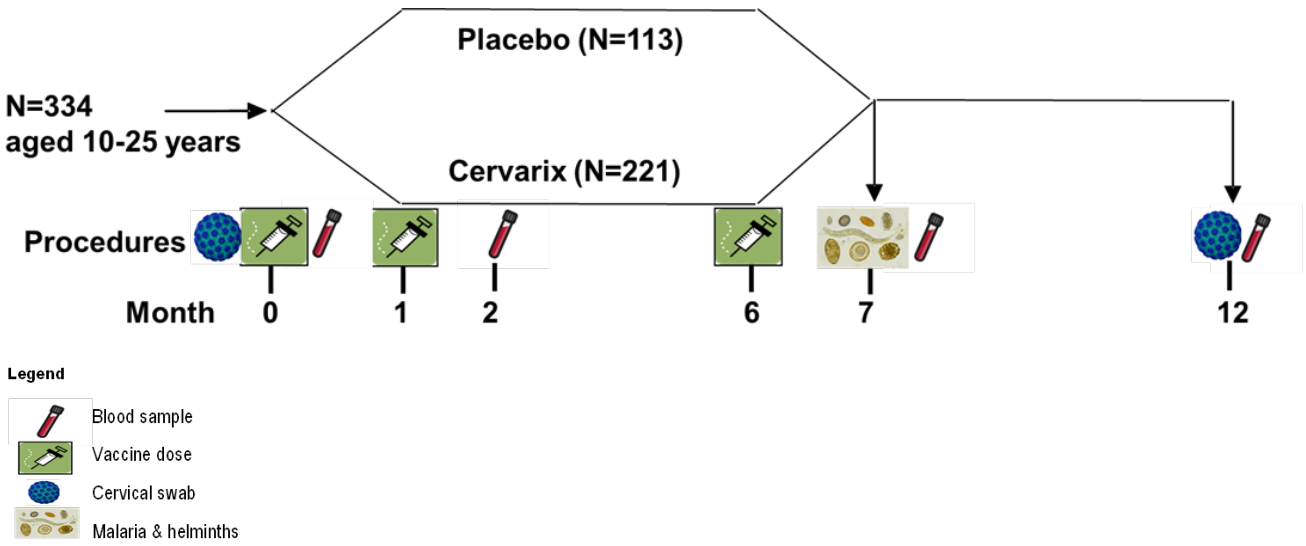
The HPV-021 trial was a double-blind, randomised, placebo-controlled, multi-centre immunogenicity and safety trial of the 2-valent vaccine, Cervarix, conducted between October 2007 and July 2010 in Tanzania and Senegal.[77] The design of the main trial was specified by the trial sponsor, GSK Biologicals. However, we were able to design and nest

supplementary studies within the main trial in Tanzania to answer additional questions. I was a co-investigator on the supplementary studies.

3.3.1 Methods

The trial enrolled healthy, HIV-negative females aged 10-25 years. Participants were randomly assigned (2:1) to receive either 3 doses of Cervarix or a placebo vaccine at 0, 1 and 6 months (Figure 2). Participants were seen at month (M)1, 2, 4, 6, 7, 8, 10 and 12, and blood samples for immunogenicity were collected at M0 (before vaccination) and at M2, 7 and 12. HPV16 and HPV18 IgG antibody concentrations were measured by ELISA at the GSK central laboratory in Belgium.

Figure 2: Design of GSK HPV-021 trial



Although, ideally, we wanted to know about malaria and helminth infections at the time of vaccination, this would have meant having to treat any infections that were identified, irrespective of whether the participant had any clinical symptoms, which would have potentially impacted on the vaccine immune response. The trial sponsor (GSK) also specifically requested that this was not done, so that immunogenicity and safety could be assessed under more 'real world' conditions. Therefore, we tested for these infections at M7, one month after the last vaccine dose, when collection of a blood sample for immunogenicity was already planned as part of the main trial. At that visit, we collected a finger-prick blood sample to be examined for malaria by light microscopy, and a urine

sample for the diagnosis of *Schistosoma haematobium*. Participants were also asked to collect stool samples on 3 consecutive days in the week after the M7 visit. The samples were examined for *Schistosoma mansoni*, *Ascaris*, *Trichuris*, *Strongyloides*, *Taenia* spp and hookworm using the Kato-Katz method.[107] Participants with malaria parasitaemia or any helminth infection were offered treatment following Tanzanian national treatment guidelines.

The primary objective of the study was to determine whether the presence and/or burden of malaria and helminth infections affected HPV16 and HPV18 antibody concentrations one month (M7) and 6 months (M12) after the last vaccine dose. The analysis was restricted to participants in the HPV vaccine arm. Helminth infection intensities were classified into light, moderate and heavy according to WHO guidelines.[108] HPV16 and HPV18 antibody concentrations were log10-transformed for analysis. Linear regression was used to compare mean log-transformed IgG antibody concentrations between participants with and without any helminth infection, and with and without malaria. Regression coefficients and 95%CIs were back-transformed to obtain geometric mean concentration (GMC) ratios and their 95%CIs. All regression models were adjusted for participant age and number of vaccine doses received. The analysis of the effect of malaria on vaccine immune responses also controlled for the presence of helminth infections, and vice versa.

The sample size for the supplementary studies was fixed based on the main trial design. The trial aimed to enrol around 670 participants, with half of those in Tanzania. Therefore, we expected to have around 220 participants in the HPV vaccine arm for our analysis. Assuming a 10% loss to follow-up at M7, 20% of participants with malaria or a helminth infection, and a standard deviation of 0.50–0.60 log10 antibody concentrations (based on other HPV vaccine studies), we had >90% power to detect a 50% decrease in antibody GMCs.[92]

3.3.2 Results

The trial enrolled 334 participants in Tanzania, of whom 221 were randomised to the HPV vaccine arm so eligible for inclusion in the analysis. Overall, 199/221 participants (90.0%) attended the M7 visit and 206/221 (93.2%) attended the M12 visit. At M7, 10.5% tested

positive for malaria parasitaemia and 31.6% for any helminth infection. The majority (84.8%) of helminth infections were light intensity. Only 8 participants were co-infected with malaria and helminths and only 6 had more than one helminth infection.

There was some evidence that participants with malaria parasitaemia had higher HPV16 antibody GMCs at M7 than those without malaria. After controlling for age, number of vaccine doses received, and any helminth infection, HPV16 GMCs were around 50% higher in participants with than without malaria (GMC ratio=1.47, 95%CI=1.00-2.18). HPV18 GMCs were around 20% higher in those with malaria but the difference was not statistically significant (GMC ratio=1.18, 95%CI=0.79–1.76). At M12, there was still some evidence that HPV16 antibody GMCs were higher in participants who had malaria at M7 than those who did not (GMC ratio = 1.43, 95%CI 0.86-2.37). There was no evidence of a difference in HPV16 or HPV18 antibody GMCs in participants with and without helminth infection, at either visit. At M7, antibody GMCs were highest in those with moderate/heavy helminth infections; however, this difference was not statistically significant and was no longer apparent at M12.

3.3.4 Strengths and limitations

A strength of the HPV-021 study was that it was the first trial of HPV vaccine in SSA and the first study to examine the effect of malaria and helminth infections on HPV vaccine antibody responses in any setting. We found no evidence of an adverse effect of these infections on HPV vaccine immune responses.

Limitations of the study included its small sample size, and although malaria and helminth infections were common, the numbers were relatively small. There were not enough participants who were co-infected with malaria and helminths, or who had more than one helminth infection, to be able to assess the impact of multiple infections. In addition, the majority of helminth infections were light so our power to detect an adverse effect related to the intensity of infection was limited.

We measured malaria and helminth infection one month after the last dose, which may not reflect infection status at the time of vaccination; therefore, we were unable to assess the impact of infection at the time of vaccination on subsequent immune responses. The follow-

up time was also relatively short, so we could not assess the effect of these infections on long-term immune responses to HPV vaccination. Other limitations included that it was an observational study, so the findings could be affected by unmeasured confounders. We adjusted for age and number of doses received, but we had limited data on other potential confounders (e.g. nutritional status)

Lastly, the HPV-021 study provided information about the effect of malaria and helminths on the HPV16/18 antibody responses after 3 doses of vaccine, which was the recommended schedule at that time. With the WHO approval of a 1-dose schedule, the effect of malaria on antibody responses after a single dose, where antibody concentrations are substantially lower than with 3 doses, was an important question that could not be addressed by this study.

3.4 DoRIS trial (papers 4 and 5)

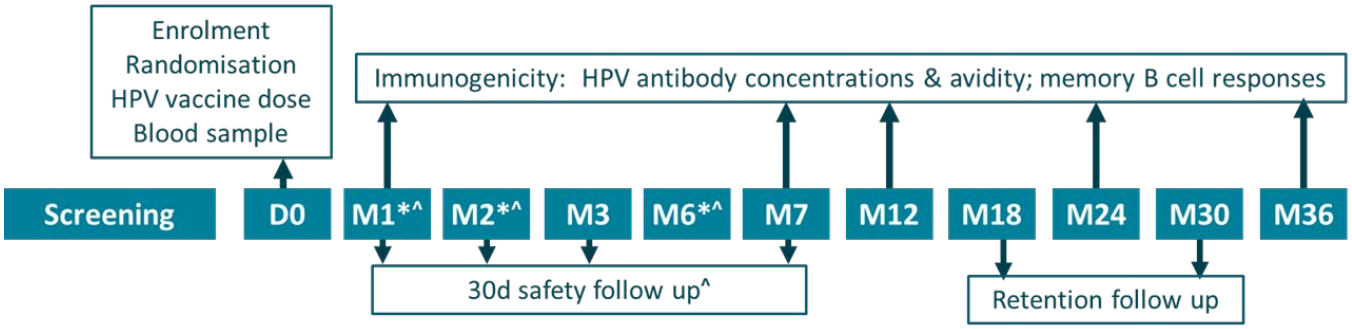
The DoRIS (Dose Reduction Immunobridging and Safety Study of two HPV vaccines in Tanzanian girls) study was the first randomised trial of the single-dose HPV vaccine schedule in girls in the primary target age range for vaccination and the first randomised trial of the 2-dose schedule in Africa. We enrolled participants between March 2017 and January 2018, and the trial is still ongoing. I am a joint principal investigator on this trial, along with Deborah Watson-Jones.

3.4.1 Methods

A detailed description of the trial procedures is provided in the trial design publication in Appendix 2. We enrolled 930 girls from primary and secondary schools in Mwanza. Girls were eligible for inclusion if they were healthy, aged 9-14 years, HIV-negative and planning to reside in Mwanza for 36 months. Participants were randomly allocated to one of six arms (155 per arm) comprising three different dose schedules of two different HPV vaccines: three doses over six months, two doses given six months apart, or a single dose of either Cervarix or Gardasil-9. Blood samples for immunogenicity assays (Appendix 3) were collected at M0, 1, 7, 12, 24 and 36 (Figure 3). To evaluate the effect of malaria on vaccine immune responses, we collected blood samples at each vaccination visit, and from all girls at M6, and stored these for later testing for malaria by PCR. The original trial was funded for

36 months of follow-up; however, we were later able to secure funding for an extended follow-up of participants in the 1 and 2 dose arms to 9 years post-first dose (M108). Blood samples for immunogenicity assays will be collected at M60, 84 and 108. The M84 visits are currently underway, with the final M108 visits expected in March 2027.

Figure 3. Design of DoRIS trial



The overall aim of the DoRIS trial was to determine whether a single dose of HPV vaccine, when given to girls aged 9-14 years, produces immune responses that are likely to be effective in preventing cervical cancer. We expected that antibody concentrations after 1 dose would be significantly lower than those after 2 or 3 doses, based on results from observational studies.[97,109] Therefore, our primary objective was to demonstrate non-inferiority of HPV16/18-specific seropositivity (rather than antibody GMCs) at M24 following 1 dose of HPV vaccine compared with 2 or 3 doses of the same vaccine. The M24 timepoint was chosen for several reasons. First, the observational data from earlier studies in Costa Rica (CVT) and in India (IARC/India study) showed that antibody responses after 1 dose reached a plateau around 12 months and remained stable thereafter, so there was strong scientific rationale for using the M24 timepoint.[109,110] Second, there was growing interest among policy makers in the single-dose schedule and in early results from the DoRIS trial.

The DoRIS trial also had a co-primary immunobridging objective, which was to demonstrate non-inferiority of HPV16/18 GMCs at M24 after 1 dose of vaccine compared with historical cohorts of women who received a single dose and in whom efficacy had been demonstrated. This was to determine if a single dose in the target age of vaccination could generate immune responses that would be likely to be efficacious against persistent HPV16/18 infection. These historical cohorts were from the CVT and the IARC/India trial. The

IARC/India trial was a cluster-randomised trial designed to compare the efficacy of 2 doses vs 3 doses of the 4-valent vaccine Gardasil in females aged 10-18 years.[110] Enrolment started in 2009; however, recruitment and vaccination were suspended after 10 months by the Indian government for reasons unrelated to the trial. As a result, some participants received only 1 dose by default. Participants continued to be followed up as originally planned. The initial results were published in January 2016, showing a similar frequency of incident HPV16/18 infections in participants who received 1, 2 or 3 doses, and no persistent HPV 16/18 infections over 4 years.[110]

The DoRIS trial sample size (155 per arm) was intended to give ≥90% power to demonstrate non-inferiority of HPV16/18-specific seropositivity at M24, comparing 1 dose with 2 or 3 doses, and non-inferiority of antibody GMCs, comparing the 1 dose arms in DoRIS with 1 dose recipients in the CVT and India trials. We assumed 20% loss to follow-up over 36 months and <5% DNA/seropositive at baseline, so expected to have 130 girls per arm at M24 for the primary per-protocol analyses. We assumed that the true proportion seropositive in each arm was 99%, and the true ratio comparing antibody GMCs was 1.0. We used a non-inferiority margin of –5% for seropositivity, i.e. the lower bound of the two-sided 95%CI for the difference (1 dose – 2 doses) had to be above –5%, implying that seropositivity with the 1-dose schedule was not decreased by more than 5.0%. For antibody GMCs, we used a non-inferiority margin of 0.50, i.e. the lower bound of the two-sided 95%CI for the GMC ratio (DoRIS/historical cohort) had to be above 0.50, implying that antibody concentrations in DoRIS were not reduced by more than 50% compared with the CVT and India trial. These non-inferiority margins were chosen based on those used in other HPV vaccine trials.[93,111]

Full details of the statistical methods are provided in papers 4 and 5, and briefly summarised here. For the immunobridging analyses, antibody concentrations were log10-transformed, and the difference in log10 concentrations at M24 between the two groups (1 dose DoRIS minus comparison cohort) and its 95% CI was calculated; the GMC ratio and its 95% CI were obtained by back-transformation. We conducted the primary analyses in the per-protocol cohort: girls who received the allocated doses of vaccine, had blood samples taken within the protocol-specified windows, and were HPV antibody and DNA negative at enrolment for the specific genotype under analysis. We then repeated the analyses in the total vaccinated cohort, to explore the robustness of our results.

3.4.2 Results

I will briefly describe the results in papers 4 and 5, which concern the primary and secondary endpoints at M24. In Chapter 4, I will present some additional DoRIS results that are relevant to this commentary.

Retention at M24 was 99%, with 918/930 girls attending as scheduled. We included 856 girls (93% of those attending) in the per-protocol analysis of HPV16 at M24, and 831 (91%) in the per-protocol analysis of HPV18. In the per-protocol cohort, all but 2 participants (1 in each of the 1 dose arms) were seropositive for HPV16 at M24. Non-inferiority of HPV16 seropositivity was met for 1 dose compared with 2 or 3 doses of both vaccines. HPV18 seropositivity was slightly lower, and 6 participants in the per-protocol cohort were not seropositive for HPV18 at M24. The non-inferiority criteria for HPV18 seropositivity comparing 1 dose with 2 or 3 doses were not met for either vaccine. However, ≥98% girls in the 1 dose arms were HPV18 seropositive at M24.

As expected, antibody GMCs were considerably higher among girls in the 2 and 3 dose arms than the 1 dose arms. For both vaccines and HPV genotypes, antibody concentrations among the 2 dose and 3 dose recipients peaked at M7 and declined thereafter through M24. However, among the 1 dose recipients, antibody concentrations remained relatively constant over time from M12 through M24 for both vaccine types.

In contrast with antibody GMCs, we found no evidence of a difference between the 1, 2 and 3 dose arms in geometric mean (GM) antibody avidity for HPV16 or HPV18, for either vaccine. Avidity is a measure of antibody quality and reflects the degree of antibody affinity maturation. GM avidity index ratios were around 1.0 for all comparisons, with the lower limit of the 95%CI >0.90 in all but 1 comparison (HPV18 GM avidity index ratio comparing 1 dose vs 3 doses of Cervarix=0.93, 95%CI=0.88-0.97).

In our immunobridging analysis, HPV16/18 antibody concentrations at M24 in the 1 dose Cervarix arm were non-inferior to those among 1 dose recipients in CVT, with GMC ratios (DoRIS/CVT) of 1.30 (95% CI=1.00-1.68) for HPV16 and 1.23 (95% CI=0.95-1.60) for HPV18. Non-inferiority was also met for seropositivity, with a difference (DoRIS-CVT) of 0.4% (95% CI= -3.1-5.1) for HPV16 and -0.4% (95% CI = -4.4-4.4) for HPV18. HPV16/18 antibody

concentrations in the 1 dose Gardasil-9 arm were significantly higher than those among participants who received 1 dose of Gardasil in the India trial, with GMC ratios (DoRIS/India) of 2.05 (95% CI=1.61-2.61) for HPV16, and 2.57 (95% CI=2.02-3.27) for HPV18. Seropositivity rates at M24 after a single dose of Gardasil-9 were also higher than in the India trial with a difference (DoRIS-India) of 6.9% (95% CI= 2.4-13.1) for HPV16 and 21.0% (95% CI=13.5-29.5) for HPV18.

The immunogenicity and immunobridging results were similar among the total vaccinated cohort and the per-protocol cohort, for both vaccines and both HPV genotypes.

3.4.3 Strengths and limitations

Strengths of the DoRIS trial are that it was the first trial to provide a range of information about reduced dose schedules of HPV vaccine. It was the first randomised trial of the single-dose schedule in girls in the target age group for vaccination and the first trial of the 2-dose schedule in SSA. It was also the first trial of Gardasil-9 in SSA and the first study in SSA to measure HPV antibody avidity or memory B cell responses with any dose of HPV vaccine.

Other strengths included that it was conducted in Tanzania, which bears one of the highest cervical cancer burdens in the world, and where HPV vaccine is most needed. The representativeness of the trial setting and study population allows the results to be generalisable to other parts of SSA. The trial had excellent retention at 2 years and nearly all participants were vaccinated and had blood samples collected according to the protocol-defined windows.

A strength of the immunobridging study is the comparison to two population groups where efficacy had been shown for up to 10 years, demonstrating the reproducibility of immune responses after a single dose in different geographic regions and for different HPV vaccines.

A critical aspect of immunobridging studies is the assay that is used to measure antibody concentrations. The DoRIS coinvestigators included Dr Ligia Pinto, the head of the Frederick National Laboratory for Cancer Research HPV Immunology Laboratory. The Frederick laboratory has substantial expertise in HPV serology and has participated in numerous studies of HPV vaccine responses.[112-114] We tested the samples from the DoRIS, CVT and

India trials together in the same batch at the Frederick laboratory, using their validated VLP ELISA assay, to minimise potential variability and allow robust comparisons between the studies.

One of the major limitations of the DoRIS results that are included in my analytical commentary is the relatively short period of follow-up of 24 months. Since girls in the target age range for vaccination may not become sexually active for many years, information on the longer-term durability of immune responses is essential. A main shortcoming of the immunobridging study was that neither the CVT nor the IARC/India trials were designed to test the efficacy of a single dose, and instead were post-hoc analyses of observational data where participants did not complete the scheduled doses. As observational studies, they are subject to bias and potential confounding, if participants who received 1 dose are different from those who completed their dose schedules. Such biases may be less likely for the India trial, since vaccination was stopped by the government, resulting in a 1 dose group by default. However, more robust evidence through a randomised trial designed to test single-dose efficacy was lacking at the time of our study. Some of these limitations have been addressed by later work described in Chapter 4.

Chapter 4. Discussion of findings and contribution to knowledge

In this chapter, I will discuss the findings of my studies on HPV epidemiology and HPV vaccines, how these findings have contributed to an understanding of these fields, and how they have informed subsequent research.

4.1 HPV epidemiology studies

The studies of HPV epidemiology in Mwanza provided some of the first information on the prevalence, incidence and persistence of genotype-specific HPV infection in healthy girls and young women in East Africa. At the time that these studies were planned, there were very limited data on HPV infection among women in SSA. In addition, much of the information that existed was from populations that were potentially at higher risk of HPV infection, such

as women attending family planning clinics, pregnant women, or women who were HIV-positive. Data from adolescents were particularly scarce.

The HPV-021 epidemiology study found one of the highest HR HPV prevalences (55%) and incidences (51/100 person-years for HR HPV) described globally for young, apparently low-risk, females in the general population. To put this in context, a study in Uganda of young women (median age 20 years) attending a sexual health clinic reported a HR HPV incidence of 21/100 person-years, whilst a population-based study in Uganda found a HR HPV incidence of 11/100 person-years among women aged <25 years.[115,116] Our results suggested that HPV transmission was extremely efficient among young women in the Mwanza setting.

The RHASA study was one of the first to examine HPV infection in adolescent girls in SSA around the age of sexual debut. Despite the limitations of its cross-sectional design, it provided valuable information on the genotypes which are acquired soon after initiation of sexual activity. It was also the first to characterise the vaginal microbiome in this population. We showed that HPV prevalence was strongly associated with the presence of non-optimal microbiota and that disturbances of the vaginal flora around the time of sexual debut may be an important factor determining the vulnerability of adolescent girls in this setting to HPV and other STIs.

Across our 2 studies, we found the most prevalent HR genotypes to be HPV16, HPV45 and HPV58, in contrast with global estimates at that time that found HPV16 and HPV18 to be the most prevalent in women with normal cytology.[55] The prevalence of HPV16 and HPV18, the HR genotypes targeted by the licensed HPV vaccines at that time, among girls aged ≤18 years was still low relative to other genotypes. Our studies thus provided useful data to model the impact of extending the age range of HPV vaccination through a 'catch-up' campaign in this region.

In the HPV-021 study, we found that 27% of genotype-specific HPV infections detected at enrolment were still present after 12 months, with no difference between HR and LR types. This was in contrast to a study in Zimbabwe in women aged 18-49 years (mean age 27), which reported a similar proportion of HPV infections that were still present at 12 months

(30%), but persistence was much higher with HR than LR genotypes (37% vs 22%, respectively).[117]

Estimates of persistence and clearance are difficult to compare between studies, in part because the interval between testing can have a large impact on the results. More frequent testing may detect infections that would be missed with longer intervals. A study of young women aged 18-21 in the USA that collected samples every 4 months found that 19% of genotype-specific HPV infections that had apparently cleared were redetected within a year.[25] A systematic review of approaches to calculating HPV clearance showed that estimates varied widely depending on how clearance was defined, person-years were calculated, and whether prevalent infections were included.[118] The authors identified 54 theoretically possible approaches to estimate clearance rates.

We were unable to estimate the duration, or clearance rate, of genotype-specific infections in the HPV-021 study because we sampled at only two timepoints. Furthermore, since the study was nested in an HPV vaccine trial and only 24 participants in the placebo arm contributed samples at both timepoints, we could not obtain reliable estimates of HPV16/18 incidence in the unvaccinated population. We subsequently designed another study (the HPV natural history study) to address some of the limitations of the HPV-021 epidemiology and RHASA studies.[119] The natural history study 503 enrolled girls aged 15-16 years, and collected samples for HPV testing every 3 months over 18 months, allowing us to obtain a more accurate estimate of genotype-specific incidence, as well as estimates of duration and clearance. The study formed the research project for an LSHTM PhD student (Catherine Houlihan). I was her statistical advisor and conducted some of the analyses.

In that study, we found that HPV infection was acquired, and cleared, rapidly in young women after their first reported sex (Appendix 4). The overall incidence of HR HPV infections was 66/100 person-years (95%CI:45–95), which was considerably higher than had been reported in other studies. Median duration of new HR HPV genotype-specific infections was 6.0 months. Among girls who were HPV-negative at visits before their reported sexual debut ('HPV naïve'), median time from reported sexual debut to HPV infection was 4.9 months. This duration of HR HPV infection was shorter than in previous

studies. A 2013 meta-analysis found a weighted median duration of HR types of 9.3 months, with a range from 6-15 months, although there were no data from SSA.[27]

Taken together, the data from our HPV epidemiology studies in Mwanza showed that, without HPV vaccination or screening programmes in place, it was likely that cervical cancer would continue to contribute significantly to female morbidity and mortality in the region. We shared our data from these studies with HPV researchers in the United States who were developing mathematical models of the natural history of HPV infection and cervical carcinogenesis in low-resource settings.[120] Our data also contributed to the IARC HPV Information Centre estimates of the HPV burden in SSA.[121]

4.2 HPV vaccines

Although there were the limitations, as described in Chapter 3, to the HPV-021 study, it provided reassurance that malaria and helminth infections, which are common in many parts of SSA, did not have an obvious negative impact on vaccine antibody responses. At the time of that study, no country in SSA had yet introduced HPV vaccines so these data were important for policymakers in countries where these infections were endemic.

There was some evidence that participants with malaria at M7 had higher HPV16 antibody concentrations at that timepoint, although the difference had reduced by M12. The mechanism behind this observation and its clinical significance are unclear. A study in Uganda among girls aged 10-16 years found weak evidence of an increase in HPV18 antibody concentrations at 24 months after vaccination among participants with malaria antibodies, indicating previous malaria exposure, although the timing of the malaria infection relative to vaccination was unknown.[122]

At the time of our findings from the HPV-021 study, we speculated that the polyclonal antibodies induced by malaria infection may have enhanced production of antibodies to the HPV vaccine. However, we were unable to assess whether there was any impact on antibody quality, as measured by avidity, or on durability of the antibody response. Therefore, when designing the DoRIS trial, we collected blood samples at each vaccination visit, to examine the impact of malaria at the time of vaccination (rather than after the last dose) on subsequent immune responses. The results were presented at the IUSTI

conference in September 2022 and provide a somewhat different picture from our earlier findings in the HPV-021 study.

Malaria was common in the DoRIS trial, with 148/620 (23.9%) participants in the 1 and 2 dose arms testing positive for malaria parasitaemia by PCR at the time of vaccination, although only 2 participants had symptoms of clinical malaria. We found no evidence of an effect of malaria at the time of vaccination on HPV16/18 antibody GMCs or avidity in the 1 dose arms of either vaccine, up to M36. However, in contrast with our findings from the HPV-021 study, there was some evidence that HPV16 antibody GMCs and avidity in the 2 dose Cervarix arm were lower in participants with malaria than those without, although no effect was seen for HPV18 antibody responses nor was there any evidence of an effect in the 2 dose Gardasil-9 arm (unpublished data; Table 2).

The clinical significance of these findings is uncertain. The magnitude of the differences between participants with and without malaria was relatively small, particularly for antibody avidity. HPV16/18 antibody GMCs in participants with malaria in the 2 dose arms were still significantly higher than among 1 dose recipients without malaria. Given that single-dose HPV vaccine antibody concentrations appear to be sufficient to confer protection, it seems likely that vaccine efficacy would not be reduced in individuals with malaria. We are currently evaluating the effect of malaria on HPV16/18 antibody responses at M60. An ongoing randomised trial in Uganda examining the effect of intermittent preventive malaria treatment on immune responses to various vaccines, including HPV vaccine, may provide further information.[123]

Table 2. Comparisons of antibody concentrations and antibody avidity at 36 months, between participants with and without malaria parasitaemia at the time of vaccination in DoRIS trial (per-protocol cohort[1])

	1 dose			2 doses		
	N	GMC[2] (95% CI) (IU/mL)	GM avidity index[2] (95% CI)	N	GMC[2] (95% CI) (IU/mL)	GM avidity index[2] (95% CI)
HPV16						
Cervarix						
No malaria	123	21 (18-25)	3.0 (2.9-3.1)	105	131 (114-115)	3.1 (3.1-3.2)
Malaria[3]	23	19 (12-30)	3.0 (2.9-3.2)	36	96 (74-126)	3.0 (2.8-3.1)
Ratio (malaria/no malaria)		0.91 (0.60-1.38)	1.01 (0.94-1.09)		0.74 (0.55-1.00)	0.95 (0.91-0.99)
Gardasil-9						
No malaria	114	13 (12-16)	2.9 (2.8-3.0)	100	84 (70-101)	3.0 (2.9-3.1)
Malaria[3]	26	12 (9-16)	3.0 (2.8-3.1)	40	79 (58-108)	3.0 (2.9-3.1)
Ratio (malaria/no malaria)		0.82 (0.55-1.20)	1.01 (0.94-1.09)		0.94 (0.69-1.28)	0.99 (0.95-1.03)
HPV18						
Cervarix						
No malaria	121	9 (8-11)	1.8 (1.7-1.9)	102	40 (34-47)	1.9 (1.8-1.9)
Malaria[3]	18	8 (4-15)	1.9 (1.8-2.0)	38	41 (31-53)	1.8 (1.7-1.9)
Ratio (malaria/no malaria)		0.84 (0.53-1.33)	1.03 (0.91-1.16)		1.02 (0.74-1.40)	0.97 (0.91-1.04)
Gardasil-9						
No malaria	107	6 (5-7)	2.1 (2.0-2.1)	99	22 (18-27)	2.1 (2.1-2.2)
Malaria[3]	24	5 (4-7)	2.0 (1.8-2.2)	36	19 (13-26)	2.0 (1.9-2.2)
Ratio (malaria/no malaria)		0.77 (0.50-1.18)	0.95 (0.88-1.03)		0.87 (0.62-1.24)	0.97 (0.92-1.02)

[1]Participants who were antibody negative and DNA negative at baseline for the HPV genotype under analysis. [2]Antibody geometric mean concentration (GMC) or geometric mean (GM) avidity index. [3]Malaria parasitaemia at any vaccination visit.

An important driver for my research on HPV vaccines was the potential advantage of a single-dose schedule in terms of cost, delivery and acceptability, especially for countries with limited resources. If a single dose could be shown to produce immune responses in young girls in SSA that were likely to be protective against HPV infection and related disease, this had the potential to facilitate the introduction of HPV vaccination programmes in LMIC and increase global HPV vaccination coverage.

In 2018, the WHO Director General announced his commitment to eliminate cervical cancer. Following this call, a Global Strategy for elimination of cervical cancer was drafted in 2020, with a proposed target that 90% of girls aged ≤15 years will have been vaccinated for HPV by 2030.[2] However, in 2021, only 37% of LMIC had HPV vaccination programmes in place and global vaccination coverage in girls aged ≤15 was only 21%.[3] It was estimated that less than a third of the world's population of girls aged 9-14 years, the target age for HPV vaccination, lived in countries providing HPV vaccines, and 60% of cervical cancer cases occurred in countries that have not yet introduced HPV vaccination.[124] Barriers to HPV vaccine introduction included the costs and logistical challenges of delivering a multi-dose schedule to young girls.[89] In addition, a global HPV vaccine shortage since 2018 constrained the supply of vaccines worldwide.[125]

The DoRIS trial provided the first randomised trial data on the single-dose schedule in girls in the target age group for vaccination. Our immunogenicity data supported observations from non-randomised studies that a single dose of HPV vaccine in girls in the target age range for vaccination can induce strong and sustained antibody responses to two years post-vaccination. HPV 16/18 antibody GMCs after 1 dose reached a plateau by M12 that was sustained to M24. Similar kinetics of the antibody response after a single dose were seen in the 1 dose recipients in the CVT and IARC/India trial, where antibody concentrations have remained stable up to 11 years.[126,127] Such long-term stability of antibody concentrations suggests that a single dose of HPV vaccine induces a pool of long-lived plasma cells which continue to produce antibodies for years, without the need for a booster dose. We have since reported that HPV16/18 antibody GMCs after 1 dose in DoRIS remain stable up to M60 (Appendix 5), further supporting the durability of the immune response in young girls, and in the SSA setting.

We failed to demonstrate non-inferiority of HPV18 seropositivity comparing 1 dose with 2 or 3 doses of either vaccine at M24, and in our later analysis at M60. The significance of undetectable anti-HPV18 antibodies in vaccinated individuals is unclear. An extended follow-up of women aged 16-23 years who received 3 doses of Gardasil in a randomised trial found that 35% no longer had detectable HPV18 antibodies at 5 years, despite sustained efficacy against persistent HPV18 infection and related disease.[128]. Similarly, among women who received 3 doses of Gardasil in the FUTURE trial in Finland, 15% had no detectable HPV18 antibody at 2-4 years, despite sustained VE of >90% against persistent HPV18 infection.[129]

The mechanism for protection among subjects who become nominally HPV18 seronegative several years after vaccination is still unclear. The lack of detectable HPV18 antibodies may relate in part to the sensitivity of the assay used and the value that is used for the assay cut-off.[114], However, in our immunobridging analysis that used a validated VLP ELISA that has been used in a number of HPV vaccine trials, only 77% of the 1 dose group in the IARC/India trial were seropositive for HPV18 at M24 yet efficacy against HPV18 infection did not differ between the 1, 2 and 3 dose groups.[130,131] It is unclear whether a minimum serum antibody concentration must be maintained for protection or whether exposure to the virus can activate memory B cells to produce neutralising antibody locally in the genital tract. These data suggest that some of the vaccine's protection is likely to be mediated through immune memory.

In line with our hypotheses when planning the DoRIS trial, we found no difference in antibody avidity between the 3 dose groups of either vaccine. A study in the Netherlands of girls who were vaccinated with Cervarix through the national programme found that HPV16 antibody avidity at 5 years did not differ between 1, 2 and 3 doses, and HPV18 avidity was higher in the single dose recipients.[132] In the IARC/India trial, HPV16 and HPV18 antibody avidity at 18 months was slightly, but statistically significantly, higher in the 1 dose than the 3 dose group (avidity index ratio for HPV16 =1.10, 95%CI=1.01-1.19; HPV18=1.11, 95%CI=1.01-1.22).[110] The observed higher avidity with a single dose in those studies was not expected, and the reasons for this are unclear. Nevertheless, these findings indicate that a single dose of HPV vaccine is sufficient to induce affinity maturation without the need for a second dose.

In our immunobridging study, our finding that single-dose antibody GMCs in DoRIS were not significantly different from those in the CVT was unexpected, given the older age of women in the CVT (median 20 years vs 10 years in DoRIS). One explanation may be that the very potent adjuvant in Cervarix overrides any effect of younger age when only a single dose is given. Another explanation may be that vaccination may boost a women's responses to previous natural infection. A small study to investigate the effect of vaccination on memory B cell responses in women who were HPV seropositive from natural infection found that 40% had a sharp increase in neutralising antibody levels at 1 week post vaccination with a single dose, suggesting an anamnestic (immune memory) response to vaccination.[133] Boosting of vaccine antibody responses may also have occurred during exposure to HPV through sexual activity. The CVT participants were all sexually active at enrolment, whereas only 1.5% of DoRIS participants reported having passed sexual debut by M24. The HPV viral load through sexual exposure is thought to usually be too low to induce an anamnestic response.[104,128] However, in the IARC India trial (girls aged 10-18 years at the time of vaccination), a small increase in HPV16/18 antibody GMCs in single-dose recipients was noted between M36 and M120; the authors speculated that this may have been a result of a boosting effect as girls became sexually active.[127] We are currently examining the effect of sexual debut on single-dose HPV16/18 antibody responses at M60 in DoRIS.

By 2018, the CVT and IARC/India trial had published results showing comparable efficacy against incident HPV16/18 infection in the 1, 2 and 3 dose groups up to 7 years of follow-up.[130,134] The Single-Dose HPV Vaccine Evaluation Consortium (of which I am a member), coordinated by PATH, was formed in 2018 to evaluate the accumulating data on the single-dose HPV vaccination schedule.[135] When we began recruitment for the DoRIS trial, it was the only randomised trial of the single-dose schedule; however, by 2019 there were 5 other trials designed to formally evaluate the single-dose schedule that were in progress or due to begin soon (Appendix 6). In October 2019, WHO SAGE met to review the evidence regarding single dose efficacy. At that point, they felt that the existing evidence, still based on observational studies alone, was not sufficient to support a change in the current WHO recommendation.[136]

In December 2021, the WHO SAGE Working Group met again to review further results from single dose studies. We were invited to present the DoRIS results at that meeting, along

with researchers working on other studies of the single-dose schedule. In that same month, we also presented our results to the UK Joint Committee on Vaccination and Immunisation (JCVI). The JCVI had discussed the potential for a single-dose schedule at a meeting in June 2020 but agreed that they would like to see more data on Gardasil-9 before providing advice, since the UK HPV vaccination programme was moving to Gardasil-9.

In their March 2022 report, the SAGE Working Group noted that the immunogenicity results from DoRIS strongly supported the conclusion that efficacy of a single dose in young girls would be comparable to that demonstrated in young women.[137] They also noted that HPV16 and HPV18 seropositivity at M24 after 1 dose was very high with both vaccines, and that non-inferiority of seropositivity was "just missed" for HPV18, and noted the dependence on the assay sensitivity and cut-off points for seropositivity. The UK JCVI commented that the stability of HPV16/18 antibody responses up to 24 months after a single dose of Gardasil-9 was "very reassuring".[138] We were asked to present the DoRIS results again at a WHO SAGE meeting in April 2022, and to the UK JCVI again in May 2022. At these meetings, in addition to the results at M24, we presented the immunogenicity results up to M36, the results from the malaria analysis, and an immunobridging comparison to the first randomised trial designed to test the efficacy of a single dose, the KEN SHE trial.

The KEN SHE trial was planned after DoRIS had started. It was conducted among sexually-active women aged 15-20 years in Kenya; participants were randomly allocated to 1 dose of Cervarix, Gardasil-9 or a control meningococcal vaccine.[139] We had early discussions with the KEN SHE investigators, and they added a M24 blood sample to their protocol so that we could bridge the DoRIS results to theirs; the publication arising from this analysis is in Appendix 7. Vaccine efficacy against incident persistent HPV16/18 infection in KEN SHE at M36 was ≥97.5% for both HPV vaccines.[140] In our immunobridging analysis, HPV16 and HPV18 antibody GMCs at M24 after 1 dose in DoRIS were non-inferior to those in KEN SHE, for both vaccines.

The KEN SHE immunobridging analysis provided several important advances over our immunobridging to the CVT and India trial. First, we were comparing immune responses in DoRIS to those from a randomised trial with direct and rigorous evidence of 1 dose efficacy rather than to observational studies, thus providing the strongest evidence of likely

protection afforded by the single-dose regimen in young girls. It also provided the first immunobridging comparison for Gardasil-9. The trials were both conducted in East Africa and in some areas where malaria is endemic.

At the April 2022 meeting, based on a review of all available evidence including the DoRIS trial, SAGE made the initial recommendation that countries be allowed to choose between a 1- or 2-dose schedule for 9–14-year-old girls.[74] This recommendation was from a public health perspective, because the protection conferred by a single dose was comparable to that with 2 doses, and the single-dose schedule was easier to implement and less resource-intensive. Several modelling studies showed that the increased coverage that could be achieved with a single dose, and resulting herd immunity compared with 2 doses would compensate for any theorical decrease in efficacy.[137,141,142] The UK JCVI made similar recommendations in August 2022.[143] In December 2022, WHO released a statement that, that as an off-label option, a single-dose schedule can be used in females and males aged 9–20 years.[144] This WHO recommendation was endorsed by the Pan American Health Organization Technical Advisory Group and WHO's Regional Office for Africa in 2023 and 2024, respectively.

Since then, a number of countries have switched to a single-dose schedule in their national programmes, including Australia (February 2023) and the UK (October 2023). In September 2023, Nigeria became the first country in Africa to introduce a national HPV vaccination programme with the single-dose schedule. Tanzania will switch to single-dose delivery in 2024, in part because of our work. As of September 2024, 58 countries were delivering a single-dose schedule in their HPV vaccination programmes, including 17 countries in SSA.[3,145,146]

Chapter 5. Remaining knowledge gaps

In this chapter, I will discuss some of the remaining knowledge gaps for single-dose HPV vaccination and research that is addressing these.

Although WHO endorsed a single-dose HPV vaccine schedule in 2022 for males and females aged 9-20 years, there are limited data on the immunogenicity, and no data on efficacy, of a single dose in males. Comparable antibody concentrations and efficacy have been shown in males and females with multi-dose HPV vaccine schedules, but there has only been one small study of the single-dose schedule in males.[147-150] The study examined HPV16/18 antibody responses over 24 months after a single dose of Gardasil-9 in 50 boys and 130 girls aged 9-11 years in the United States.[151] HPV16/18 antibody GMCs at M24 were 10-15% lower in boys than in girls, although the difference was not statistically significant, and GMCs were stable from 12 to 24 months in both sexes.

We are currently conducting a cluster-randomised trial of single-dose vaccination in boys in Tanzania, the Add-Vacc trial, and I am a co-PI of this study. Add-Vacc was designed to measure the impact of adding single-dose male HPV vaccination to routine female HPV vaccination on population prevalence of HPV vaccine-genotypes, and will also collect immunogenicity data. Overall, 26 communities (clusters) were randomly allocated 1:1 to the control arm (2 doses of Gardasil offered to females aged 14 years through the Tanzanian national programme) or the intervention (a one-off campaign to offer a single dose of Gardasil to males aged 14-18 years, alongside routine female vaccination through the national programme). The outcome will be evaluated through cross-sectional surveys of HPV prevalence in men and women aged 18-21 years in the 26 communities at baseline and 36 months. In addition, we enrolled a cohort of 200 vaccinated boys who will be followed for 2 years to measure vaccine immunogenicity and safety. We completed vaccination of males in March 2024, and the final HPV prevalence survey will be conducted in 2026-2027.

This trial will allow us to address a number of key questions in the field of cervical cancer control. This will be the first time that a gender-neutral vaccination approach has been evaluated in SSA. It will allow us to determine whether male vaccination given alongside female vaccination in this setting provides additional benefit in reducing population HPV prevalence. Our cohort of 200 boys will provide important data on the single-dose immune response in males, and the only data on HPV vaccine immunogenicity in males in SSA, for any dose schedule. We will compare the immunogenicity results from Add-Vacc to those from the single-dose arms in DoRIS, to assess whether antibody responses are similar. Add-Vacc will also provide an estimate of the impact of the Tanzanian national programme in

females only, by comparing HPV prevalence in the baseline and M36 population surveys in the control communities. Measuring the impact of national HPV vaccine delivery in different settings is essential for informing and understanding vaccination strategies for cervical cancer elimination.

We will also explore whether the gender-neutral strategy improves acceptability and uptake of HPV vaccination in girls. Mathematical modelling has suggested that increasing coverage for girls could have a greater impact on cervical cancer than extending vaccination to boys; however, coverage in girls would need to be 90% to achieve elimination.[152,153] Such high coverage is unlikely to be achievable in much of SSA. In Tanzania, for example, data from our baseline survey for Add-Vacc suggest that coverage in girls is <40%. If gender-neutral vaccination increases acceptability of HPV vaccination, the impact may be greater than can be achieved through female vaccination alone. For example, modelling studies have shown that addition of male vaccination with moderate coverage (40-60%) for both sexes will lead to similar falls in HPV prevalence as 80% coverage in females alone.[152,153]

One of the research priorities for HPV vaccination recommended by WHO in 2022 was on widening the age range for the single-dose schedule, both to children <9 years old, and adults >20 years old.[144] As mentioned in Chapter 3, one of the challenges for HPV vaccination programmes is the cost of delivering the vaccine to girls in the primary target age group (9-14 years). This is an age group that is not usually targeted for health interventions, so few LMIC have optimal delivery platforms. Other challenges to delivering to school-aged girls are potential stigma when vaccinating young girls against a sexually transmitted infection and the fact that many girls do not attend school. Girls who are not in school may be more at risk of HPV infection, and have less access to cervical cancer screening, than those who attend school, and therefore suffer disproportionately if not vaccinated. Delivery of HPV vaccines could be simplified, less expensive and achieve higher and more equitable coverage if delivered to infants and young children as part of routine Essential Programme on Immunization (EPI) vaccinations.

Existing data on HPV vaccination in older children provide a strong rationale for exploring single-dose HPV vaccination within routine childhood immunisations. Studies have consistently demonstrated higher HPV vaccine antibody GMCs in children aged 9-14 years

compared with adults given the same number of doses.[77,90,91,93] A small study in girls aged 4-6 years (n=74 receiving HPV vaccine) found that the safety and tolerability of 2 doses of HPV vaccine (Cervarix) were similar to the control vaccines (measles-mumps-rubella and diphtheria-tetanus-acellular-pertussis), and that antibody GMCs were higher than those in historical controls of females aged 15-25 years who received 3 doses of Cervarix.[154,155] An ongoing trial in The Gambia (the HANDS trial, Appendix 6) is examining immune responses after 1 or 2 doses of Gardasil-9 in girls aged 4-8 years and 9-14 years, compared with those after 3 doses in females aged 15-26 years. Early unpublished results from this trial have shown that antibody GMCs in girls aged 4-8 years are higher than or similar to GMCs in girls aged 9-14 years, and significantly higher than those aged 15-26 years, after the same number of doses. The safety and tolerability of the vaccine is also comparable in the two younger age groups.

However, there are no data on the immunogenicity or safety of HPV vaccines in children <4 years old, and no data on the persistence of antibody responses in children <9 years old. This is a critical knowledge gap since the vaccine must provide protection from infancy to sexual debut and beyond. Few vaccines are able to provide this type of durable protection. However, as discussed in Chapters 3 and 4, antibody concentrations in girls who received a single dose of HPV vaccine at age 10-14 years in the IARC/India trial have remained stable for 11 years, with sustained efficacy.[127,131] Including HPV vaccine within childhood EPI vaccination becomes realistic if the same antibody kinetics are demonstrated when the vaccine is delivered in early childhood.

Another research priority is for further evidence on the longer-term immunogenicity and efficacy of the single-dose HPV vaccine schedule. The KEN SHE trial demonstrated that the single-dose schedule had >97% efficacy against persistent HPV16/18 infection at M36.[140] However, the evidence for longer term protection, and for protection against HPV-related disease (vs persistent HPV infection), still comes from observational studies. KEN SHE has been extended and will continue to follow participants to 54 months. Two ongoing randomised trials of single-dose efficacy in Costa Rica (ESCUDDO and PRISMA, Appendix 6) will also follow women for up to 5 years. These trials are assessing efficacy against persistent cervical infection, and not HPV-related disease. Although persistent infection with

oncogenic HPV is a necessary prerequisite for cervical cancer, some researchers have argued the need for studies with disease endpoints.

In March 2024, Merck announced their plans for a 9-year trial to evaluate the efficacy of a single dose of HPV vaccine against persistent infection with HPV vaccine genotypes and HPV-related external genital and cervical disease.[156] The trial will be conducted in females aged 16-26 years. They are also planning a 9-year trial to evaluate single-dose efficacy against persistent anogenital infection and external genital disease in males. MITU is being considered as a potential site for this study and I have been involved in these discussions.

In conclusion, I consider that the research I have done on HPV epidemiology has advanced knowledge about HPV infection in SSA. My work on HPV vaccination in collaboration with colleagues has provided insights into the immunogenicity and likely protection of a single dose when given to girls in the target age range for vaccination, and supported the recommendations for a single-dose HPV vaccine schedule. This recommendation has encouraged more countries to introduce these highly efficacious vaccines. I hope that my ongoing research in HPV vaccines will continue to inform the use of the single-dose schedule and to contribute to the global efforts to meet the WHO cervical cancer elimination goals.

SECTION 2: RESEARCH PUBLICATIONS

Paper 1. High prevalence and incidence of human papillomavirus in a cohort of healthy young African female subjects

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Student ID Number	2301598	**Title**	Ms
First Name(s)	Kathryne		
Surname/Family Name	Baisley		
Thesis Title	HPV epidemiology and HPV vaccination in East Africa		
Primary Supervisor	Deborah Watson-Jones		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

Where was the work published?	Sexually Transmitted Infections		
When was the work published?	2013		
If the work was published prior to registration for your research degree, give a brief rationale for its inclusion	PhD by prior publication		
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Stage of publication	Choose an item.

SECTION D – Multi-authored work

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)	This paper presents results from a sub-study nested in the first trial of HPV vaccine in Africa. The trial was conducted in Tanzania and Senegal, and there were a number of supplementary studies as part of the trial in Tanzania. I was a co-investigator on the supplementary studies, and contributed to the conception of the idea and the development of the study design, protocol and questionnaires. I was also the study statistician, had overall responsibility for the study data management, wrote the statistical analysis plan, analysed the data and interpreted the results. For this paper, in addition to the analyses, I contributed to the drafting of the manuscript and to the reponse to the reviewers during the peer-review process.

SECTION E

Student Signature	[redacted]
Date	14 October 2024

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Date	17 October 2024

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Paper 1 High prevalence and incidence of human papillomavirus in a cohort of healthy young African female subjects

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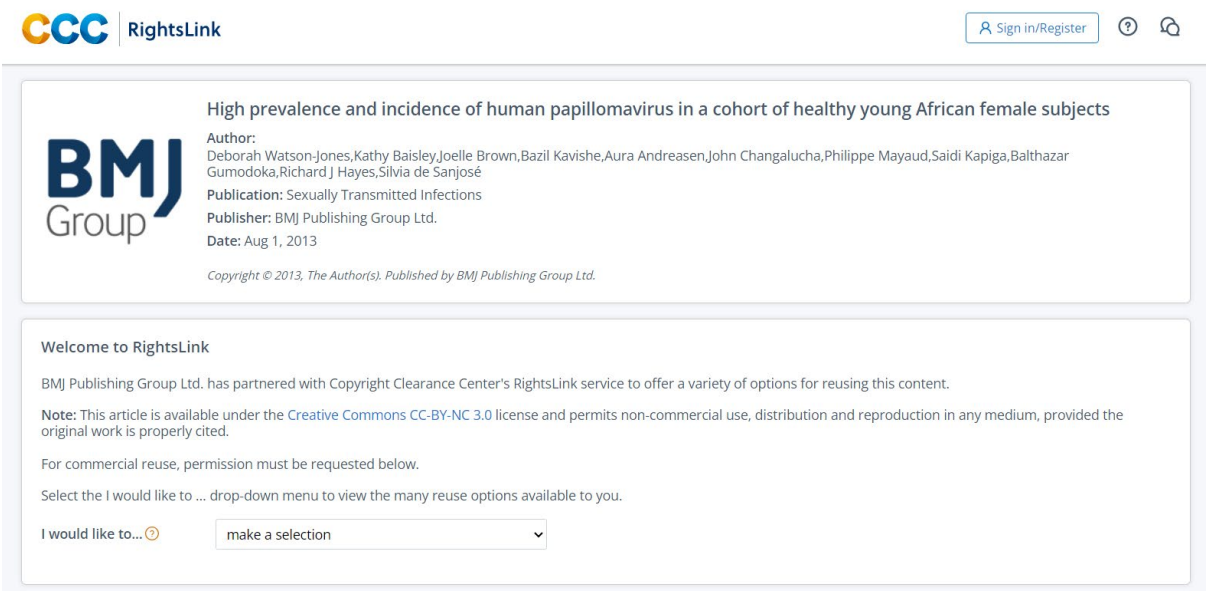

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ORIGINAL ARTICLE

High prevalence and incidence of human papillomavirus in a cohort of healthy young African female subjects

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The results from this study have been presented in part at the 27th International Papillomavirus Conference, Berlin, 17–22 September 2011 (Watson-Jones D, Brown, J, de Sanjosé S, *et al.* Cervical HPV prevalence and genotypes in Tanzanian girls and women. (Abstract P-02.24)).

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ABSTRACT

Objectives We measured the prevalence and incidence of human papillomavirus (HPV) infection in young female subjects recruited for a safety and immunogenicity trial of the bivalent HPV-16/18 vaccine in Tanzania.

Methods Healthy HIV negative female subjects aged 10–25 years were enrolled and randomised (2:1) to receive HPV-16/18 vaccine or placebo ($Al(OH)_3$ control). At enrolment, if sexually active, genital specimens were collected for HPV DNA, other reproductive tract infections and cervical cytology. Subjects were followed to 12 months when HPV testing was repeated.

Results In total 334 participants were enrolled; 221 and 113 in vaccine and control arms, respectively. At enrolment, 74% of 142 sexually active subjects had HPV infection of whom 69% had >1 genotype. Prevalent infections were HPV-45 (16%), HPV-53 (14%), HPV-16 (13%) and HPV-58 (13%). Only age was associated with prevalent HPV infection at enrolment. Among 23 girls who reported age at first sex as 1 year younger than their current age, 15 (65.2%) had HPV infection. Of 187 genotype-specific infections at enrolment, 51 (27%) were present at 12 months. Overall, 67% of 97 sexually active participants with results at enrolment and 12 months had a new HPV genotype at follow-up. Among HPV uninfected female subjects at enrolment, the incidence of any HPV infection was 76 per 100 person-years.

Conclusions Among young women in Tanzania, HPV is highly prevalent and acquired soon after sexual debut. Early HPV vaccination is highly recommended in this population.

INTRODUCTION

The primary cause of cervical cancer is persistent infection with high risk (HR) human papillomavirus (HPV) genotypes. East Africa has one of the highest rates of cervical cancer in the world.[1] Reviews of global age-specific prevalence show a high prevalence of HPV in young sexually active women,[2–4] but there are few data on the epidemiology of HPV infection in sexually active girls and young women aged <25 years in East Africa, where prevalences have been reported as high as 55% in Mozambique.[3] We measured the burden of HPV infection and risk factors for infection in a cohort of HIV negative girls and young women aged 10–25 years in Tanzania recruited for a safety and immunogenicity trial of a prophylactic HPV vaccine.[5] These data will be used to help to inform recommended age of HPV vaccination in a future national vaccination programme.

METHODS

Study design

This substudy was nested within a Phase IIIb immunogenicity and safety study of the HPV-16/18 AS04-adjuvanted vaccine. This double-blind, randomised, controlled trial (NCT00481767) was conducted in Dakar, Senegal and Mwanza, Tanzania, with eligible female subjects being randomly assigned (2:1) to receive either three doses of vaccine (vaccine group) or Al(OH)3 (control group).[6] Trial results have been published elsewhere.[6] The HPV substudy was conducted between October 2007 and July 2010 in Mwanza.[6] The trial and the substudy were approved by the ethics committees of the National Institute for Medical Research (NIMR), Tanzania and the London School of Hygiene & Tropical Medicine.

Study participants

Study participants were recruited from schools, colleges and family planning clinics in Mwanza and invited to attend an eligibility screening visit 1 month before enrolment. They were eligible if they were aged 10–25 years, HIV negative, not pregnant, had ≤6 lifetime sexual partners, were free of health problems, had no history of neurological disorders and, if sexually active, were willing to use contraception or abstain from sex for 30 days before vaccination until 2 months after completion of vaccination. This was requested for all participants and contraception was provided at the research clinic (the majority (75%) of sexually active women used hormonal contraception throughout the study). Participants were asked for written consent or, if illiterate, for witnessed thumb-printed informed consent. Parental/guardian consent was obtained for participants aged below 18 years.

Follow-up procedures

Participants were followed to month 12. HPV vaccine (Cervarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) or the control injection was given at months 0, 1 and 6.

Specimen collection

Blood samples were collected at the screening visit (day 30) for HIV and syphilis. At enrolment

(month 0), before vaccination, participants were interviewed about sexual activity and, if sexually active, symptoms of reproductive tract infections. A genital examination was performed on participants who reported ever being sexually active. Vaginal swabs were taken for bacterial vaginosis (BV) and Trichomonas vaginalis (TV). A Papanicolou smear was taken and an endocervical swab was collected for Neisseria gonorrhoeae (NG) and Chlamydia trachomatis (CT). An ectocervical swab and endocervical swab were also taken for HPV DNA testing at enrolment and month 12 from participants who reported ever being sexually active. Syndromic reproductive tract infection treatment was provided and treatment was offered for NG, CT, TV and symptomatic BV diagnosed on laboratory testing.

Laboratory tests

Cervical swabs for HPV DNA testing were frozen at −20°C and sent to the Catalan Institute of Oncology in Barcelona where they were genotyped for 37 different HPV genotypes by the Roche Linear Array assay (Roche, Branchburg, USA) according to the manufacturer's instructions. The PCR reaction included an additional primer pair targeting the human β-globin gene as an internal control. Genotyping was performed in an automated system, Auto-LiPA 48 (Tecan Austria GmbH, distributed by Innogenetics). HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 and -68 were considered HR genotypes; all other genotypes were considered low risk (LR).[7]

Papanicolou smears were processed and results recorded from a single reading in Mwanza. Endocervical swabs were tested for NG and CT by PCR (AMPLICOR, Roche, Branchburg, USA) in Mwanza. Gram stained vaginal smears were examined for *Candida albicans* spores and for BV using the Nugent score while TV was diagnosed by culture (InPouch TV, BioMed Diagnostics, San Jose, California, USA).

HIV serology was determined using two rapid tests, Determine HIV-1/2 (Alere Medical Co., Matsudo-shi, Chiba, Japan) and SD Bioline HIV-1/2 3.0 (SD Standard Diagnostics, Inc. Hagal-dong, Kyonggi-do, Korea). Positive, indeterminate or discordant results were confirmed by an HIV Ag–Ab combination ELISA (Murex Biotech, Dartford, UK) and Uni-Form II Ag–Ab micro ELISA (bioMérieux, Basingstoke, UK). Discordant samples on ELISA were tested for P24 antigen (Biorad, Genetic Systems, UK). Serum samples were tested for syphilis by the rapid plasma reagin test (Immutrep, Omega Diagnostics, Alva, UK) and *Treponema pallidum* particle agglutination assay (Fujirebio, Tokyo, Japan).

Statistical analysis

Data were double entered and verified in DMSys (SigmaSoft International), and analysed using STATA V.11.0 (StataCorp LP; College Station, Texas, USA).

The trial aimed to enrol 333 participants in Mwanza (222 and 111 participants in the vaccine and control arms, respectively). Enrolment was age-stratified, with a third of participants in the 15–25 years age-stratum and the remainder in the 10–14 years age-stratum.

Cohort characteristics at enrolment were tabulated and HPV genotype prevalence was calculated among sexually active participants. The number of new infections (genotype not present at enrolment, present at month 12), persistent infections (same genotype at enrolment and month 12) and cleared infections (positive for the genotype at enrolment but negative for that genotype at month 12) were tabulated by treatment arm and overall. Type-specific persistence and clearance were calculated among women who were infected with the genotype at enrolment. Type-specific cumulative incidence was calculated among those who were negative for the genotype at enrolment. The proportion of women with any new HPV infection, any persistence and any clearance were calculated among all women who had samples at both time points.

The incidence rate (per 100 person-years) of any HPV genotype, and any HR genotype, was calculated among women who were negative for all genotypes, or negative for all HR genotypes, respectively, at enrolment. Person-years at risk were calculated from date of enrolment until date of HPV acquisition, assumed to occur mid-way between the last negative and first positive results.

Risk factors associated with prevalent HPV infection at enrolment among sexually active participants were analysed using logistic regression to estimate OR and 95% CI. Participants who were positive for any HPV genotype were classed as 'infected'; those negative for all HPV genotypes were classed as 'uninfected'. Age was considered an a priori confounder, and so was included in all models. Factors that were associated with HPV infection at $p<0.20$ in the age-adjusted analysis were considered for inclusion in a multivariable model; those remaining independently associated at $p<0.10$ were retained. After age-adjustment, no other variables were associated with HPV infection at $p<0.10$, and so no further model building was done.

RESULTS

Cohort screening, enrolment and follow-up

In total, 587 participants attended the screening visit. Of 379 eligible female subjects, 334 (88.1%) were enrolled (221 and 113 in the vaccine and control arms, respectively); 45 refused, 15 had moved away, 16 did not attend the visit and 25 were not enrolled because the enrolment target had been reached. The median age of enrolled participants was 18 years (IQR 13–19).

Overall, 308/334 (92.2%) participants attended the month 12 visit; 206 (93.2%) in the vaccine arm and 102 (90.3%) in the placebo arm ($p=0.34$).

Reasons for not completing follow-up included withdrawal of consent (10), moved away (4), temporary travel (5), being untraceable (3) and unknown reason (4).

Cohort description at enrolment

Approximately half (46.5%) of 334 enrolled participants had secondary school or higher education; 78.2% were currently students. Most (87.4%) were single. Only 2 (0.6%) had ever smoked and 4 (1.3%) had vulval genital warts. No cervico-vaginal warts were observed.

At enrolment, 142 (42.5%) participants reported having passed their sexual debut; median reported age at first sex was 16 years (IQR 15–17), and 75 (52.8%) reported >1 lifetime sexual partner. Two-thirds (66.0%) of sexually active women reported never using condoms. Cervical and vaginal samples were available for 117 (82.4%) and 125 (88.0%) participants, respectively. One participant had congenital absence of a cervix and samples for NG, CT and HPV were taken from the vaginal vault. There were no cases of low or high grade squamous intraepithelial lesions on cervical cytology. Overall 27.4% had BV, 12.8% had TV, 5.1% had CT and 2.6% had NG. Two participants (1.4%) had active syphilis.

Prevalence of HPV at enrolment by genotype, age and recent sexual debut

Overall 73.5% (86/117; 95% CI 64.5 to 81.2) of sexually active participants with HPV results at enrolment had HPV infection (table 1). Assuming that girls who had not had sex were HPV

Table 1 HPV prevalence at enrolment and at 12 months among sexually active subjects

	Enrolment			12 months		
	Control n (%)	Vaccine n (%)	Total n (%)	Control n (%)	Vaccine n (%)	Total n (%)
Swab results available/sexually active	33/47 (70.2)	84/95 (88.4)	117/142 (82.4)	37/45 (82.2)	85/91 (93.4)	122/136 (89.7)
Any HPV type						
Yes	25 (75.8)	61 (72.6)	86 (73.5)	25 (67.6)	66 (77.6)	91 (74.6)
Any high risk HPV						
Yes	18 (54.5)	46 (54.8)	64 (54.7)	12 (32.4)	50 (58.8)	62 (50.8)
HPV 16/18						
Yes	4 (12.1)	13 (15.5)	17 (14.5)	3 (8.1)	5 (5.9)	8 (6.6)
Number of HPV genotypes						
None	8 (24.2)	23 (27.4)	31 (26.5)	12 (32.4)	19 (22.4)	31 (25.4)
1	7 (21.2)	20 (23.8)	27 (23.1)	11 (29.7)	18 (21.2)	29 (23.8)
2	9 (27.3)	16 (19.0)	25 (21.4)	2 (5.4)	19 (22.4)	21 (17.2)
3	4 (12.1)	11 (13.1)	15 (12.8)	4 (10.8)	14 (16.5)	18 (14.8)
4 or more	5 (15.2)	14 (16.7)	19 (16.2)	8 (21.6)	15 (17.6)	23 (18.9)
Swab results available at both visits/sexually active at both visits				24/39 (61.5)	73/84 (86.9)	97/123 (78.9)
Any new HPV type						
Yes				16 (66.7)	49 (67.1)	65 (67.0)
Any new high risk HPV						
Yes				7 (29.2)	34 (46.6)	41 (42.3)
Any new HPV-16/18						
Yes				2 (8.3)	1 (1.4)	3 (3.1)
Any persistent HPV						
Yes				10 (41.7)	22 (30.1)	32 (33.0)
Any persistent high risk HPV						
Yes				5 (20.8)	16 (21.9)	21 (21.6)

HPV, human papillomavirus.

negative, the overall cohort HPV prevalence was 27.8% (86/309). In total, 54.7% (64/117) of sexually active participants were infected with HR genotypes. The most common (figure 1) were HPV-45 (16.2%), HPV-16 (12.8%) and HPV-58 (12.8%). Seventeen participants (14.5%) were infected with either HPV-16 or -18. The most common LR genotype was HPV-53 (13.7%).

In sexually active female subjects, HPV prevalence was 36% (4/11) in those aged ≤16 years, increased to 86% (18/21) in 19–20-year-olds, then declined to 64% (18/28) in those aged ≥23 years (table 2). Assuming that female subjects who were not sexually active were HPV negative, cohort HPV prevalence was 3% in those aged ≤16 years, then showed a similar trend of rapid

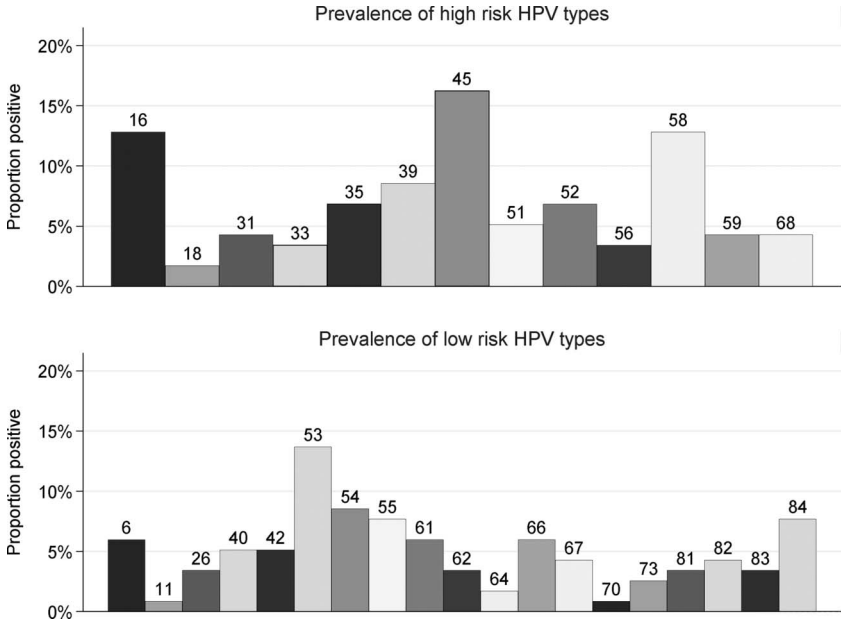

Figure 1 Prevalence of human papillomavirus (HPV) by genotype in 117 sexually active girls at enrolment.

Table 2 Cervical HPV infection at enrolment and associated factors among 117 sexually active subjects

	No with HPV/number sexually active (%)	Unadjusted OR (95% CI)	Age-adjusted OR (95% CI)
Sociodemographic factors			
Age group (years)		p=0.02	p=0.02
≤16	4/11 (36.4)	0.13 (0.03 to 0.60)	0.13 (0.03 to 0.60)
17–18	26/32 (81.2)	1	1
19–20	18/21 (85.7)	1.38 (0.31 to 6.27)	1.38 (0.31 to 6.27)
21–22	20/25 (80.0)	0.92 (0.25 to 3.46)	0.92 (0.25 to 3.46)
23+	18/28 (64.3)	0.42 (0.13 to 1.35)	0.42 (0.13 to 1.35)
Religion		p=0.97	p=0.66
Catholic	36/49 (73.5)	1	1
Other Christian	26/ 36 (72.2)	0.94 (0.36 to 2.47)	0.84 (0.29 to 2.42)
Muslim	24/32 (75.0)	1.08 (0.39 to 3.01)	1.47 (0.49 to 4.45)
Education level		p=0.72	p=0.78
Less than primary	11/14 (78.6)	1	1
Primary	32/46 (69.6)	0.62 (0.15 to 2.59)	0.59 (0.13 to 2.73)
Secondary or above	43/57 (75.4)	0.84 (0.20 to 3.44)	0.64 (0.13 to 3.10)
Marital status		p=0.51	p=0.31
Single	57/76 (75.0)	1	1
Married	27/39 (69.2)	0.75 (0.32 to 1.76)	0.66 (0.20 to 2.16)
Separated/divorced	2/2 (100)	–	–
Number of children ever had		p=0.76	p=0.63
None	45/62 (72.6)	1	1
1	22/31 (71.0)	0.92 (0.36 to 2.40)	0.71 (0.22 to 2.32)
2 or more	19/24 (79.2)	1.44 (0.46 to 4.45)	1.32 (0.32 to 5.41)
Behavioural factors			
Lifetime partners		p=0.82	p=0.70
1	35/47 (74.5)	1	1
2–3	36/48 (75.0)	1.03 (0.41 to 2.60)	0.76 (0.27 to 2.17)
4–5	15/22 (68.2)	0.73 (0.24 to 2.23)	0.59 (0.17 to 2.07)
Age at first sex (years)		p=0.19	p=0.53
≤14	10/17 (58.8)	1	1
15–16	35/48 (72.9)	1.88 (0.59 to 5.99)	1.53 (0.41 to 5.71)
17–18	31/37 (83.8)	3.73 (1.02 to 13.72)	2.66 (0.58 to 12.20)
19+	9/14 (64.3)	1.26 (0.29 to 5.42)	1.18 (0.20 to 6.97)
Time since first sex (years)		p=0.40	p=0.74
≤1 year	19/29 (65.5)	1	1
2–3 years	26/34 (76.5)	1.71 (0.57 to 5.15)	1.29 (0.38 to 4.41)
4–5 years	21/25 (84.0)	2.76 (0.74 to 10.29)	2.23 (0.49 to 10.17)
>5 years	20/29 (69.0)	1.17 (0.39 to 3.51)	1.27 (0.28 to 5.81)
Condom use		p=0.05	p=0.11
Never	53/79 (67.1)	1	1
Sometimes	11/12 (91.7)	5.40 (0.66 to 44.08)	4.69 (0.54 to 40.51)
Often/always	22/26 (84.6)	2.70 (0.84 to 8.64)	2.64 (0.76 to 9.16)
Using hormonal contraception at screening		p=0.08	p=0.17
No	52/76 (68.4)	1	1
Yes	34/41 (82.9)	2.24 (0.87 to 5.78)	2.07 (0.71 to 5.97)
Clinical factors			
Ectopy		p=0.45	p=0.33
None	64/89 (71.9)	1	1
<20%	19/23 (82.6)	1.86 (0.57 to 6.00)	2.17 (0.63 to 7.52)
20%–50%	3/5 (60.0)	0.59 (0.09 to 3.72)	0.53 (0.08 to 3.75)
Age at menarche (years)		p=0.21	p=0.29
≤13	14/24 (58.3)	1	1
14	22/31 (71.0)	1.75 (0.57 to 5.36)	1.38 (0.41 to 4.70)
15	23/29 (79.3)	2.74 (0.82 to 9.19)	2.86 (0.77 to 10.59)
16+	27/33 (81.8)	3.21 (0.97 to 10.68)	2.78 (0.74 to 10.48)
Vaginal flora		p=0.64	p=0.88
Negative	50/65 (76.9)	1	1
Positive (BV)	22/32 (68.8)	0.66 (0.26 to 1.70)	0.83 (0.30 to 2.30)
Intermediate	14/20 (70.0)	0.70 (0.23 to 2.14)	0.75 (0.23 to 2.46)

Continued

Table 2 Continued

	No with HPV/number sexually active (%)	Unadjusted OR (95% CI)	Age-adjusted OR (95% CI)
Chlamydia trachomatis or Neisseria gonorrhoeae		p=0.92	p=0.52
Negative	80/109 (73.4)	1	1
Positive	6/8 (75.0)	1.09 (0.21 to 5.70)	1.80 (0.28 to 11.37)
Trichomonas vaginalis		p=0.22	p=0.76
Negative	77/102 (75.5)	1	1
Positive	9/15 (60.0)	0.49 (0.16 to 1.50)	0.82 (0.22 to 2.97)
Syphilis serology*			
Negative	84/114 (73.7)		
Past infection	0/1 (–)	–	–
Active infection	2/2 (100)	–	–

*Negative defined as negative on both TPPA and RPR. Past infection defined as positive on TPPA and negative on RPR. Active infection defined as positive on both TPPA and RPR.
BV, bacterial vaginosis; HPV, human papillomavirus; RPR, rapid plasma reagin; TPPA, *Treponema pallidum* particle agglutination assay.

increase with age, followed by a gradual decline (see online supplementary figure S1).

Among HPV infected participants, 68.6% (59/86) were infected with >1 genotype.

Of six participants who reported age at sexual debut as their current age, 4 (66.6%) were HPV infected. Among 23 girls who reported age at first sex as 1 year younger than their current age, 15 (65.2%) had HPV infection.

Factors associated with prevalent HPV infection at enrolment

In the unadjusted analysis (table 2), HPV prevalence was higher among participants who reported sometimes or often using condoms than among those who reported never using condoms (p=0.05). There was some evidence that HPV prevalence was higher among participants using hormonal contraception (combined oral contraceptives, depot medroxyprogesterone acetate or implants) at screening (p=0.08). There was no evidence of an association with lifetime partners, age at first sex or marital status, education, religion, parity and other STIs.

In the adjusted analysis, only age remained significantly associated with HPV infection at p<0.10. Compared with participants aged 17–18 years, those aged <17 years had lower odds of HPV infection (adjusted OR 0.19, 95% CI 0.04 to 0.90). The odds of infection were also lower in female subjects aged 23 years and above (adjusted OR 0.55, 95% CI 0.16 to 1.90). There was weak evidence of an association with reported condom use after adjusting for age (p=0.11).

Incidence, persistence and clearance of HPV infection over 12 months

At month 12, 136/308 (44.3%) participants reported being sexually active. In all, 13 reported becoming sexually active during follow-up, of whom 9 (69.2%) were HPV-infected at 12 months, and five had HR genotypes. Overall, HPV prevalence at 12 months was 74.6% (9/122 sexually participants with HPV results; 95% CI 65.9 to 82.0; table 1).

Of 187 genotype-specific infections at enrolment, 51 (27.2%) were present at month 12; persistence was similar for HR and LR genotypes (table 3). HR genotype persistence was 30.4% (7/23) and 27.0% (17/63) in the control and vaccine arms, respectively (p=0.75). LR genotype persistent infections were non-significantly higher in the control (36.4%, 12/33) compared with the vaccine arm (22.1%, 15/68; p=0.13). Overall, 33.9% (19/56) and 24.4% (32/131) of all infections were still present at month 12 in the control and vaccine arms, respectively (p=0.18).

Cumulative incidence of HR genotypes ranged from 1% to 12%, and was highest for HPV-51 (12.9%), HPV-39 (12.1%) and HPV-35 (8.7%) in the vaccine arm, and HPV-51 (9.1%), HPV-16 (4.8%) and HPV-58 (4.8%) in the control arm. LR genotypes cumulative incidence ranged from 2% to 10%, being highest for HPV-66 (8.7%), HPV-67 (8.5%) and HPV-61 (7.4%) in the vaccine arm, and HPV-53 (21.7%), HPV-6 (13.6%) and HPV-61 (9.1%) in the control arm.

In the control arm, there was one new HPV-16 and one new HPV-18 infection. In the vaccine arm, there was one new HPV-18 infection; the subject received two doses of vaccine.

Among HPV uninfected participants at enrolment, the incidence of any HPV infection was 76 (95% CI 46 to 126) per 100 person-years. Among those negative for all HR genotypes, the incidence of HR HPV infection was 51 (95% CI 46 to 126) per 100 person-years. Among those negative for all LR genotypes, the incidence of LR HPV infection was 54 (95% CI 35 to 82) per 100 person-years.

Of 97 participants who had HPV results at both time points, 65 (67.0%) were infected with a new HPV genotype by month 12 (table 1), 32 (33%) had persistent infection with ≥1 genotype and 62 (63.9%) had cleared ≥1 genotype by month 12. Only 11/70 (15.7%) participants who were infected at enrolment had cleared all their infections by month 12.

DISCUSSION

An extremely high prevalence of HPV infection was observed in HIV negative sexually active girls and young women with normal cervical cytology in Tanzania. HPV-45, -16, -58 and -52 were the most prevalent types. The most common types worldwide in women with normal cytology in a large meta-analysis also included HPV-16 and -58 as well as other HPV genotypes that were less common in our study (HPV-18, -52, -31).[4] In women with normal cervical cytology and in cervical cancer cases, HPV-45 was reported to be more common in sub-Saharan Africa and Latin America than other regions.[4 8]

The peak in HPV prevalence in young sexually active girls followed by a decrease in prevalence in older female subjects has been described in other studies[3 9 10] but our study also adds information on acquisition of infection in the years following sexual debut.

In the present study, infection with multiple HPV types was common and observed in over 50% of the sexually active cohort. Younger age (<30 years) has been associated with multiple cervical HPV infections in many studies, including population-based studies in Colombia and a trial cohort in the

Table 3 Cumulative HPV incidence over 1 year, persistence and clearance among 97 women with results available at enrolment and 12 months, by HPV genotype

	Control (N=24 women)				Vaccine (N=73 women)				Total (N=97 women)			
	Infected at visit 1	Persistent*	Cleared*	New†	Infected at visit 1	Persistent*	Cleared*	New†	Infected at visit 1	Persistent*	Cleared*	New†
High risk												
HPV-16	3	–	3 (100%)	1 (4.8%)	11	4 (36.4%)	7 (63.6%)	0 (–)	14	4 (28.6%)	10 (71.4%)	1 (1.2%)
HPV-18	1	–	1 (100%)	1 (4.4%)	0	–	–	1 (1.4%)	1	–	1 (100%)	2 (2.1%)
HPV-31	0	–	–	0 (–)	3	1 (33.3%)	2 (66.7%)	3 (4.3%)	3	1 (33.3%)	2 (66.7%)	3 (3.2%)
HPV-33	1	–	1 (100%)	0 (–)	2	1 (50.0%)	1 (50.0%)	2 (2.8%)	3	1 (33.3%)	2 (66.7%)	2 (2.1%)
HPV-35	1	1 (100%)	–	0 (–)	4	2 (50.0%)	2 (50.0%)	6 (8.7%)	5	3 (60.0%)	2 (40.0%)	6 (6.5%)
HPV-39	1	–	1 (100%)	1 (4.4%)	7	1 (14.3%)	6 (85.7%)	8 (12.1%)	8	1 (12.5%)	7 (87.5%)	9 (10.1%)
HPV-45	4	–	4 (100%)	0 (–)	13	1 (7.7%)	12 (92.3%)	3 (5.0%)	17	1 (5.9%)	16 (94.1%)	3 (3.8%)
HPV-51	2	1 (50.0%)	1 (50.0%)	2 (9.1%)	3	1 (33.3%)	2 (66.7%)	9 (12.9%)	5	2 (40.0%)	3 (60.0%)	11 (12.0%)
HPV-52	1	1 (100%)	–	1 (4.4%)	7	2 (28.6%)	5 (71.4%)	4 (6.1%)	8	3 (37.5%)	5 (62.5%)	5 (5.6%)
HPV-56	2	–	2 (100%)	1 (4.6%)	0	–	–	3 (4.1%)	2	–	2 (100%)	4 (4.2%)
HPV-58	3	1 (33.3%)	2 (66.7%)	1 (4.8%)	9	3 (33.3%)	6 (66.7%)	0 (–)	12	4 (33.3%)	8 (66.7%)	1 (1.2%)
HPV-59	2	1 (50.0%)	1 (50.0%)	0 (–)	2	–	2 (100%)	3 (4.2%)	4	1 (25.0%)	3 (75.0%)	3 (3.2%)
HPV-68	2	2 (100%)	–	1 (4.6%)	2	1 (50.0%)	1 (50.0%)	6 (8.5%)	4	3 (75.0%)	1 (25.0%)	7 (7.5%)
All high risk infections‡	23	7 (30.4%)	16 (69.6%)	9	63	17 (27.0%)	46 (73.0%)	48	86	24 (27.9%)	62 (72.1%)	57
Low risk												
HPV-6	2	–	2 (100%)	3 (13.6%)	4	1 (25.0%)	3 (75.0%)	3 (4.4%)	6	1 (16.7%)	5 (83.3%)	6 (6.6%)
HPV-11	0	–	–	0 (–)	1	–	1 (100%)	4 (5.6%)	1	–	1 (100%)	4 (4.2%)
HPV-26	1	–	1 (100%)	1 (4.4%)	3	–	3 (100%)	2 (2.9%)	4	–	4 (100%)	3 (3.2%)
HPV-40	2	–	2 (100%)	1 (4.6%)	3	–	3 (100%)	2 (2.9%)	5	–	5 (100%)	3 (3.3%)
HPV-42	1	–	1 (100%)	0 (–)	4	1 (25.0%)	3 (75.0%)	3 (4.4%)	5	1 (20.0%)	4 (80.0%)	3 (3.3%)
HPV-53	1	1 (100%)	–	5 (21.7%)	10	2 (20.0%)	8 (80.0%)	4 (6.4%)	11	3 (27.3%)	8 (72.7%)	9 (10.5%)
HPV-54	5	3 (60.0%)	2 (40.0%)	2 (10.5%)	3	1 (33.3%)	2 (66.7%)	4 (5.7%)	8	4 (50.0%)	4 (50.0%)	6 (6.7%)
HPV-55	2	1 (50.0%)	1 (50.0%)	1 (4.6%)	6	1 (16.7%)	5 (83.3%)	2 (3.0%)	8	2 (25.0%)	6 (75.0%)	3 (3.4%)
HPV-61	2	1 (50.0%)	1 (50.0%)	2 (9.1%)	5	2 (40.0%)	3 (60.0%)	5 (7.4%)	7	3 (42.9%)	4 (57.1%)	7 (7.8%)
HPV-62	0	–	–	1 (4.2%)	4	2 (50.0%)	2 (50.0%)	3 (4.4%)	4	2 (50.0%)	2 (50.0%)	4 (4.3%)
HPV-64	0	–	–	0 (–)	1	–	1 (100%)	2 (2.8%)	1	–	1 (100%)	2 (2.1%)
HPV-66	3	2 (66.7%)	1 (33.3%)	1 (4.5%)	4	–	4 (100%)	6 (8.7%)	7	2 (28.6%)	5 (71.4%)	7 (7.8%)
HPV-67	2	–	2 (100%)	1 (4.6%)	2	–	2 (100%)	6 (8.5%)	4	–	4 (100%)	7 (7.5%)
HPV-70	1	–	1 (100%)	1 (4.4%)	0	–	–	1 (1.4%)	1	–	1 (100%)	2 (2.1%)
HPV-73	1	–	1 (100%)	1 (4.4%)	1	–	1 (100%)	1 (1.4%)	2	–	2 (100%)	2 (2.1%)
HPV-81	3	1 (33.3%)	2 (66.7%)	1 (4.8%)	1	–	1 (100%)	3 (4.2%)	4	1 (25.0%)	3 (75.0%)	4 (4.3%)
HPV-82	1	–	1 (100%)	0 (–)	3	1 (33.3%)	2 (66.7%)	2 (2.9%)	4	1 (25.0%)	3 (75.0%)	2 (2.2%)
HPV-83	1	1 (100%)	–	2 (8.7%)	2	1 (50.0%)	1 (50.0%)	4 (5.6%)	3	2 (66.7%)	1 (33.3%)	6 (6.4%)
HPV-84	4	1 (25.0%)	3 (75.0%)	0 (–)	3	–	3 (100%)	5 (7.1%)	7	1 (14.3%)	6 (85.7%)	5 (5.6%)
CP6108	1	1 (100%)	–	1 (4.4%)	8	3 (37.5%)	5 (62.5%)	3 (4.6%)	9	4 (44.4%)	5 (55.6%)	4 (4.6%)
All low risk infections‡	33	12 (36.4%)	21 (63.6%)	24	68	15 (22.1%)	53 (77.9%)	65	101	27 (26.7%)	74 (73.3%)	89

*Among those infected with that serotype at visit 1 (enrolment).
†Cumulative incidence among those uninfected with that serotype at enrolment.
‡Total number of genotype-specific infections among 97 women (24 in control and 73 in vaccine arm).
HPV, human papillomavirus.

UK.[11 12] This may be due to a lack of natural immunity to HPV during the initial years of sexual activity but may also be due to numbers and characteristics of sexual partners.

A number of factors including age, number of sexual partners, age at menarche, hormonal contraceptives, HIV infection and smoking have been associated with HPV infection.[9 11 13] Only age was a significant risk factor for HPV infection in this study. Younger age at sexual debut was not associated with HPV infection although this was associated with HR HPV in population-based studies in Nigeria and Uganda.[14 15] Cigarette smoking, rare in our study population, was associated with HR HPV in the above Ugandan study and has been associated with prevalent and persistent HPV in other countries.[15–17] Although our OR point estimates suggest that there may be an association with

factors such as hormonal contraception, or more frequent condom use, these results should be interpreted with caution as our power to detect a significant association was low, and both variables may be a marker of more frequent sexual intercourse or with higher risk partners. Furthermore, since HPV infection is common, the OR should not be interpreted as a risk ratio; for example, with 68% prevalence in those not using hormonal contraception, an OR of 2 reflects a 19% increase in risk.

Cumulative HPV incidence in sexually active young women is high in developed countries. One US study found a cumulative 36 month incidence of 43% in college students.[18] HPV incidence was also high in our study and infection with new HPV types was acquired in two-thirds of sexually active participants over 1 year. This may be an underestimate of the true incidence since some undetected HPV infections may have been acquired and lost between enrolment and 12 months. A recent study of 380 Ugandan women followed for a median of 18.5 months found an HPV incidence of 30.5/100 person-years with a higher incidence of HR than LR types.[19] Reasons for the high incidence reported in our study are unclear since we have limited data on type and age of sexual partners but our results provide an indication of the high infection pressure for HPV in this setting.

Transmission of HPV appears extremely efficient in the early years of sexual activity in this population, with around two-thirds of girls acquiring HPV infection within the first few years following sexual debut. Although over a quarter of participants experienced persistent HPV infection, a predictor for cervical lesion development,[20] most infections were transitory and 73% of HPV genotype-specific infections were cleared within 12 months. Similar clearance rates have been observed in studies in developed countries.[21] The median duration of infection in young sexually active girls in a US study was 8 months.[18] A Brazilian study found that 12-month clearance was higher for LR HPV than for HR HPV types[22] but this was not seen in our study or in a Colombian study.[23] Our study was not powered to measure the effect of vaccine on HPV incidence or persistence.

A high prevalence of HPV infection in young women does not necessarily translate to a high rate of persistent infection, a prerequisite for development of precancerous and cancerous lesions, since most women should clear their HPV infections. The high infection pressure for HPV in this setting means some persistent infections will develop and are likely to lead to higher rates of cervical cancer than observed in developed countries since there is an absence of adequate screening and treatment programmes. The risk of developing cervical cancer will obviously be increased with HIV infection. Recent data suggest that HPV-16 and -18 are associated with 70% or more of cervical cancer cases in most of the world including sub-Saharan Africa.[13 24–26] Given the absence of widespread screening programmes in East Africa, our data therefore suggest that primary prevention through HPV vaccination before sexual debut is an important public health intervention to control this disease.

The strengths of our study are its prospective design which included young women around the age of sexual debut and our testing for many HPV genotypes. Limitations include that we did not sample HPV in girls who did not report sexual activity, so limiting our scope for analysis of the association of HPV infection with number of sexual partners. In addition, since under-reporting of sexual activity with face-to-face interviews has been well documented among young women in Africa,[27 28] and HPV has been detected in 2% of vaginal samples from virgins in the USA,[29] by not sampling all subjects we could have underestimated the prevalence of HPV infection. This study was not representative of all young women in our setting since HIV positive participants and participants with >6 lifetime sexual partners, who might be at higher risk of HPV infection, were excluded and so our observed prevalence and incidence of HPV is likely to be conservative. Participants were only followed for 12 months, so limiting our ability to detect clearance or persistence of genotype-specific HPV infection. Last, our small sample size gave us limited ability to detect associations of HPV with sexual behaviour and other variables. For risk factors with prevalences of less than 25%, we had less than 70% power to detect even very strong associations (eg, an OR=3) with HPV infection.

In conclusion, we found an extremely high prevalence and incidence of HPV infection in young HIV negative Tanzanian female subjects. The high rates of HPV infection and poor access to cervical screening services have led to Tanzania having one of the highest rates of cervical cancer in the world[1] and therefore it is positive news that Tanzania is planning a national HPV vaccination programme.[30] Since sexual activity was reported in girls aged 14 years and above in this cohort, and because prevalent HPV infection rises quickly after sexual debut,[31] and vaccination is most efficacious in female subjects before they acquire HPV infection, ideally girls <14-years-old should be targeted for vaccination in this population.

Key messages

- Young Tanzanian women in Mwanza have a very high prevalence and incidence of human papillomavirus (HPV) infection, the primary cause of cervical cancer.
- HPV is rapidly acquired after sexual debut.
- HPV vaccination represents an opportunity for primary prevention and should be provided prior to initiation of sexual intercourse.

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Contributors DW-J, RJH and PM conceived the study. DWJ prepared the protocol and had overall responsibility for supervision and conduct of the study. SK supervised the study activities and BG provided clinical supervision. JB coordinated the study and BK supervised the trial teams and data collection. Data analysis was done by KB. Laboratory analysis was supervised by JC and AA in Mwanza and by SdS in Barcelona. DWJ prepared the first draft of the manuscript. All authors commented on and contributed to the final version of the manuscript.

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Provenance and peer review Not commissioned; externally peer reviewed.

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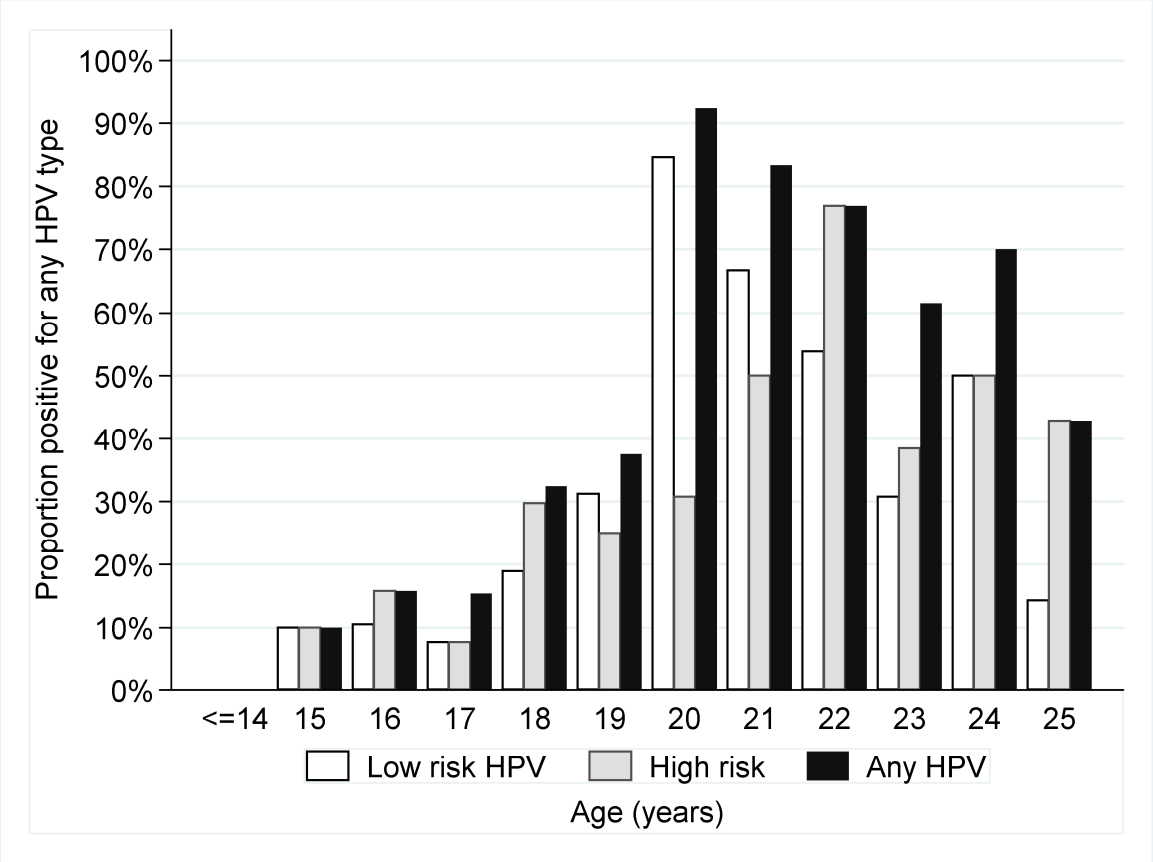
Supplemental Figure 1. Prevalence of HPV infection (any type) by age[1]



[1] Prevalence in all girls, assuming that girls who report being non-sexually active are negative for all serotypes. No girls under age 13 reported being sexually active.

Paper 2. HPV prevalence around the time of sexual debut in adolescent girls in Tanzania

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ORIGINAL RESEARCH

HPV prevalence around the time of sexual debut in adolescent girls in Tanzania

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ABSTRACT

Objectives Cervical cancer is the leading cause of cancer-related mortality among women in sub-Saharan Africa (SSA). Data on human papillomavirus (HPV) epidemiology in adolescent girls in SSA are essential to inform HPV vaccine policy recommendations for cervical cancer prevention. We assessed the burden of HPV infection, and risk factors for infection, among adolescent girls around the time of sexual debut.

Methods Cross-sectional study of secondary school girls aged 17–18 years in Tanzania. Consenting participants provided samples for HPV and STI testing. Vaginal swabs were tested for 37 HPV genotypes by Roche Linear Array. Logistic regression was used to identify factors associated with HPV infection. Y chromosome was tested as a marker of recent condomless sex.

Results 163/385 girls (42.3%) reported previous penetrative sex. HPV was detected in 125/385 (32.5%) girls, including 84/163 (51.5%) girls reporting previous sex and 41/222 (18.5%) reporting no previous sex. High-risk (HR) genotypes were detected in 70/125 (56.0%) girls with HPV infection. The most common HR genotype was HPV-16 (15/385; 3.9%). The prevalence of other HR HPV vaccine genotypes was between 0.8% and 3.1%. Among 186 girls who reported no previous sex, were negative for Y chromosome, and had no STI, 32 (17%) had detectable HPV. *Lactobacillus* sp and bacterial vaginosis-associated bacteria were negatively and positively associated, respectively, with HPV.

Conclusions HPV prevalence among adolescent girls around the time of sexual debut was high. However, prevalence of most vaccine genotypes was low, indicating that extending the age range of HPV vaccination in this region may be cost-effective.

INTRODUCTION

Cervical cancer is the leading cause of cancer mortality among women in sub-Saharan Africa (SSA), and East Africa bears one of the highest burdens, with an age-standardised incidence of 40/100 000 and mortality rate of 30/100 000.[1] Almost all cervical cancers can be attributed to persistent infection with one of 13 high-risk (HR) genotypes of human papillomavirus (HPV).[2] In addition to its oncogenic potential, HPV may also be an important cofactor in HIV acquisition.[3]

Infection with up to 7 hours and two low-risk HPV genotypes can be prevented with HPV vaccination.[4] However, the vaccine offers less protection once an HPV genotype has been acquired. Vaccination is recommended before first sex since the predominant mechanism of HPV acquisition is thought to be through penetrative sex.[5] HPV incidence increases rapidly after first sex and with changes of sexual partner, although most infections are cleared within 12 months.[6 7] Reviews of global age-specific HPV prevalence show the highest prevalence in women aged <25 years.[8] Most women are assumed to be HPV negative before first sex; however, some studies have detected HPV in girls and young women who report no previous penetrative sex.[9 10]

The few published studies on HPV infection in adolescent girls in SSA suggest that HPV prevalence may be very high at a young age. A study in Tanzania found HPV prevalences of 73% in sexually active girls aged 14–18 years, and one in Uganda in girls aged 12–24 found a prevalence of 75%.[11 12] A recent study in Tanzanian girls aged 15–16 years who reported no previous penetrative sex found an HPV prevalence of 8%.[13] The prevalence of non-optimal vaginal microbiota, including bacterial vaginosis (BV), is particularly high in SSA.[14] The vaginal microbiome may modulate susceptibility to HPV infection, as well as other STIs and HIV.[15]

There is an important need for data on HPV epidemiology in adolescent girls in SSA in order to inform HPV vaccine policy recommendations, to help allocate scarce public health resources efficiently and achieve the greatest public health gains, especially as vaccine supplies are currently constrained.[16] Policymakers in SSA may not be able to draw conclusions from other settings because of differences in demographic structure, sexual behaviour, HPV genotype distribution and cofactors such as HIV infection. As part of a cross-sectional study of the vaginal microbiota of girls aged 17–18 years in secondary schools in Tanzania, over half of whom reported no previous sex, we measured the burden of HPV infection and risk factors for infection.

METHODS

Study design

The study design and procedures have been reported previously.[17] Briefly, this was a cross sectional survey in Mwanza, north-western Tanzania. Girls

were enrolled between November 2013 and June 2014 from government-funded secondary schools; selected schools had at least 25 girls in the target age range. Inclusion criteria were being aged 17–18 years, resident in Mwanza and planning to stay in Mwanza for 1 month after enrolment. Exclusion criteria were being outside the age range; being unwilling/unable to provide informed assent/consent (or parent unable/unwilling to provide informed consent, if aged 17); being temporarily in Mwanza, or planned travel within 1 month after enrolment.

Girls were interviewed about sociodemographics, hygiene practices and sexual behaviours. Participants provided five self-administered vaginal swabs in the presence of a nurse who assisted them if needed. Blood and urine samples were collected. Participants were offered HIV voluntary counselling and testing, with referral for care if positive. Laboratory results for treatable STI and free treatment as required were provided to participants within 2 weeks.

Laboratory methods

Laboratory procedures have been described previously.[17] HPV genotyping used the Linear Array HPV Genotyping assay (Roche Molecular Systems, USA), which detects 37 genotypes. DNA was extracted using the AmpliLute Liquid Media Extraction Kit (Roche Molecular Systems), and amplified using the Linear Array HPV Genotyping Test. Generated amplicons were detected using the Linear Array Detection Kit. PCR reaction in this assay is based on a multiplex system, including human β-globin amplification primers, as an internal control for specimen quality. Specimens consistently negative for β-globin amplification were excluded since it was assumed that vaginal sampling was unsuccessful, or the extraction or amplification failed. DNA extraction, amplification and typing were performed in different rooms and included negative processing controls.

Vaginal swabs were tested for *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG) by in-house real-time PCR.[17] Concentrations of *Lactobacillus crispatus*, *L. gasseri*, *L. jensenii*, *L. iners*, *L. vaginalis*, *Gardnerella vaginalis* and *Atopobium vaginae* were measured using quantitative PCR as previously described,[18] with DNA extraction by the QIAamp DNA Mini Kit. Primers and probes were from Eurogentec (Belgium) and PCRs were run on the QIAGEN Rotor Gene. Gram-stained vaginal smears were examined for BV using the Nugent score. *Trichomonas vaginalis* (TV) was diagnosed by culture (InPouch TV, BioMed Diagnostics, USA). Serum samples were tested for herpes simplex virus type 2 (HSV-2) antibodies by ELISA (Kalon Biological, UK). Syphilis was determined by Immutrep Rapid Plasma Reagin (Omega Diagnostics, Scotland) and *Treponema pallidum* particle agglutination assay (SERODIA, Fujirebio, Japan). Blood samples were tested with Determine HIV1/2 rapid test (Alere, Japan), then Uni-Gold HIV (Trinity Biotech, Ireland) if reactive. If both tests were reactive, the result was deemed positive. If tests were discordant, the sample was tested with HIV1/2 Stat-Pak (Chembio, USA), and deemed positive if reactive. Swabs from girls who reported no previous sex were tested for Y chromosome using an in-house real-time PCR, as a marker of recent condomless sex.[19] Y chromosome can be detected for up to 15 days, so may provide a rough measure of reporting bias.

Except for Y chromosome testing, all tests were done at the National Institute for Medical Research (NIMR) laboratory, Mwanza. Quality assurance (QA) and Y chromosome testing were performed by the STI Reference Laboratory at ITM Antwerp.

Statistical methods

Questionnaire data were double-entered into OpenClinica (Akaza Research, USA), and analysed using STATA V.14.0 (StataCorp, USA).

Participant characteristics, and the number of infections of each HPV genotype, were tabulated among girls who reported no previous penetrative sex and those who had passed sexual debut (termed 'sexually active'). Socioeconomic status was measured using a deprivation score, based on household ownership of three items: 1=car (least deprived); 2=television, without car; 3=mobile phone, without car or television; 4=none of the three items (most deprived). We used logistic regression to estimate ORs and 95% CIs for factors associated with any HPV infection among all girls. Potential determinants of HPV infection were examined using a conceptual framework with three levels: sociodemographic, behavioural and biological factors. Age was considered an a priori confounder and included in all models. First, sociodemographic factors whose age-adjusted association with HPV infection was significant at $p<0.10$ were included in a multivariable model; those remaining associated at $p<0.10$ were retained. Behavioural factors were then added to this model one by one. Those that were associated with HPV at $p<0.10$, after adjusting for sociodemographic factors, were retained if they remained significant at $p<0.10$. Associations with biological factors were determined in a similar way. This strategy allowed us to assess the effects of variables at each level of the framework, adjusted for more distal variables. We used a similar approach to examine sexual behaviour factors associated with HPV infection among girls who reported being sexually active.

Ethical considerations

The Institutional Review Board of the Institute of Tropical Medicine in Antwerp (867/13), the Ethics Committee of the University Teaching Hospital in Antwerp (13/14/147), the Lake Zone Institutional Review Board in Mwanza (MR/53/100/86) and the National Ethics Committee of the NIMR Coordinating Committee (NIMR/HQ/R.8a/Vol.IX/1544) approved the study protocol. All participants provided written informed consent/assent; written parental consent was required for participants <18 years.

RESULTS

Characteristics of study participants

We identified 26 eligible secondary schools; 24 participated in the study. A total of 1210 girls aged 17–18 years were registered on the school lists; 802 (66%) were located and their parents invited to a meeting about the study. Four hundred and thirty-nine parents (55%) attended the meeting and 421 (96%) agreed to their daughter's participation. Four hundred and one out of 421 (95%) girls consented/assented and were enrolled (50% of those located; 33% of those on the school lists). Of these, 385 (97%) had HPV results and were included in the analysis.

Overall, 222 (58%) participants reported never having had penetrative sex. Of those who reported previous sex, 61% (99) had passed sexual debut in the past year. Sexually active girls were older than those who reported no previous sex (51% vs 39% aged 18 years, respectively; $p=0.02$); however, there was no evidence of a difference in other sociodemographic characteristics (table 1). Nearly all participants (381; 99%) had passed menarche, at a median age of 14 years (IQR 14–15). Among girls who reported no previous sex, 20 (9%) reported non-penetrative sexual contact with a male partner (eg, kissing, genital touching).

Table 1 Characteristics at enrolment of 385 adolescent girls attending secondary school in Mwanza, Tanzania

	Report no previous penetrative sex (n=222) n (column %)	Report previous penetrative sex (n=163) n (column %)	All girls (n=385) n (column %)
Sociodemographic			
Age (years)			
17	135 (61)	80 (49)	215 (56)
18	87 (39)	83 (51)	170 (44)
Tribe			
Sukuma	99 (45)	70 (43)	169 (44)
Non-Sukuma	123 (55)	93 (57)	216 (56)
Religion			
Catholic	97 (44)	87 (53)	184 (48)
Other Christian	88 (40)	54 (33)	142 (37)
Muslim	32 (14)	20 (12)	52 (14)
Other	5 (2)	2 (1)	7 (2)
Secondary school form			
Form 1	2 (1)	1 (1)	3 (1)
Form 2	47 (21)	21 (13)	68 (18)
Form 3	127 (57)	99 (61)	226 (59)
Form 4	46 (21)	42 (26)	88 (23)
Who lives with			
Mother in household	146 (66)	98 (60)	244 (63)
Father but not mother	12 (5)	12 (7)	24 (6)
Neither mother or father	64 (29)	53 (33)	117 (30)
Deprivation score			
1 (least deprived)	16 (7)	8 (5)	24 (6)
2	88 (40)	76 (47)	164 (43)
3	110 (50)	73 (45)	183 (48)
4 (most deprived)	8 (4)	6 (4)	14 (4)
Behavioural			
Ever drank alcohol			
Yes	6 (3)	5 (3)	11 (3)
Ever kissed with tongues			
Yes	19 (9)	76 (47)	95 (25)
Ever engaged in genital touching*			
Yes	3 (1)	40 (25)	43 (11)
Ever had oral sex†			
Yes	1 (<1)	9 (6)	10 (3)
Ever had anal sex			
Yes	0	2 (1)	2 (1)
Passed menarche			
Yes	218 (98)	163 (100)	381 (99)
Ever cleansed inside vagina			
Yes	16 (7)	42 (26)	58 (15)

*Sexual touching with a man/boy where a girl touched his penis with her hand, he touched her vagina with his hand or he rubbed his penis on her legs/buttocks/genitals but did not have vaginal sex.
†Ever had a man/boy put his penis in a girl's mouth, or he licked/sucked the girl's genitals.

The overall prevalence of any STI was 21% among girls who reported previous sex (TV 9%, CT 5%, NG 2%, MG 4%, HSV-2 3%, HIV 0%), and 7% among girls who reported no previous sex (TV 1%, CT <1%, NG 1%, MG 1%, HSV-2 2%, HIV 1%). BV prevalence among girls who reported previous sex was 33%, and 19% among those reporting no previous sex.

Prevalence of HPV genotypes

Three hundred and eighty-seven out of 401 girls provided vaginal swabs for HPV testing (six were pregnant, eight refused); β-globin was detected in 385/387 specimens. The prevalence of any HPV infection was 32.5% (125/385); 64/125 girls (51.2%) with HPV infection had >1 genotype. The most prevalent HR genotypes were HPV-16 (3.9%), HPV-39 and HPV-52 (both 3.1%), and HPV-58 (2.9%) (figure 1). HPV-18 was detected in three girls (0.8%). Seventeen girls (4.4%) were infected with HPV-16 and/or HPV-18, the HR genotypes targeted by all HPV vaccines. Fifty-three girls (13.8%) were infected with a genotype targeted by the new 9-valent HPV vaccine, Gardasil-9.

HPV prevalence varied by self-reported sexual behaviour (online supplementary table S1; online supplementary figures S1 and S2). HPV was detected in 84/163 (51.5%) sexually active girls and 41/222 (18.5%) girls who reported no penetrative sex. HR HPV was detected in 47 (28.8%) sexually active girls, and 23 (10.4%) who reported no penetrative sex. Among those with HR HPV, 31.9% of sexually active girls and 13.0% of those who reported no penetrative sex were infected with >1 hour genotype. The most common HR genotypes among sexually active girls were HPV-52 (6.7%), HPV-16 (5.5%), HPV-39 and HPV-68 (both 4.9%). Among girls who reported no penetrative sex, the most common HR genotypes were HPV-16 and HPV-58 (both 2.7%).

Among the 222 girls who reported no penetrative sex, 19 (9%) were either positive for Y chromosome (n=7) or had laboratory-confirmed STI other than HPV (n=12). HPV prevalence among the 186 girls who reported no sexual contact nor penetrative sex, and had no evidence of Y chromosome nor any STI, was 17.2% (n=32).

Factors associated with HPV infection

In the unadjusted analysis among all girls, there was some evidence of an association of HPV with increasing deprivation score, and strong evidence of an association with sexual behaviour (penetrative sex, kissing, engaging in genital touching; table 2). There was also evidence of an association with vaginal cleansing and menstrual hygiene. After adjusting for age, deprivation score and penetrative sex, there was still strong evidence of an association with menstrual hygiene (p=0.004), with participants who used cloths instead of commercial pads having the lowest odds of HPV infection, and those who used only underwear having the highest odds of infection. There was also weak evidence of an association with vaginal cleansing (adjusted OR (aOR)=1.70, 95% CI 0.92 to 3.16, p=0.09). After adjusting for age, deprivation score, penetrative sex, menstrual hygiene and vaginal cleansing, there was evidence of an association with gonorrhoea (aOR=5.70, 95% CI 0.91 to 35.6), MG (aOR=6.01, 95% CI 1.08 to 33.6), HIV (aOR=10.4, 95% CI 0.90 to 121.5) and BV (aOR=1.93, 95% CI 1.13 to 3.29).

Among sexually active girls, after adjusting for age and deprivation score, there was evidence of an association of HPV with having >1 lifetime partner (aOR=2.63, 95% CI 1.22 to 5.69), an older first partner and a first partner who had concurrent partners (online supplementary table S2). There was also evidence of an association with time since sexual debut, with HPV infection highest among those whose sexual debut was 1–2 years ago (aOR=2.29, 95% CI 1.01 to 5.21, relative to those with sexual debut <1 year ago).

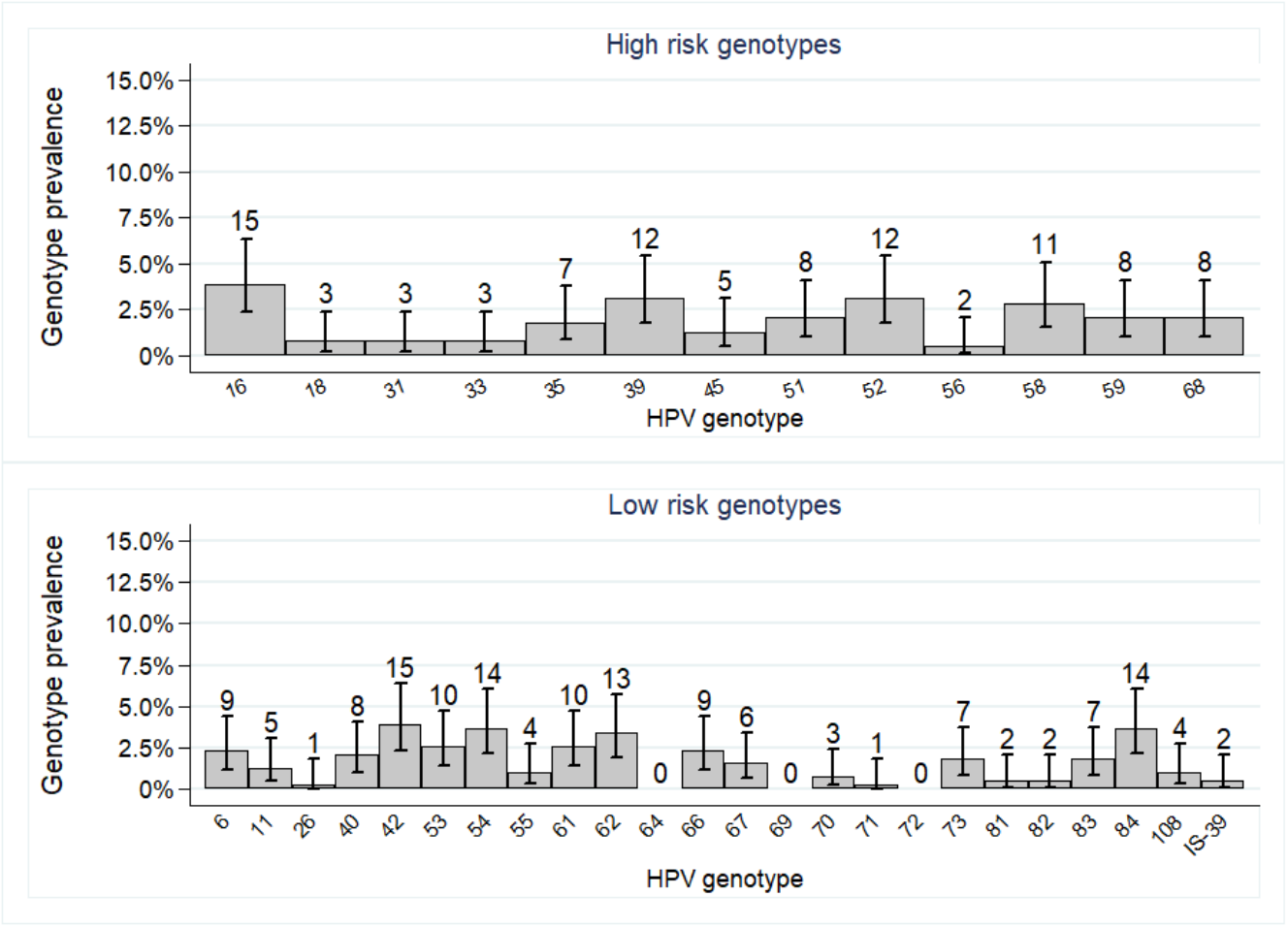

Figure 1 HPV genotype-specific prevalence among 385 girls attending secondary school in Mwanza, Tanzania. Vertical lines indicate 95% CIs and numbers are raw frequencies. HPV, human papillomavirus.

Association with vaginal microbiota

Among all girls, after adjusting for age, deprivation score and penetrative sex, HPV infection was positively associated with *A. vaginae* (aOR=2.19, 95% CI 1.32 to 3.64; p=0.002) and G. *vaginalis* (aOR=1.79, 95% CI 1.05 to 3.04) (table 3). In contrast, HPV infection had an inverse association with several *Lactobacillus* spp, including *L. crispatus* (aOR=0.48, 95% CI 0.29 to 0.80; p=0.005) and *L. jensenii* (aOR=0.44, 95% CI 0.27 to 0.73; p=0.001) and, to a lesser extent, *L vaginalis*. The same trends were seen for the association with high levels (>1 million cells/mL) of each bacterial species.

DISCUSSION

We found a high prevalence of HPV infection among adolescent Tanzanian girls around the time of reported sexual debut. Over half of sexually active participants and one-fifth of those who reported no penetrative sex had HPV infection, and 56% of those infected had an HR genotype. Multiple HPV genotype infections were also very common, with 51% of HPV-infected girls having >1 genotype. These findings are consistent with previous studies of HPV in sexually active adolescents in the USA and in Africa.[11 12 20]

HPV-16 was the most prevalent HR genotype (3.9%). The prevalence of HPV-18 was <1% and of other vaccine genotypes was <3%, except for HPV-52 (targeted by Gardasil-9), which was 3.1%. Similarly, a study in Mozambique found HPV-52, HPV-58 and HPV-16 were the most common HR genotypes in young women.[21] Also, a large meta-analysis of HPV prevalence worldwide found HPV-16 to be the most common genotype among women in SSA, followed by HPV-52.[8]

The Director General of the WHO recently announced a goal to eliminate cervical cancer.[22] Currently, vaccination strategies target girls in an age range considered to be presexual debut, typically 9–14 years. The optimum upper age limit for vaccination will depend on different factors including HPV vaccine genotype prevalence, age of sexual debut, cost per dose of vaccine, availability of cervical cancer screening and HPV transmission dynamics. Screening for cervical cancer is extremely limited in Tanzania. Previous studies of HPV epidemiology in Tanzania have shown one of the highest reported HPV incidences, and a high prevalence of vaccine-related genotypes among young women aged 20–25 years, so the cost-effectiveness of vaccinating girls up to 17 years is likely to be high.[11] Over half the girls in our study reported no previous penetrative sex, and most were not yet infected with vaccine-related genotypes. These findings suggest that a catch-up strategy that goes beyond the multiyear cohort approach of vaccinating 9–14 year-olds,[23] by offering vaccination to girls aged 15–17 years, could help reduce HPV acquisition at a critical time after sexual debut. Furthermore, this would contribute towards the goal of cervical cancer elimination

Table 2 Factors associated with any HPV infection among 385 adolescent girls attending secondary school in Mwanza, Tanzania

	n with HPV/total n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*
Sociodemographic			
Age (years)		P=0.40	
17	66/215 (30.7)	1	
18	59/170 (34.7)	1.20 (0.78 to 1.84)	
Tribe		P=0.85	
Sukuma	54/169 (32.0)	1	
Non-Sukuma	71/216 (32.9)	1.04 (0.68 to 1.60)	
Religion		P=0.60	
Catholic	59/184 (32.1)	1	
Other Christian	51/142 (35.9)	1.19 (0.75 to 1.88)	
Muslim	15/52 (28.8)	0.86 (0.44 to 1.69)	
Other	0/7 (0.0)	–	
Who lives with		P=0.86	
Mother in household	79/244 (32.4)	1	
Father but not mother	9/24 (37.5)	1.25 (0.53 to 2.99)	
Neither mother or father	37/117 (31.6)	0.97 (0.60 to 1.55)	
Deprivation score			
1 (least deprived)	6/24 (25.0)		
2	51/164 (31.1)	P=0.09	
3	59/183 (32.2)	1.32 (0.95 to 1.82)	
4 (most deprived)	9/14 (64.3)		
Behaviour			
Ever drank alcohol		P=0.28	P=0.24
No	123/374 (32.9)	1	1
Yes	2/11 (18.2)	0.45 (0.10 to 2.13)	0.41 (0.08 to 2.05)
Ever kissed with tongues		P=0.006	P=0.82
No	83/290 (28.6)	1	1
Yes	42/95 (44.2)	1.98 (1.23 to 3.19)	0.94 (0.54 to 1.63)
Ever engaged in genital touching†		P=0.003	P=0.64
No	102/342 (29.8)	1	1
Yes	23/43 (53.5)	2.71 (1.42 to 5.14)	1.19 (0.59 to 2.40)
Ever had oral sex‡		P=0.07	P=0.55
No	119/375 (31.7)	1	1
Yes	6/10 (60.0)	3.23 (0.89 to 11.65)	1.51 (0.39 to 5.87)
Ever had vaginal sex		P<0.001	P<0.001
No	41/222 (18.5)	1	1
Yes	84/163 (51.5)	4.69 (2.97 to 7.42)	4.81 (3.02 to 7.66)
Menstrual hygiene§		P=0.002	P=0.004
Pads only (±pants)	68/202 (33.7)	1	1
Cloths only (±pants)	12/71 (16.9)	0.40 (0.20 to 0.80)	0.45 (0.22 to 0.94)
Pants only	15/29 (51.7)	2.11 (0.96 to 4.63)	2.75 (1.17 to 6.46)
Cloth and pads (±pants)	30/79 (38.0)	1.21 (0.70 to 2.07)	1.16 (0.65 to 2.08)
Ever cleansed inside vagina		P=0.001	P=0.09
No	95/327 (29.1)	1	1
Yes	30/58 (51.7)	2.62 (1.48 to 4.62)	1.70 (0.92 to 3.16)
Biological			
HSV-2		P=0.45	P=0.48
Negative	121/376 (32.2)	1	1
Positive	4/9 (44.4)	1.69 (0.44 to 6.39)	1.70 (0.40 to 7.29)
Chlamydia trachomatis		P=0.15	P=0.76
Negative	120/376 (31.9)	1	1
Positive	5/9 (55.6)	2.67 (0.70 to 10.11)	1.25 (0.31 to 5.02)
Neisseria gonorrhoeae		P=0.03	P=0.05
Negative	120/378 (31.7)	1	1
Positive	5/7 (71.4)	5.37 (1.03 to 28.10)	5.70 (0.91 to 35.56)

Continued

Table 2 Continued

	n with HPV/total n (%)	Crude OR (95% CI)	
Trichomonas vaginalis		P=0.02	P=0.79
Negative	115/368 (31.3)	1	1
Positive	10/17 (58.8)	3.14 (1.17 to 8.46)	1.16 (0.39 to 3.45)
Active syphilis (RPR+/TPPA+)			
Negative	125/385 (32.9)	–	–
Positive	0 (–)	–	–
HIV		P=0.24	P=0.06
Negative	123/382 (32.2)	1	1
Positive	2/3 (66.7)	4.21 (0.38 to 46.89)	10.44 (0.90 to 121.5)
Mycoplasma genitalium		P=0.005	P=0.02
Negative	118/376 (31.4)	1	1
Positive	7/9 (77.8)	7.65 (1.57 to 37.39)	6.01 (1.08 to 33.56)
Bacterial vaginosis¶		P=0.002	P=0.05
Normal	71/261 (27.2)	1	1
Intermediate	9/28 (32.1)	1.27 (0.55 to 2.93)	0.94 (0.36 to 2.44)
BV	45/95 (47.4)	2.41 (1.48 to 3.92)	1.93 (1.13 to 3.29)

*Behavioural factors adjusted for age (a priori), deprivation score and vaginal sex. Biological factors adjusted for age, deprivation score, vaginal sex, menstrual hygiene and vaginal cleansing.
†Sexual touching with a man/boy where a girl touched his penis with her hand, he touched her vagina with his hand or he rubbed his penis on her legs/buttocks/genitals but did not have vaginal sex.
‡Ever had a man/boy put his penis in a girl's mouth, or he licked/sucked the girl's genitals.
§Sanitary products used for menstrual hygiene; most girls who reported using pads or cloths also reported wearing pants (underwear). Restricted to girls who have passed menarche (n=381).
¶Missing data for one girl.
BV, bacterial vaginosis; HPV, human papillomavirus; HSV-2, herpes simplex virus type 2; RPR, Rapid Plasma Reagin; TPPA, Treponema pallidum particle agglutination.

by decreasing the proportion of females in the population who would otherwise acquire persistent HPV infection, a necessary prerequisite for cervical cancer.

The Tanzanian national programme is currently vaccinating with Gardasil, which covers 2/13 (15%) circulating HR genotypes in our study population, but only one of the more common ones (HPV-16). In contrast, Gardasil-9 would cover 7/13 (54%) circulating HR genotypes, including the three most common ones. Although Gardasil and Cervarix offer some cross-protection for other genotypes (Gardasil against HPV-31, and Cervarix against HPV-31, HPV-33 and HPV-45), these cross-protected genotypes were less common in our population. Therefore, Gardasil-9 may offer the best coverage given the distribution of HR genotypes in our setting.

HPV transmission appears extremely efficient in the early years of sexual activity in our setting, with >60% of girls whose sexual debut was 1–2 years ago being infected with HPV. HPV prevalence among girls who report no penetrative sex was also high (18.5%), and substantially higher than in studies in industrialised countries among women who reported no previous sex. A cross-sectional study in Sweden in women aged 10–25 years who reported no previous sex found a prevalence of 1.5%.[10] A longitudinal study in USA found HPV in only 1.7% of samples from women aged 18–20 who never had sex.[9] Our prevalence is also higher than in a longitudinal study in Tanzanian girls aged 15–16 years who reported no penetrative sex over 18 months, which found HPV in only 11.6% of samples.[24] Lack of disclosure is likely to be part of the explanation for the high HPV prevalence in girls in our study who denied previous sexual activity. This is supported by our finding that 9% of girls who reported no previous sex were positive for Y chromosome and/or an STI (excluding HPV). In Tanzania, girls who are still in school may be particularly reluctant to disclose sexual activity, since potential consequences include expulsion, physical punishment or social exclusion.[25]

Alternative explanations for HPV infection in girls who report no previous sex include mother-to-child transmission, non-penetrative sexual contact or transmission via fomites.[26 27] A recent study in Mwanza showed a high prevalence of HPV DNA in oral washes and fingertip samples from adolescent girls, and on surfaces in their bathrooms.[28] Nevertheless, even with potential reporting errors, our findings of a very high HPV prevalence in girls who report no penetrative sex are important.

After adjusting for potential confounders, we found a strong inverse association between HPV and D-lactic-acid and H_2O_2-producing *Lactobacillus* spp, including *L. crispatus* and *L. jensenii*, key constituents of optimal vaginal microbiota. Furthermore, there was a strong positive association between HPV infection and anaerobic bacterial species *G. vaginalis* and *A. vaginae*. These species are characteristic of BV, which has been associated with increased susceptibility to STI and HIV.[29] A recent meta-analysis found that disturbance in the vaginal microbiota away from a *Lactobacillus*-dominated environment was associated with increased risk of HPV acquisition and persistence, and related cervical disease.[30]

Strengths of our study include detailed interviews given by trained nurses experienced in adolescent sexual behaviour research. Collection of vaginal swabs, although self-administered, was observed by nurses; all but two specimens contained β-globin, indicating successful sampling and specimen processing. The Roche Linear Array used for HPV genotyping has a high sensitivity and specificity; all laboratory assays were conducted according to standard operating procedures with external QA at an internationally recognised reference laboratory.

Table 3 Association of bacterial species with any HPV infection among 385 adolescent girls attending secondary school in Mwanza, Tanzania

	n with HPV/total n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*	Adjusted OR (95% CI)†
Presence/absence				
Atopobium vaginae‡		P<0.001	P<0.001	P=0.002
Absent	49/220 (22.3)	1	1	1
Present	63/139 (45.3)	2.89 (1.82 to 4.59)	2.30 (1.40 to 3.76)	2.19 (1.32 to 3.64)
Gardnerella vaginalis§		P<0.001	P=0.02	P=0.03
Absent	31/145 (21.4)	1	1	1
Present	87/209 (41.6)	2.62 (1.62 to 4.25)	1.87 (1.12 to 3.14)	1.79 (1.05 to 3.04)
Lactobacillus vaginalis¶		P<0.001	P=0.03	P=0.04
Absent	59/131 (45.0)	1	1	1
Present	64/242 (26.4)	0.44 (0.28 to 0.69)	0.58 (0.36 to 0.93)	0.60 (0.37 to 0.98)
*L. crispatus***		P<0.001	P=0.005	P=0.005
Absent	55/120 (45.8)	1	1	1
Present	60/238 (25.2)	0.40 (0.25 to 0.63)	0.49 (0.30 to 0.81)	0.48 (0.29 to 0.80)
L. gasseri††		P=0.91	P=0.86	P=0.76
Absent	98/300 (32.7)	1	1	1
Present	24/75 (32.0)	0.97 (0.56 to 1.67)	1.06 (0.59 to 1.89)	1.10 (0.61 to 1.99)
L. iners		P=0.08	P=0.20	P=0.37
Absent	16/68 (23.5)	1	1	1
Present	106/311 (34.1)	1.68 (0.92 to 3.08)	1.51 (0.79 to 2.89)	1.35 (0.69 to 2.63)
L. jensenii¶		P<0.001	P<0.001	P=0.001
Absent	83/198 (41.9)	1	1	1
Present	37/175 (21.1)	0.37 (0.23 to 0.59)	0.44 (0.27 to 0.71)	0.44 (0.27 to 0.73)
>1 000 000/mL				
A. vaginae‡		P<0.001	P<0.001	P=0.001
No	55/239 (23.0)	1	1	1
Yes	57/120 (47.5)	3.03 (1.90 to 4.83)	2.37 (1.44 to 3.92)	2.33 (1.39 to 3.91)
G. vaginalis§		P<0.001	P=0.02	P=0.02
No	47/195 (24.1)	1	1	1
Yes	71/159 (44.7)	2.54 (1.61 to 4.00)	1.83 (1.12 to 2.98)	1.83 (1.10 to 3.03)
L. vaginalis¶		P=0.57	P=0.75	P=0.68
No	91/269 (33.8)	1	1	1
Yes	32/104 (30.8)	0.87 (0.53 to 1.41)	0.92 (0.54 to 1.55)	0.89 (0.52 to 1.53)
*L. crispatus***		P<0.001	P=0.009	P=0.01
No	59/132 (44.7)	1	1	1
Yes	56/226 (24.8)	0.41 (0.26 to 0.64)	0.52 (0.32 to 0.85)	0.52 (0.32 to 0.87)
L. gasseri††		P=0.57	P=0.41	P=0.34
No	106/331 (32.0)	1	1	1
Yes	16/44 (36.4)	1.21 (0.63 to 2.34)	1.35 (0.67 to 2.73)	1.42 (0.69 to 2.91)
L. iners‡‡		P=0.05	P=0.24	P=0.52
No	20/85 (23.5)	1	1	1
Yes	102/294 (34.7)	1.73 (0.99 to 3.01)	1.43 (0.79 to 2.59)	1.22 (0.66 to 2.25)
L. jensenii¶		P<0.001	P<0.001	P<0.001
No	94/238 (39.5)	1	1	1
Yes	26/135 (19.3)	0.37 (0.22 to 0.60)	0.40 (0.24 to 0.68)	0.38 (0.22 to 0.65)

*Adjusted for age (a priori), deprivation score and vaginal sex.
†Adjusted for age (a priori), deprivation score, vaginal sex, menstrual hygiene and vaginal cleansing.
‡Missing data for 26 girls.
§Missing data for 31 girls.
¶Missing data for 12 girls.
**Missing data for 27 girls.
††Missing data for 10 girls.
‡‡Missing data for six girls.
HPV, human papillomavirus.

Limitations include the cross-sectional design, which makes it difficult to assess causality or to measure past HPV infection, since individual genotype-specific infections may be rapidly cleared.[7] Face-to-face interviews may have increased social desirability bias in responses; the inclusion of parents and recruitment from schools may have compounded this issue. We enrolled girls who were still in school; many girls in this age range in Tanzania are no longer in school. Unpublished demographic and health

survey (DHS) data from the Mwanza region in 2017 showed 41% of girls aged 17–18 years were still in school. Furthermore, only 33% of 1210 girls on the school lists were enrolled, mostly because they were not found at the school or their parents could not be located, which suggests possible selection bias. National DHS data show that young women with secondary education have an older age at first sex, and later age at first birth.[31] Therefore, we may have underestimated HPV prevalence among all women in this age group. However, our findings of the distribution of HPV genotypes, and factors associated with HPV, are consistent with other studies in SSA, and may be more broadly generalisable.

In conclusion, we found a high prevalence of HPV infection, and HR genotypes, among adolescent girls in the early years after becoming sexually active, and among girls who reported no penetrative sex. HPV vaccination in Tanzania is currently offered to 14-year-old girls through a national vaccination programme. The prevalence of most vaccine-related genotypes was low, indicating that extending the age range of HPV vaccination through a catch-up campaign in this region, with one of the highest rates of cervical cancer worldwide and limited facilities for screening, may be cost-effective.

Key messages

- Human papillomavirus (HPV) infection among adolescent girls attending secondary school in Tanzania was high (32.5%), and high-risk (HR) oncogenic genotypes were detected in over half the girls with HPV infection.
- HPV infection was inversely associated with *Lactobacillus* spp, key constituents of optimal vaginal microbiota.
- The Tanzanian national programme is vaccinating with Gardasil, which protects against 2/13 (15%) HR genotypes circulating in our study population, including the most prevalent one (HPV-16).
- Extending the age range of vaccination in this region, where cervical cancer screening is extremely limited, may be cost-effective.

Handling editor Nigel Field

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Contributors Conception or design of the work: DWJ, AB, JC, TC, SN, KJB, SF, AA, RJH. Data collection: JI, SN, AA. Data analysis and interpretation: KJB, CHH, RJH, AB, DWJ. Drafting the article: KJB, DWJ. Critical revision of the article: KJB, AA, SF, TC, AB, DWJ. Final approval of the version to be published: DWJ, AB, RJH, CHH, SF, TC, JC, SN, JI, AA, KJB.

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Supplementary Table S1. HPV genotype prevalence among 385 adolescent girls, by whether or not report having passed sexual debut

	Report not having passed sexual debut (N=222) n (column %)	Report having passed sexual debut (N=163) n (column %)	All girls (N=385) n (column %)
High risk (HR) genotypes			
HPV 16	6 (2.7%)	9 (5.5%)	15 (3.9%)
HPV 18	0 (0.0%)	3 (1.8%)	3 (0.8%)
HPV 31	1 (0.5%)	2 (1.2%)	3 (0.8%)
HPV 33	0 (0.0%)	3 (1.8%)	3 (0.8%)
HPV 35	3 (1.4%)	4 (2.5%)	7 (1.8%)
HPV 39	4 (1.8%)	8 (4.9%)	12 (3.1%)
HPV 45	3 (1.4%)	2 (1.2%)	5 (1.3%)
HPV 51	2 (0.9%)	6 (3.7%)	8 (2.1%)
HPV 52	1 (0.5%)	11 (6.7%)	12 (3.1%)
HPV 56	0 (0.0%)	2 (1.2%)	2 (0.5%)
HPV 58	6 (2.7%)	5 (3.1%)	11 (2.9%)
HPV 59	1 (0.5%)	7 (4.3%)	8 (2.1%)
HPV 68	0 (0.0%)	8 (4.9%)	8 (2.1%)
Any HR infection	23 (10.4%)	47 (28.8%)	70 (18.2%)
>1 HR genotype[1]	3 (13.0%)	15 (31.9%)	18 (25.7%)
All HR infections[2]	***27***	***70***	***97***
Low risk (LR) genotypes			
HPV 6	1 (0.5%)	8 (4.9%)	9 (2.3%)
HPV 11	2 (0.9%)	3 (1.8%)	5 (1.3%)
HPV 26	0 (0.0%)	1 (0.6%)	1 (0.3%)
HPV 40	1 (0.5%)	7 (4.3%)	8 (2.1%)
HPV 42	3 (1.4%)	12 (7.4%)	15 (3.9%)
HPV 53	2 (0.9%)	8 (4.9%)	10 (2.6%)
HPV 54	8 (3.6%)	6 (3.7%)	14 (3.6%)
HPV 55	1 (0.5%)	3 (1.8%)	4 (1.0%)
HPV 61	3 (1.4%)	7 (4.3%)	10 (2.6%)
HPV 62	1 (0.5%)	12 (7.4%)	13 (3.4%)
HPV 64	0 (0.0%)	0 (0.0%)	0 (0.0%)
HPV 66	1 (0.5%)	8 (4.9%)	9 (2.3%)
HPV 67	2 (0.9%)	4 (2.5%)	6 (1.6%)
HPV 69	0 (0.0%)	0 (0.0%)	0 (0.0%)
HPV 70	0 (0.0%)	3 (1.8%)	3 (0.8%)
HPV 71	0 (0.0%)	1 (0.6%)	1 (0.3%)
HPV 72	0 (0.0%)	0 (0.0%)	0 (0.0%)
HPV 73	4 (1.8%)	3 (1.8%)	7 (1.8%)
HPV 81	0 (0.0%)	2 (1.2%)	2 (0.5%)
HPV 82	0 (0.0%)	2 (1.2%)	2 (0.5%)
HPV 83	1 (0.5%)	6 (3.7%)	7 (1.8%)
HPV 84	1 (0.5%)	13 (8.0%)	14 (3.6%)
HPV 108	1 (0.5%)	3 (1.8%)	4 (1.0%)
HPV is39	1 (0.5%)	1 (0.6%)	2 (0.5%)
Any LR infection	26 (11.7%)	70 (42.9%)	96 (24.9%)
>1 LR genotype[1]	5 (19.2%)	29 (41.4%)	34 (35.4%)
All LR infections[2]	***33***	***113***	***146***

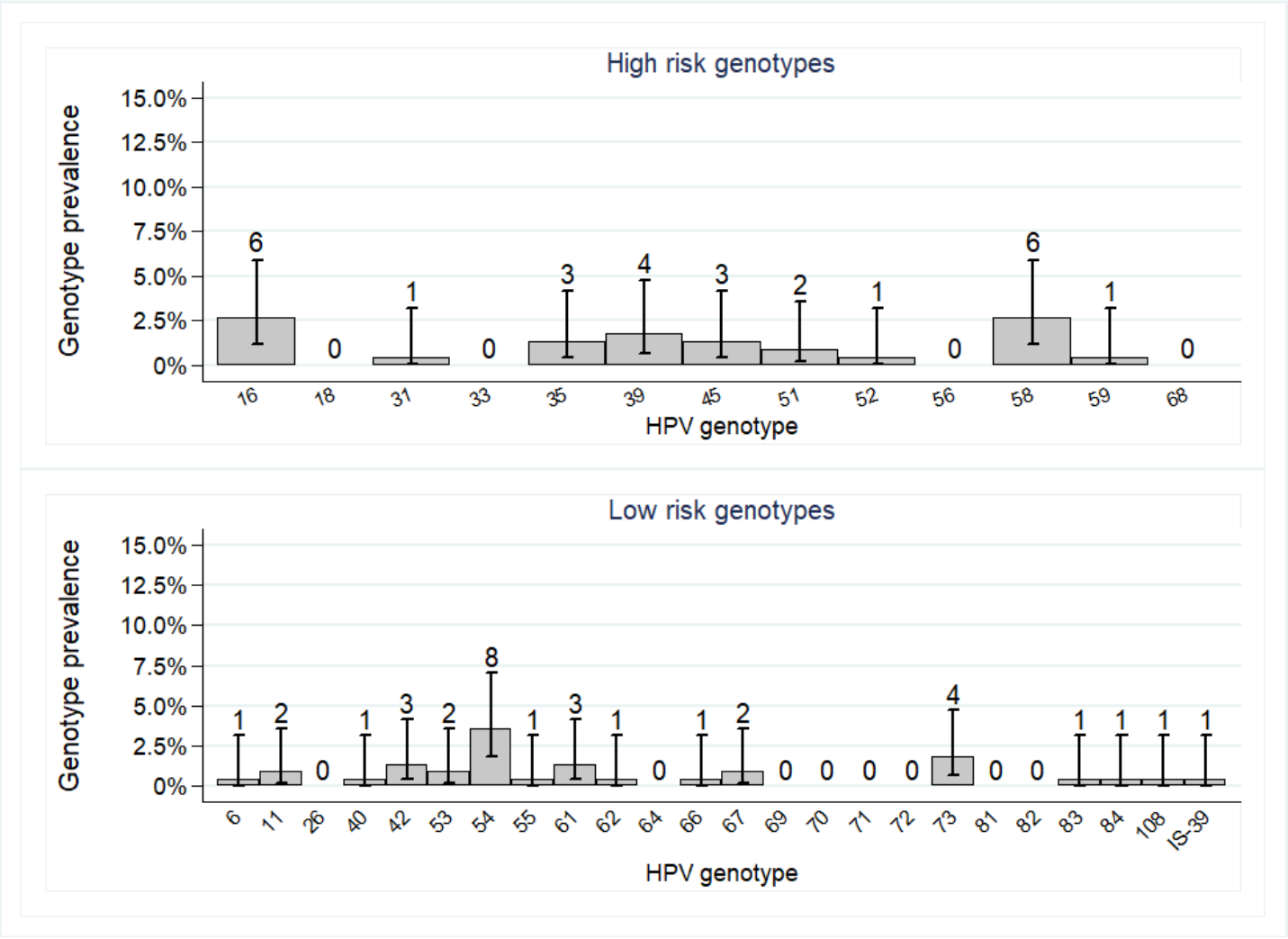
Any HPV infection	41 (18.5%)	84 (51.5%)	125 (32.5%)
>1 HPV infection[1]	13 (31.7%)	51 (60.7%)	64 (51.2%)
All HPV infections[2]	***60***	***183***	***243***

[1]Denominator is girls with group-specific (HR or LR) HPV infection, and any HPV infection. [2]Total number of group-specific (HR or LR), and total number of HPV infections, among 385 girls.

Supplementary Table S2. Behavioural factors associated with any HPV infection among adolescent girls attending secondary school in Mwanza, Tanzania, who report having passed sexual debut

	N with HPV / total N (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[1]
Lifetime partners		P=0.007	P=0.01
1	56 / 123 (45.5%)	1	1
2+	28 / 40 (70.0%)	2.79 (1.30-5.99)	2.63 (1.22-5.69)
Timing of sexual debut		P=0.15	P=0.13
<1 year ago	23 / 54 (42.6%)	1	1
1 year – < 2years ago	28 / 45 (62.2%)	2.22 (0.99-4.98)	2.29 (1.01-5.21)
≥2 years ago	32 / 63 (50.8%)	1.39 (0.67-2.89)	1.44 (0.69-3.03)
Description of first sex		P=0.78	P=0.80
No or little coercion	60 / 118 (50.8%)	1	1
Coercion or force	24 / 45 (53.3%)	1.10 (0.56-2.20)	1.09 (0.54-2.19)
Age difference with first partner		P=0.07	P=0.04
Same age (+/-1 yr)	17 / 47 (36.2%)	1	1
2-4 yrs older	37 / 67 (55.2%)	2.18 (1.01-4.68)	2.68 (1.20-6.01)
5+ years older	16 / 27 (59.3%)	2.57 (0.97-6.78)	2.39 (0.88-6.50)
Don't know[2]	14 / 21 (66.7%)	–	–
Condom use with first partner		P=0.41	P=0.36
Always/usually	33 / 69 (47.8%)	1	1
Rarely/never	49 / 90 (54.4%)	1.30 (0.70-2.44)	1.35 (0.71-2.57)
Can't remember[2]	2 / 3 (66.7%)	–	–
First partner has concurrent partners		P=0.02	P=0.03
No	25 / 61 (41.0%)	1	1
Yes	21 / 32 (65.6%)	2.75 (1.13-6.70)	2.68 (1.09-6.59)
Don't know[2]	38 / 69 (55.1%)	–	–
Ever had sex for gifts?		P=0.90	P=0.73
No	77 / 149 (51.7%)	1	1
Yes	7 / 14 (50.0%)	0.94 (0.31-2.80)	0.82 (0.27-2.50)

[1]Adjusted for age (a priori) and deprivation score. [2]'Don't know' responses considered missing data and not included in analysis.


High risk genotypes
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Low risk genotypes
Genotype prevalence
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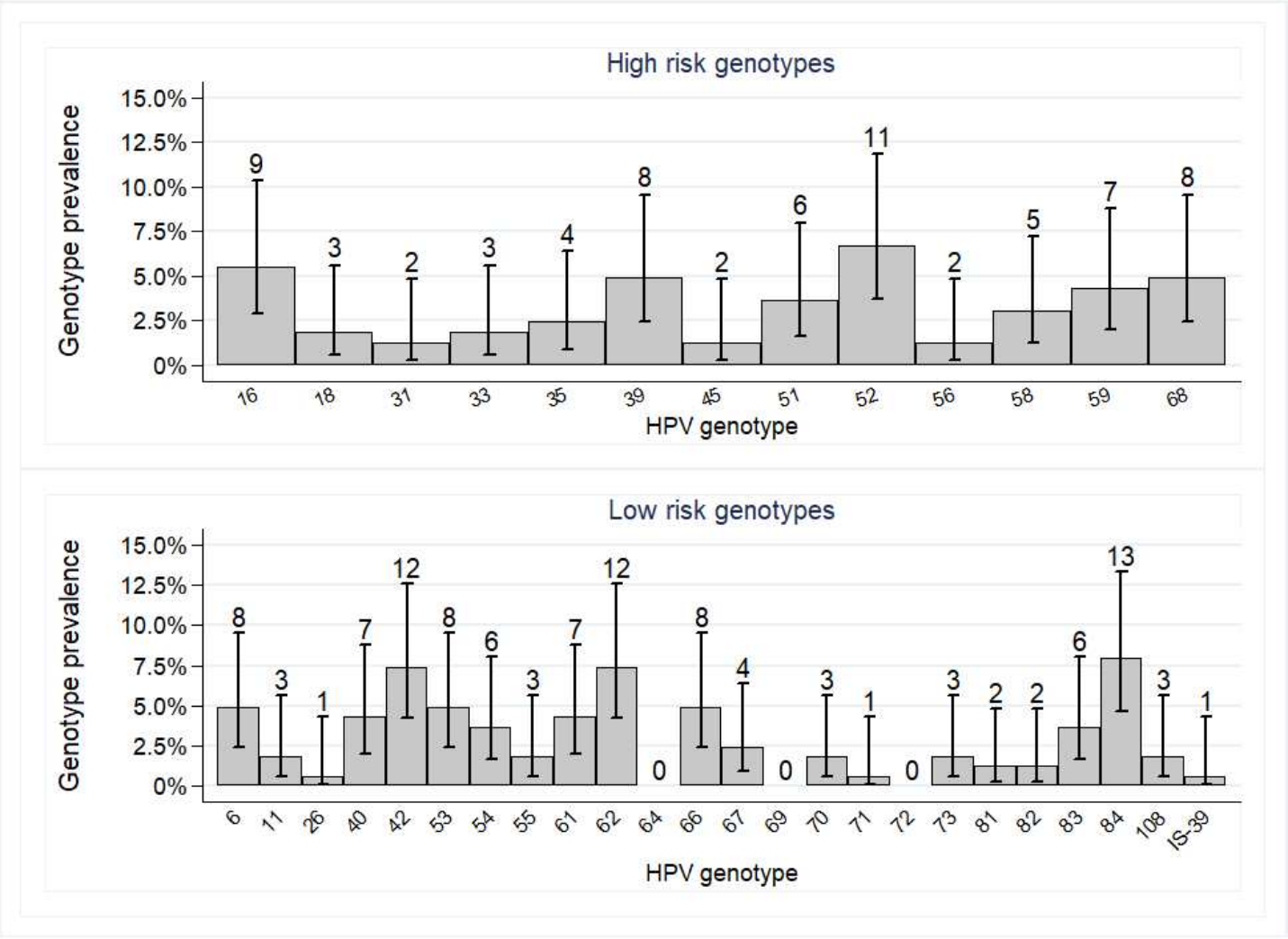
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Paper 3. Impact of malaria and helminth infections on immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in Tanzania

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Primary Supervisor	Deborah Watson-Jones		

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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)	This paper presents results from a sub-study nested in the first trial of HPV vaccine in Africa (as described for the first paper). The trial was conducted in Tanzania and Senegal, and there were a number of supplementary studies as part of the trial in Tanzania. I was a co-investigator on the supplementary studies, and contributed to the conception of the idea and the development of the study design, protocol and questionnaires. I was also the study statistician, had overall responsibility for the study data management, wrote the statistical analysis plan, analysed the data and interpreted the results. For this paper, in addition to the analyses, I contributed to the drafting of the manuscript and to the reponse to the reviewers during the peer-review process.

SECTION E

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Impact of malaria and helminth infections on immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in Tanzania

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Impact of malaria and helminth infections on immunogenicity of the human papillomavirus-16/18 AS04-adjuvanted vaccine in Tanzania ☆☆

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ABSTRACT

Background:

Endemic malaria and helminth infections in sub-Saharan Africa can act as immunological modulators and impact responses to standard immunizations. We conducted a cohort study to measure the influence of malaria and helminth infections on the immunogenicity of the bivalent HPV-16/18 vaccine.
Methods:

We evaluated the association between malaria and helminth infections, and HPV-16/18 antibody responses among 298 Tanzanian females aged 10–25 years enrolled in a randomized controlled trial of the HPV-16/18 vaccine. Malaria parasitaemia was diagnosed by examination of blood smears, and helminth infections were diagnosed by examination of urine and stool samples, respectively. Geometric mean antibody titres (GMT) against HPV-16/18 antibodies were measured by enzyme-linked immunosorbent assay.
Results:

Parasitic infections were common; one-third (30.4%) of participants had a helminth infection and 10.2% had malaria parasitaemia. Overall, the vaccine induced high HPV-16/18 GMTs, and there was no evidence of a reduction in HPV-16 or HPV-18 GMT at Month 7 or Month 12 follow-up visits among participants with helminths or malaria. There was some evidence that participants with malaria had increased GMTs compared to those without malaria.
Conclusions:

The data show high HPV immunogenicity regardless of the presence of malaria and helminth infections. The mechanism and significance for the increase in GMT in those with malaria is unknown.

☆☆ This study was registered under ControlledTrials.com (ISRCTN90378590).

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1. Introduction

Human papillomavirus (HPV) genotypes 16 and 18 are estimated to cause 70% of cervical cancers worldwide [1]. Over 85% of the global burden of cervical cancer occurs in developing countries and Tanzania reports one of highest rates of cervical cancer in Africa [2]. Potent, durable HPV vaccine efficacy will be essential if the vaccine is introduced for the control of cervical cancer. Endemic infections in sub-Saharan Africa, such as malaria and helminth infections, act as immunological modulators, and have been found to adversely impact immune response to standard immunizations, such as antituberculosis vaccine bacillus Calmette–Guerin (BCG), typhoid fever, tetanus and polio vaccines [3–9]. Studies to evaluate the effect of HPV vaccines in populations whose immunological system may be challenged by multiple co-infections such as malaria and helminth infections are needed [10,11]. We conducted a study to measure the influence of malaria parasitaemia and helminth infection on the immunogenicity of HPV-16/18 vaccine (GlaxoSmithKline (GSK) Biologicals SA). This study was nested within a cohort recruited for a Phase IIIb immunogenicity and safety trial of the HPV-16/18 vaccine (the HPV 021 trial) conducted in Tanzania and Senegal among HIV-negative girls and young women aged 10–25 years [12].

2. Methods

The HPV 021 trial (NCT00481767) and the malaria/helminth study were conducted from October 2007 to July 2010 in Mwanza, Tanzania, one of the two participating HPV-021 trial centres. GSK Biologicals was the funding source for the studies. Both studies were approved by the ethics committees of the National Institute for Medical Research (NIMR), Tanzania and the London School of Hygiene & Tropical Medicine (LSHTM), United Kingdom. The helminth/malaria study was registered under ControlledTrials.com (ISRCTN90378590).

The HPV 021 trial was a double-blind, randomized, placebo-controlled phase IIIb trial. Eligible participants were randomly assigned (2:1) to receive either three doses of HPV-16/18 AS04-adjuvanted vaccine (vaccine group) or Al(OH)3 (placebo group) at 0,1 and 6 months. After enrolment (Month 0), participants returned to the clinic at Months 1, 2, 4, 6, 7, 8, 10 and 12 for follow-up visit procedures. Participants were tested for malaria and helminth infections at the Month 7 visit, one month after the scheduled vaccine dose 3. In addition, participants could attend government health services for investigation and management of any illnesses between booked study visits. A record was kept of investigations and treatments given through these other health services.

The primary objective of this analysis was to evaluate the association of malaria parasitaemia and helminth infection with antibody responses against HPV-16 and HPV-18 one month (Month 7) and six months (Month 12) after the last scheduled vaccine dose in African females aged 10–25 years.

Potential participants were recruited from schools, colleges and family planning clinics in Mwanza, and invited to attend a screening visit for eligibility approximately one month prior to enrolment. Prior to screening, informed consent was obtained from participants aged 18–25 years. For participants aged 10–17 years, we sought consent from a parent or legally authorized representative, as well as assent from the participant. Participants were eligible for enrolment if they were aged 10–25 years at the time of first vaccination, HIV negative, not pregnant, had not had more than six lifetime sexual partners, were free of obvious health problems as established by medical history and examination, had no history of neurologic disorders and were willing to use contraception or to abstain from sex if sexually active for 30 days prior to vaccination and for two months after completion of vaccination. The enrolment was age-stratified, with one-third of participants in the 10–14 years age-stratum and the remainder in the 15–25 years age-stratum.

Study procedures for the HPV 021 trial have been described in detail elsewhere [12]. In brief, the HPV vaccine and placebo were administered intramuscularly into the deltoid muscle of the non-dominant arm at the Month 0 visit and again at Month 1 and Month 6 visits. Sociodemographic characteristics were collected at Month 0 in face-to-face interviews using standardized questionnaires. Blood samples were collected at Months 0, 2, 7 and 12 to evaluate antibody responses against HPV-16 and HPV-18 by enzyme-linked immunosorbent assay (ELISA). In order to test for helminth infection and malaria parasitaemia at Month 7, participants provided (i) a blood sample for the diagnosis of malaria, (ii) a first void urine sample for the diagnosis of *Schistosoma haematobium* and (iii) three separate stool samples (during the week following the Month 7 visit) for the diagnosis of *Schistosoma mansoni, Ancylostoma duodenale* (hookworm), *Strongyloides stercoralis, Ascaris lumbricoides, Trichuris trichiura and Taenia* spp. Participants who tested positive for malaria or helminth infections were provided with treatment by study clinicians at a subsequent study visit.

2.1. Laboratory testing

2.1.1. Malaria

Pairs of thick and thin peripheral blood films from each patient were stained with Giemsa stain in Mwanza, and examined by light microscopy at NIMR in Mwanza, and confirmed at LSHTM. Each thick film was scanned under oil immersion for at least 5 min and the presence of asexual malaria parasites or sexual gametocytes was recorded. Where parasites were seen, the number per 200 white blood cells (WBC) on the thick film was counted and multiplied by 40 to give number of parasites per microliter (parasite density, assuming 8000 WBC per μL as per World Health Organization recommendations for Africa) [13]. In thin films, parasite detection (where possible) and species confirmation was done by scanning for a similar duration.

2.1.2. Helminths

A 10 mL aliquot from each urine sample was filtered through 25 mm, 12 μm Millipore filters on Swinnex filter holders. After filtration, the filter was placed onto a glass slide using blunt forceps adding a drop of saline and a glass coverslip. The filter was then examined at the NIMR laboratory under light microscopy for the eggs of *S. haematobium.*

Stool samples were examined at the NIMR laboratory for quantitative egg counts for *S. mansoni*, hookworm, *S stercoralis, A. lumbricoides, T. trichiura* and *Taenia* spp. using the Kato-Katz method [14,15]. The stool samples were first homogenised by passing through a sieve, and then a 41.7 mg template was used. The faecal portion was covered with a cellophane square that had been soaked in malachite green and glycerol. The sample was examined immediately and then again after 24 h. Eggs were counted and expressed as eggs per gram of faeces. For quality control, a random sample of 10% of positive and negative stool slides were sent to the Uganda Virus Research Institute/Medical Research Council laboratories in Entebbe for repeat Kato-Katz testing.

In addition, charcoal culture was used to confirm *S. stercoralis* in a subset of samples. Approximately 50 mg of unfixed fresh faeces were mixed with distilled water in a 20 mL universal tube [16]. To this suspension an equal volume of granulated hardwood charcoal was added. After mixing, the suspension was placed over a wet disc of filter paper in a petri dish and stored in the dark at room temperature. The petri dishes were observed daily for the presence

of larvae for a week under a dissection microscope, adding water to the filter paper as needed.

2.1.3. HPV immunogenicity

As part of the HPV 021 trial, serological assays for immunogenicity were performed at a GSK laboratory in Belgium. ELISA was used to determine antibodies to HPV-16 and HPV-18 as described previously [17]. As there are no established immunological correlates of protection for HPV-16 or HPV-18, immunogenicity was determined in terms of seroconversion rates and geometric mean antibody titres (GMTs). Seropositivity was defined as an antibody titre greater than or equal to the assay threshold of 8 ELISA units (EU)/mL for HPV-16 and 7 EU/mL for HPV-18 [17].

2.2. Analyses

Data were double entered and verified in DMSys® (SigmaSoft International) and analysed using STATA11.0 (StataCorp LP; College Station, Texas, USA). Sociodemographic characteristics of participants attending the Month 7 visit were tabulated by infection status and overall. The prevalence of malaria parasitaemia and each helminth infection at Month 7 was tabulated by age group and overall. Helminth infection intensities were classified into light, moderate and heavy, according to WHO guidelines [18]. For each individual, the arithmetic mean of the helminth species-specific egg counts from the Kato-Katz thick stool smears was calculated and multiplied by 24, to obtain the eggs per gram of faeces (EPG). The upper limits of light and moderate infections were 100 and 400 EPG for *S. mansoni*; 2000 and 4000 EPG for hookworm; 1000 and 10,000 EPG for *T. trichiura* and 5000 and 50,000 EPG for *A. lumbricoides*, respectively. For *S. haematobium*, egg counts from urine were classified into two categories only, light (<50 eggs/10 mL of urine) and heavy (≥50 eggs/10 mL of urine or visible haematuria). There were too few participants in the vaccine-arm who were co-infected with both malaria and helminth infections ($n = 8$), or multiple helminth infections ($n = 6$) to examine the relationship between co-infection and HPV immunogenicity.

Because the anti-HPV-16 and HPV-18 IgG antibody concentrations showed skewed distributions, HPV antibody results were transformed as $\log_{10}$ (IgG concentration). Geometric mean titres (GMT, EU/mL) and 95% confidence intervals (CI) were calculated.

The analysis of HPV vaccine antibody response, and malaria and helminth infection was restricted to participants in the vaccine-arm who attended the Month 7 visit ($n = 195$) or the Month 12 visit ($n = 196$) and had immunogenicity results. Box plots were used to graphically examine the distribution of raw antibody responses by malaria and helminth infection status. Linear regression was used to compare mean log-transformed IgG antibody between participants with and without any helminth infection, and with and without malaria. Regression coefficients and confidence limits were back-transformed to express results as ratios of geometric means (GMR). These analyses controlled for potential confounding by age of participants, and number of vaccine doses received. Analyses of malaria and HPV vaccine antibody response controlled for presence of any helminth infection. Similarly, the analyses of helminth infection and HPV vaccine antibody response controlled for malaria parasitaemia. There were insufficient data to examine associations with specific helminth infections.

3. Results

3.1. Cohort screening, enrolment and follow-up

In total 587 participants attended the screening visit, and 334 were enrolled in the HPV 021 trial. Of these, 221 participants were randomized to the vaccination arm and 113 to the placebo-arm. Overall, 298 (89%) participants attended the Month 7 visit (90 and 88% in the vaccine and placebo arms, respectively) and 308 (92%) attended the Month 12 visit (93 and 90% in the vaccine and placebo arms, respectively). The most common reason for discontinuation was withdrawal of consent (4%). The majority (96%) of participants received all three vaccine or placebo doses (Table 1); number of doses received did not differ substantially between participants in the vaccine and placebo arms or between those with and without malaria and/or helminth infections (Table 1) in either trial arm.

All participants were of African origin and were HIV-seronegative at baseline. The median age of participants was 18 years (IQR = 13–19). More than three-quarters of participants (82%) were currently students. Most (89%) participants were single. Approximately one-third (37%) of participants lived in houses constructed from cement blocks, and 40% lived in homes constructed from mud bricks (Table 1). As previously reported, sociodemographic characteristics did not differ by vaccine-arm [12].

3.2. Prevalence of malaria and helminths at Month 7

At Month 7, approximately one-third (38.1%) of participants tested positive for either malaria parasitaemia or helminth infection. The prevalence of malaria parasitaemia in the entire cohort was 10.2% (Table 2) and in the vaccinated cohort was 10.5%. The prevalence of any helminth infection was 30.4% in the entire cohort (Table 2), and 31.6% in the vaccinated cohort. *S. mansoni* was the most commonly detected helminth, found in one-quarter of participants (24.0%), followed by hookworm (5.7%). *S. haematobium* was rare; only two (0.7%) participants tested positive. The prevalence of malaria parasitaemia was somewhat higher in younger participants (Table 2), although there was not strong evidence of a difference ($p = 0.24$).

Three quarters (77.9%) of *S. mansoni* infections were light infections, 17.6% were moderate and 4.4% were heavy. Of the two *S. haematobium* infections, one was light and one was heavy. All (100%) of the hookworm, *A. lumbricoides, T. trichiura and Taenia* spp. infections were categorized as light infections.

3.3. Geometric mean titres for HPV-16/18 antibody response

As previously reported, all initially seronegative participants in the vaccinated cohort seroconverted for anti-HPV-16 and -18 antibodies, and remained seropositive up to Month 7. At Month 12, all initially seronegative participants in the vaccine group remained seropositive for anti-HPV-16, and all except one (13-year-old girl) remained seropositive for anti-HPV-18 [12]. Four participants had missing antibody results at Month 7, but were seropositve for anti-HPV-16 and -18 antibodies at Month 12.

HPV immunogenicity was high at Month 7 and Month 12. Among the vaccinated cohort who attended the Month 7 visit and had antibody results ($n = 195$), the GMT HPV-16 antibody response at Month 7 was 10,786 EU/mL (95% CI 9126–12,747), and the GMT HPV-18 antibody response was 3701 EU/mL (95% CI 3156–4340) (Table 3). As previously reported, HPV-16/18 serostatus at enrolment (prior to vaccination) did not influence GMTs at Month 7 or Month 12 [12]. GMT HPV-16 and HPV-18 antibody responses at Month 7 were at least 2 fold higher in 10–14-year-olds (19,374 EU/mL, 95% CI 16,600–22,611 and 5723 EU/mL, 95% CI 4790–6839, respectively) than in 15–25-year-olds (7770 EU/mL, 95% CI 6188–9755 and 2900 EU/mL, 95% CI 2333–3605, respectively, $P < 0.001$).

Antibody responses to HPV-16/18 among 107 vaccine-arm participants without helminths or malaria parasitaemia were high. The GMT HPV-16 antibody response among helminth and malaria uninfected 10–14-year-olds at Month 7 ($N = 40$) was 18,248 EU/mL (95% CI 14,742–22,587), and for 15–25-year-olds ($N = 67$) was

Table 1
Characteristics of cohort attending for Month 7 visit.

	By infection status[a] (*N* = 273)			
	No infection (*N* = 169) *n* (%)	Any helminth[b] (*N* = 86) *n* (%)	Malaria[b] (*N* = 29) *n* (%)	Total[c] (*N* = 298) *n* (%)
Age group (years)				
10–14	62 (36.7)	25 (29.1)	14 (48.3)	107 (35.9)
15–19	74 (43.8)	48 (55.8)	10 (34.5)	138 (46.3)
20–25	33 (19.5)	13 (15.1)	5 (17.2)	53 (17.8)
Tribe[d]				
Sukuma	57 (33.9)	30 (34.9)	9 (31.0)	97 (32.7)
Non-sukuma	111 (66.1)	56 (65.1)	20 (69.0)	200 (67.3)
Religion[d]				
Catholic	79 (47.3)	41 (47.7)	15 (51.7)	137 (46.3)
Other christian	48 (28.7)	22 (25.6)	7 (24.1)	81 (27.4)
Muslim	40 (24.0)	23 (26.7)	7 (24.1)	78 (26.4)
Education level[d]				
Less than primary	47 (28.0)	27 (31.4)	11 (37.9)	87 (29.3)
Primary	39 (23.2)	20 (23.3)	7 (24.1)	70 (23.6)
Secondary	77 (45.8)	37 (43.0)	11 (37.9)	129 (43.4)
Above secondary	5 (3.0)	2 (2.3)	0 (-)	11 (3.7)
Marital status[d]				
Single	149 (88.7)	75 (87.2)	25 (86.2)	265 (89.2)
Married	19 (11.3)	11 (12.8)	3 (10.3)	31 (10.4)
Divorced/separated	0	0	1 (3.5)	1 (0.3)
Occupation[d]				
Student	137 (82.5)	67 (77.9)	24 (82.8)	243 (82.4)
Manual/clerical/other	12 (7.2)	7 (8.1)	3 (10.3)	21 (7.1)
Housewife/unemployed	17 (10.2)	12 (14.0)	2 (6.9)	31 (10.5)
Housing construction[d]				
Cement blocks	68 (41.0)	27 (31.4)	10 (34.5)	108 (36.6)
Mud bricks	64 (38.6)	34 (39.5)	12 (41.4)	118 (40.0)
Burnt bricks	26 (15.7)	14 (16.3)	6 (20.7)	50 (16.9)
Other	8 (4.8)	11 (12.8)	1 (3.5)	19 (6.4)
Vaccine doses received				
Three	160 (94.7)	85 (98.8)	29 (100)	287 (96.3)
Less than three	9 (5.3)	1 (1.2)	0	11 (3.7)

[a] Among 273 participants with complete data on all infections.
[b] Includes 11 participants who were positive for both helminth and malaria infection.
[c] Among 298 participants who attended the 7 month visit.
[d] Missing data on tribe, education and marital status for 1 participant. Missing data on religion for 2 participants. Missing data on occupation and housing construction for 3 participants.

Table 2
Prevalence[a] of helminths and malaria infection at Month 7, by age group and overall.

	10–14 years (*N* = 107) *n* (%)	15–19 years (*N* = 138) *n* (%)	20–25 years (*N* = 53) *n* (%)	All ages (*N* = 298) *n* (%)
S. mansoni[b]	20 (19.6)	42 (32.1)	6 (12.0)	68 (24.0)
Hookworm[b]	2 (2.0)	7 (5.3)	7 (14.0)	16 (5.7)
S. stercoralis[b]	0	0	0	0
A. lumbricoides[b]	2 (2.0)	0	0	2 (0.7)
T. trichiura[b]	2 (2.0)	1 (0.8)	3 (6.0)	6 (2.1)
Taenia spp.[b]	0	0	1 (2.0)	1 (0.4)
S. haematobium	1 (0.9)	1 (0.7)	0	2 (0.7)
Any helminth[c]	25 (24.5)	48 (36.6)	13 (26.0)	86 (30.4)
Malaria[d]	14 (14.1)	10 (7.5)	5 (9.6)	29 (10.2)
Number of infections[e]				
None	62 (64.6)	74 (57.8)	33 (67.4)	169 (61.9)
1	27 (28.1)	47 (36.7)	10 (20.4)	84 (30.8)
2	6 (6.3)	7 (5.5)	6 (12.2)	19 (7.0)
3	1 (1.0)	0	0	1 (0.4)

[a] Prevalence of each infection is among those without missing data for that organism.
[b] Missing helminth results for 5 participants in 10–14 years age group, 7 in the 15–19 years age group and 3 in the 20–25 years age group.
[c] Among 283 participants with complete data on all helminths.
[d] Missing malaria results from 8 participants in the 10–14 years age group, 4 particpants in the 15–19 years age group and 1 participant in the 20–25 years age group.
[e] Among 273 participants with complete data on all infections.

Table 3
Antibody responses at Month 7 and at Month 12 in vaccinated participants by helminth infection and malaria infection status.

	N	Geometric mean titre (EU/mL) (95% CI)	Unadjusted geometric mean ratio (95% CI)	Adjusted geometric mean ratio[a] (95% CI)
Month 7				
HPV-16 IgG				
Overall	195	10786 (9126–12747)	–	–
Any helminth				
No	126	10492 (8445–13036)	$P=0.27$	$P>0.99$
Yes	60	12761 (10269–15857)	1.22 (0.86–1.72)	1.00 (0.77–1.29)
Intensity of helminth infection				
None	126	10492 (8445–13036)	$P=0.48$	$P=0.72$
Light	50	12363 (9936–15383)	1.18 (0.81–1.71)	0.96 (0.73–1.26)
Moderate/heavy	10	14946 (6442–34679)	1.42 (0.69–2.95)	1.20 (0.71–2.03)
Malaria				
No	166	9750 (8082–11761)	$P=0.01$	$P=0.05$
Yes	20	20357 (14430–28720)	2.09 (1.20–3.63)	1.47 (1.00–2.18)
HPV-18 IgG				
Overall	195	3701 (3156–4340)	–	–
Any helminth				
No	126	3513 (2880–4285)	$P=0.19$	$P=0.64$
Yes	60	4392 (3418–5643)	1.25 (0.90–1.75)	1.06 (0.82–1.38)
Intensity of helminth infection				
None	126	3513 (2880–4285)	$P=0.26$	$P=0.35$
Light	50	4129 (3162–5393)	1.18 (0.82–1.68)	1.00 (0.75–1.32)
Moderate/heavy	10	5973 (2689–13268)	1.70 (0.84–3.42)	1.46 (0.85–2.51)
Malaria				
No	166	3434 (2873–4104)	$P=0.07$	$P=0.42$
Yes	20	5648 (3736–8538)	1.64 (0.97–2.80)	1.18 (0.79–1.76)
Month 12				
HPV-16 IgG				
Overall	196	2656 (2246–3140)	–	–
Any helminth				
No	129	2613 (2124–3215)	$P=0.64$	$P=0.70$
Yes	59	2843 (2171–3723)	1.09 (0.76–1.55)	0.94 (0.67–1.31)
Intensity of helminth infection				
None	129	2617 (2129–3217)	$P=0.67$	$P=0.70$
Light	49	2994 (2301–3895)	1.14 (0.78–1.67)	0.98 (0.69–1.40)
Moderate/heavy	10	2218 (745–6600)	0.85 (0.40–1.77)	0.75 (0.38–1.48)
Malaria				
No	167	2461 (2039–2971)	$P=0.05$	$P=0.16$
Yes	20	4335 (2890–6502)	1.76 (1.01–3.08)	1.43 (0.86–2.37)
HPV-18 IgG				
Overall	196	986 (834–1166)	–	–
Any helminth				
No	129	970 (781–1205)	$P=0.71$	$P=0.89$
Yes	59	1038 (802–1344)	1.07 (0.74–1.54)	0.98 (0.69–1.38)
Intensity of helminth infection				
None	129	973 (784–1207)	$P=0.83$	$P=0.85$
Light	49	1076 (806–1436)	1.11 (0.75–1.63)	1.01 (0.70–1.47)
Moderate/heavy	10	880 (453–1712)	0.90 (0.42–1.93)	0.82 (0.41–1.66)
Malaria				
No	167	952 (787–1151)	$P=0.59$	$P=0.79$
Yes	20	1109 (764–1609)	1.16 (0.66–2.05)	0.93 (0.55–1.58)

[a] Geometric mean ratio (GMR) for helminth infection adjusted for participant age, number of vaccine doses and malaria infection. GMR for malaria infection adjusted for age, number of vaccine doses and any helminth infection.

6493 EU/mL (95% CI 4606–9153). Similarly, the GMT HPV-18 antibody response among helminth and malaria uninfected 10–14-year-olds at Month 7 was 5255 EU/mL (95% CI 4109–6720), and for 15–25-year-olds was 2479 EU/mL (95% CI 1807–3399).

There was some evidence that participants with malaria parasitaemia at Month 7 had a higher GMT HPV-16 and HPV-18 antibody response (Table 3; Fig. 1). After controlling for age, number of vaccine doses received, and any helminth infection, participants with evidence of malaria had a roughly 1.5 fold higher HPV-16 GMT than participants without malaria (adjusted geometric mean ratio (GMR) = 1.47, 95% CI 1.00–2.18, $P=0.05$). Participants with malaria parasites had a 1.2 fold higher GMT HPV-18 antibody response at Month 7 compared to participants without malaria (adjusted GMR = 1.18, 95% CI 0.79–1.76, $P=0.42$).

At the Month 12 visit, there was also some evidence that the HPV-16 GMT antibody response was higher among participants with malaria parasitaemia at Month 7, adjusting for age, number of vaccine doses received, and any helminth infection (adjusted GMR = 1.43, 95% CI 0.86–2.37, $P=0.16$) (Table 3). There was no evidence of a difference in HPV-18 GMT antibody response at Month 12 between participants with malaria parasitaemia at Month 7 and those without (adjusted GMR = 0.93, 95% CI 0.55–1.58, $P=0.79$) (Table 3).

At Month 7 and Month 12, GMT antibody responses were similar in participants with and without helminth infections (Table 3). The GMR for HPV-16 antibody response at Month 7, comparing participants with and without helminth infection, was 1.00 (95% CI 0.77–1.29, $P>0.99$), after controlling for age, number of vaccine doses received and malaria parasitaemia (Table 3; Fig. 1). The adjusted GMR for HPV-18 antibody response comparing participants with and without helminth infection was 1.06 (95% CI 0.82–1.38, $P=0.64$). Similar results were seen at Month 12.

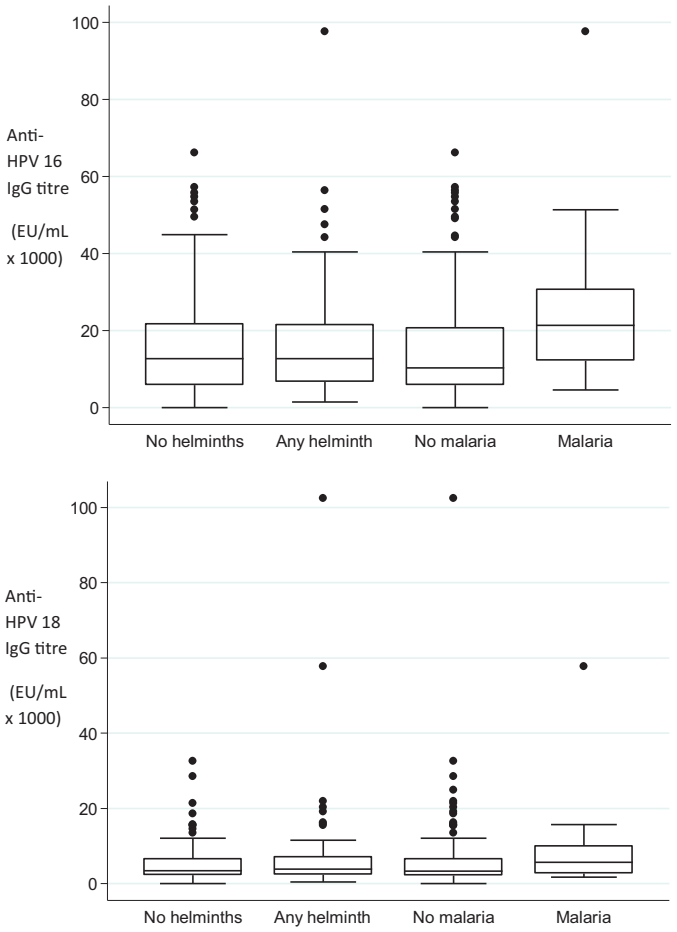

Fig. 1. Anti-HPV-16 (top) and HPV-18 (bottom) titre at 7 months after first dose of HPV vaccine in Tanzanian females aged 10–25 years, by helminth and malaria infection status. The central line represents the median; boxes represent 75th and 25th centiles; whiskers represent upper and lower adjacent values and dots represent outside values.

Although mean antibody response was highest in participants with higher intensity helminth infections, there was no evidence of a signficant difference (Table 3).

4. Discussion

This is the first study to examine the effect of malaria and helminth infections on HPV vaccine antibody responses. The incidence of cervical cancer is extremely high in many countries in sub-Saharan Africa which are considering the implementation of HPV vaccination as a cervical cancer control strategy but which also have a high prevalence of endemic malaria and helminth infections. These infections can impact immune responses to vaccinations [3–9]. Reassuringly, we found no negative impact on the immune response to the HPV-16/18 vaccine in the presence of these infections. The HPV-16/18 vaccine was highly immunogenic, especially in younger girls, as previously observed [19].

We observed some evidence of an association between malaria parasitaemia and a higher antibody response to the HPV-16/18 vaccine, which persisted adjusting for age. This association appeared weaker at Month 12 than Month 7 perhaps because there was a longer interval between the timing of the malaria and helminth tests and the antibody data. There was no observed effect of helminth infection, or intensity of helminth infection, on HPV-16/18 antibody response. The mechanism and significance of the increase in HPV-16/18 GMTs among malaria infected individuals is unclear. It is possible that malaria may induce a broader spectrum antibody response than helminths, which may potentiate the immune response to the HPV vaccine. We were unable to assess whether this observation was sustained beyond 12 months of follow-up.

As in all observational studies, these findings may be distorted by unmeasured confounders. We attempted to control for potential confounding by age and number of vaccine doses received, which produced little change in the effect estimates. This study also had a small sample size, and a relatively small number of participants with helminth and malaria infections. Results should therefore be interpreted with caution. Sensitivity of the Kato-Katz method in diagnosing helminth infections is relatively low, although we attempted to increase the sensitivity by collecting 3 stool samples from each participant [20,21]. Finally, infection diagnosed at one point during follow-up will not be representative of infection status at the time that earlier vaccine doses were administered. We were therefore unable to measure the effect of earlier infections on the response to the first and second doses of vaccine.

Both animal and human studies indicate that parasitic infections can impair long-term responses to vaccination [10,22]. Although our results are encouraging up to one year post-vaccination, because of the short-term nature of this study, our data do not allow us to evaluate whether untreated malaria or helminth infections, repeated infections or co-infections may impair long-term responses to the HPV vaccine. Longer-term follow-up of vaccinated cohorts and repeated cross-sectional surveys to assess antibody response and helminth/malaria infections in communities are warranted.

In summary, we found high HPV immunogenicity regardless of the presence of malaria and helminth infections among young girls and women in Tanzania. There was some evidence of enhanced antibody titres to HPV vaccine genotypes in participants with malaria parasitaemia. Additional research on the impact of parasitic infection on the long-term duration of protection from HPV vaccines is warranted.

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Conflict of interest statement Dr. Watson-Jones and Dr. Mayaud have received grant support through their institutions from GlaxoSmithKline Biologicals SA. During the trial, partial salary support for Drs. Watson-Jones, Andreasen, Brown and Kavishe came from GSK Biologicals. There are no other conflicts of interest.

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Paper 4 Immunogenicity and safety of one dose human papillomavirus vaccine compared with two or three doses in Tanzanian girls (DoRIS): an open-label, randomised, non-inferiority trial

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Immunogenicity and safety of one-dose human papillomavirus vaccine compared with two or three doses in Tanzanian girls (DoRIS): an open-label, randomised, non-inferiority trial

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Summary

Background An estimated 15% of girls aged 9–14 years worldwide have been vaccinated against human papillomavirus (HPV) with the recommended two-dose or three-dose schedules. A one-dose HPV vaccine schedule would be simpler and cheaper to deliver. We report immunogenicity and safety results of different doses of two different HPV vaccines in Tanzanian girls.

Methods In this open-label, randomised, phase 3, non-inferiority trial, we enrolled healthy schoolgirls aged 9–14 years from Government schools in Mwanza, Tanzania. Eligible participants were randomly assigned to receive one, two, or three doses of either the 2-valent vaccine (Cervarix, GSK Biologicals, Rixensart) or the 9-valent vaccine (Gardasil-9, Sanofi Pasteur MSD, Lyon). The primary outcome was HPV 16 specific or HPV 18 specific seropositivity following one dose compared with two or three doses of the same HPV vaccine 24 months after vaccination. Safety was assessed as solicited adverse events up to 30 days after each dose and unsolicited adverse events up to 24 months after vaccination or to last study visit. The primary outcome was done in the per-protocol population, and safety was analysed in the total vaccinated population. This study was registered in ClinicalTrials.gov, NCT02834637.

Findings Between Feb 23, 2017, and Jan 6, 2018, we screened 1002 girls for eligibility. 72 girls were excluded. 930 girls were enrolled and randomly assigned to receive one dose of Cervarix (155 participants), two doses of Cervarix (155 participants), three doses of Cervarix (155 participants), one dose of Gardasil-9 (155 participants), two doses of Gardasil-9 (155 participants), or three doses of Gardasil-9 (155 participants). 922 participants received all scheduled doses within the defined window (three withdrew, one was lost to follow-up, and one died before completion; two received their 6-month doses early, and one received the wrong valent vaccine in error; all 930 participants were included in the total vaccinated cohort). Retention at 24 months was 918 (99%) of 930 participants. In the according-to-protocol cohort, at 24 months, 99% of participants who received one dose of either HPV vaccine were seropositive for HPV 16 IgG antibodies, compared with 100% of participants who received two doses, and 100% of participants who received three doses. This met the prespecified non-inferiority criteria. Anti-HPV 18 seropositivity at 24 months did not meet non-inferiority criteria for one dose compared to two doses or three doses for either vaccine, although more than 98% of girls in all groups had HPV 18 antibodies. 53 serious adverse events (SAEs) were experienced by 42 (4·5%) of 930 girls, the most common of which was hospital admission for malaria. One girl died of malaria. Number of events was similar between groups and no SAEs were considered related to vaccination.

Interpretation A single dose of the 2-valent or 9-valent HPV vaccine in girls aged 9–14 years induced robust immune responses up to 24 months, suggesting that this reduced dose regimen could be suitable for prevention of HPV infection among girls in the target age group for vaccination.

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Introduction

Cervical cancer results in more than 340 000 potentially preventable deaths annually, with most fatalities in low-income and middle-income countries.[1] Four vaccines are licensed for the prevention of human papillomavirus (HPV), the main cause of cervical cancer. WHO cervical cancer elimination targets include 90% of girls younger than 15 years receiving a prophylactic HPV vaccine by 2030.[2] In countries that have introduced HPV vaccination, the vaccines are delivered as a multidose schedule with two doses offered to girls younger than 15 years, three doses offered to girls 15 years or older and

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See **Comment** page e1373

See **Articles** page e1485

For the KiSwahili translation of the abstract see **Online** for appendix 1

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Research in context

Evidence before this study

Several authors of this work participated in a review to collate the evidence on single dose human papillomavirus (HPV) vaccination. This review identified the absence of evidence from randomised trials and highlighted that data from Africa were also limited. A 2019 systematic review published as part of this evidence review examined the effectiveness and immunogenicity of single dose HPV vaccination among participants who received their HPV vaccine through a clinical trial. Apart from one small randomised trial examining memory B-cell responses following single dose HPV vaccination, results came from observational studies nested within three large HPV vaccine trials (Costa Rica Vaccine Trial [CVT], PATRICIA, and IARC India trial) in which participants did not complete their allocated two-dose or three-dose schedules which resulted in single dose default groups followed up for immunogenicity and efficacy against HPV infection. We did an updated search of MEDLINE, Embase, Global Health Database, and Cochrane Central Register of Controlled Trials from Aug 1, 2018, to Dec 10, 2021, using the search terms "human papillomavirus AND vaccines AND (immunogenicity OR efficacy OR effectiveness) AND dosage". From this search we identified two additional observational studies that extended the data from two of these studies and, in 2022, results were published from a randomised trial on the efficacy of single dose HPV vaccination in sexually active Kenyan women aged 15–20 years (KEN SHE study). The observational studies showed that frequency of HPV 16 and 18 incident and 12-month persistent infection and vaccine efficacy against infection endpoints was similar in women and girls who received a single dose of vaccine compared with those who received two or three doses. HPV 16 and 18 IgG antibody seropositivity was very high in all dose groups for vaccinated participants, although antibody mean concentrations were lower with one dose than with two or three doses, but remained stable over 11 and 9 years for all doses for two HPV vaccines. HPV infection endpoints were significantly lower in participants who received one vaccine dose compared to unvaccinated controls. The KEN SHE trial showed very high and non-inferior vaccine efficacy for one dose of the 2-valent and 9-valent vaccines compared with a control vaccine at 18 months after the first dose. Non-trials data include an observational cohort study of Ugandan girls who did not complete the 3-dose schedule of the 2-valent vaccine in a Government-administered HPV vaccination demonstration programme. Seroconversion was high for all doses. HPV 16 and 18 binding antibody responses were lower in girls who had received one compared with two or three doses but geometric mean concentrations for one dose recipients were not lower in these Ugandan girls compared with adult women who received one dose in the CVT and in whom efficacy had been demonstrated.

Added value of this study

This study is the first randomised clinical trial examining immune responses and safety of single dose HPV vaccine with either the 2-valent or 9-valent vaccine compared with two and three doses of the same vaccines in girls in the target age group of 9–14 years for vaccination. Antibody responses were comparable with those seen in the earlier observational studies, and were induced with both vaccines after one dose and increased after the second and third doses. Antibody geometric mean concentrations peaked at 1 month and then plateaued from month 7 for the single dose arm and peaked at month 7 then declined by month 24 for the two-dose and three-dose arms but stayed stable in the one-dose arms to 2 years. Single dose HPV 16 seropositivity at 24 months post dose was non-inferior to two and three doses and HPV 16 and HPV 18 avidity at month 24 did not differ by dose or vaccine. Both vaccines were well tolerated at all doses.

Implications of all the available evidence

A single dose of either the 2-valent or 9-valent HPV vaccine was both immunogenic and safe, with high rates of seroconversion and antibody levels stable to 2 years after vaccination and antibody kinetics similar to those seen in other settings where single-dose efficacy has been demonstrated. Higher antibody levels observed with the 2-valent vaccine compared to the 9-valent vaccine are consistent with earlier studies that also found both vaccines to be highly efficacious. A single dose of HPV vaccine would very significantly simplify vaccine delivery and reduce costs of implementing national HPV vaccination programmes, in turn potentially increasing vaccine introductions and uptake in the regions that urgently need cervical cancer prevention.

to immunocompromised individuals, and boys being offered the vaccine in some countries. Barriers to the introduction and uptake of HPV vaccination are greatest in countries that bear the highest burden of cervical cancer morbidity and mortality, particularly the cost of delivering a multidose vaccine schedule.[3] Only 15% of girls in the target age group for HPV vaccine (9–14 years) worldwide are estimated to be fully vaccinated with the currently recommended two-dose or three-dose schedules.[4] As with other primary health-care services, HPV vaccine delivery has been disrupted by the COVID-19 pandemic and, in some WHO regions, last dose coverage is less than 5%.[5]

A single dose HPV vaccine would be simpler and cheaper to deliver than a multidose schedule but evidence is needed on the immunogenicity and efficacy of a single-dose schedule. Data from several observational studies in which some participants did not complete their allocated schedules suggest that a single dose of HPV vaccine provides efficacy against incident and persistent HPV 16 or 18 infection that is similar to efficacy with two or three doses.[6] These include the IARC/India study of the

4-valent vaccine, Gardasil, and the Costa Rica Vaccine Trial (CVT) and PATRICIA trial that evaluated the 2-valent vaccine, Cervarix. In these studies, the frequency of 12-month persistent infection (a precursor for cervical cancer) with HPV 16 or HPV 18 was similar in females receiving a single dose compared with those receiving two or three doses. HPV 16 or HPV 18 IgG antibody seropositivity was high in all vaccinated groups, regardless of the number of doses received, but geometric mean concentrations (GMCs) were lower with one dose than with two or three doses. All HPV infection endpoints in these studies were significantly less frequent in participants receiving one dose compared with unvaccinated controls.[6] Protection against persistent HPV16 or HPV18 infection after a single dose of the 2-valent vaccine was sustained up to 11 years in the CVT,[7] and up to 9 years in the IARC/India study following a single dose of the 4-valent vaccine.[8]

The first randomised trial to examine the efficacy of a single dose of HPV vaccine in sexually active Kenyan women aged 15–20 years (KEN SHE) reported that, at 18 months post vaccination, the incidence of persistent HPV 16 or HPV 18 infection was 0·17/100 woman-years with both the 2-valent and the 9-valent vaccines, compared with 6·83 per 100 woman-years in the meningococcal vaccine control group.[9] Vaccine efficacy for both HPV vaccines was 97·5%.

We report the results of the DoRIS trial in Tanzania, the first randomised trial to examine immune responses after a single dose of HPV vaccine in the target age group for HPV vaccination.

Methods

Study design

This open-label, randomised, phase 3, non-inferiority, immunobridging trial of two HPV vaccines was done in Mwanza, northwestern Tanzania (Dose Reduction Immunobridging and Safety Study of two HPV vaccines in Tanzanian girls [DoRIS]). The study was approved by the Tanzanian Medical Research Coordinating Committee and the ethics committee of the London School of Hygiene & Tropical Medicine. Regulatory approval was by the Tanzania Medicines and Medical Devices Authority.

Participants

The trial protocol and procedures have been described previously.[10] Briefly, we enrolled 930 girls aged 9–14 years living in Mwanza, Tanzania. Participants from 54 Government schools in Mwanza were invited to attend a research clinic in the city, after meetings with community leaders, school heads, teachers, and parents. Written informed consent was obtained from parents or guardians with written or fingerprinted assent from participants. Eligible participants were healthy (by medical history taken by clinician and physical examination if indicated) girls who were aged 9–14 years, HIV-negative following testing at screening, planning to reside in Mwanza for 36 months, and willing to give informed assent following informed consent from a parent or guardian. Exclusion criteria were previous HPV vaccination, history of cervical lesions or genital warts, past treatment for positive cervical cancer screening, pregnancy, being immunocompromised (including HIV infection), and being unwell based on medical history, clinical examination, or laboratory tests.

Randomisation

Participants were randomly allocated (1:1:1:1:1:1) to one of six arms comprising three different dose schedules of two different HPV vaccines (three doses over 6 months, two doses given 6 months apart, or a single dose, for either the 2-valent vaccine [Cervarix, GSK Biologicals, Rixensart] or the 9-valent vaccine [Gardasil-9, Sanofi Pasteur MSD, Lyon]), using random permuted block sizes of 12, 18, and 24. An independent statistician computer-generated the randomisation list. Sequentially numbered sealed opaque envelopes concealed the allocation from the study team and participants. Once allocated, participants and clinic staff were unmasked. Participants were not masked as we did not think immune responses would be affected by girls knowing their vaccine group, and one of the trial's secondary aims was the acceptability of reduced-dose schedules.

Procedures

We evaluated two prophylactic HPV virus-like particle (VLP) vaccines, both licensed by the US Food and Drug Administration and the European Medicines Agency. The 2-valent HPV vaccine (Cervarix; GSK Biologicals) is an HPV 16 and HPV 18 VLP vaccine containing L1 major capsid proteins of HPV 16 and HPV 18 and a proprietary adjuvant system (ASO4) that is formulated with monophosphoryl-lipid A adsorbed to aluminium hydroxide. The 9-valent vaccine (Gardasil-9; Sanofi Pasteur MSD) targets 9 genotypes (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58). The vaccine has an amorphous aluminium hydroxyphosphate sulfate adjuvant and each dose contains 60 µg of HPV 16 L1 protein and 40 µg of HPV 18 L1 protein. Both vaccines have excellent efficacy for preventing HPV 16 or HPV 18 associated grade 2 or grade 3 cervical intraepithelial neoplasia and HPV 16 or 18 associated adenocarcinoma in situ in women with no previous HPV 16 or 18 infection.[11] There is no evidence of serious adverse events (SAEs) or adverse pregnancy outcomes with these vaccines.[2,11] The 2-valent vaccine demonstrates cross-protection to HPV 31, 33, and 45 infection and their sequelae.[11] The 9-valent vaccine also prevents infection and high grade cervical, vaginal, and vulval disease associated with HPV 31, 33, 45, 52, and 58.[12]

At the screening visit, after informed consent, girls were screened for eligibility, including a medical history with clinical examination if warranted, HIV testing and counselling, and a urine pregnancy test. Girls were also asked to take a test of understanding (TOU) if aged 12 years or older to demonstrate appropriate understanding

of the study. For girls younger than 12 years, a parent or guardian took the TOU. A screen failure was determined if the girl (or their parents) could not pass the TOU within three attempts.

Girls who had passed the screening process were invited to an enrolment visit within 30 days of the screening visit, at which eligibility was reconfirmed and girls were randomly allocated to one of the six arms. Blood samples were collected for immunogenicity assays and a dried blood spot was made for malaria testing by PCR. Girls were asked to provide two nurse-assisted, self-administered vaginal swabs for baseline HPV DNA testing and genotyping with the Anyplex II HPV 28 detection assay (Seegene, Seoul) done at the Catalan Institute of Oncology, Barcelona. Participants were then randomly assigned and vaccinated according to their study arm and were asked to attend the clinic 1 month after vaccination.

Subsequent vaccination visits were at 1 month after the first dose (for the second dose of the 2-valent vaccine three-dose arm) or at 2 months after the first dose (for the second dose of the 9-valent vaccine two-dose arm; appendix 2 p 9) and 6 months (girls enrolled in the two-dose and three-dose arms for either vaccine). At each vaccination visit, and at 6 months for the one-dose arms, we collected a dried blood spot for malaria testing. Participants were asked to attend the clinic 1 month after each vaccination visit for collection of information on adverse events (AEs). Whole blood samples of 15–20 mL (depending on participant's weight) were collected for immunological assays at 1, 7, 12 and 24 months after vaccination. Visit windows for vaccination and blood sampling visits were predefined in the protocol.[10]

See Online for appendix 2

All samples were processed at the Mwanza National Institute for Medical Research laboratory. HPV 16 and HPV 18 IgG concentrations were determined at the HPV Immunology Laboratory of the Frederick National Laboratory for Cancer Research in Maryland, USA, by use of an L1 VLP ELISA. This assay has previously been evaluated for monitoring antibody responses following single-dose HPV vaccination.[13] Antibody seropositivity was defined as concentrations equal to or greater than the assay threshold (1·309 IU/mL for HPV 16 and 1·109 IU/mL for HPV 18). The HPV 16 and HPV 18 specific antibody avidity index in the ELISA was determined by the ratio of antibody concentrations in serum samples treated or not treated with Guanidine-HCl (GuHCl). Serum samples were tested at a dilution that yielded an absorbance reading of 1·0 (±0·5). GuHCl was added to the samples at various concentrations (0·5–3·5 M); the GuHCl concentration that reduced the optical density by 50%, compared with sample wells without GuHCl, defined the avidity index. HPV 16 and 18 specific memory B-cell responses and immune responses to the 7 other HPV genotypes in the 9-valent vaccine are being analysed separately and results are not included here.

Outcomes

The primary outcome was non-inferiority of HPV 16 and HPV 18 specific seropositivity following one dose of HPV vaccine compared with two or three doses of the same vaccine 24 months after vaccination. This corresponded to two overall analyses: one evaluating the reduced dose schedule of the 2-valent vaccine, and one evaluating the 9-valent vaccine.

Vaccine immune responses were measured by the proportion of participants seroconverting to HPV 16 or 18, the GMC of HPV 16 and HPV 18 specific antibodies, HPV 16 and HPV 18 specific antibody avidity, and HPV 16 and HPV 18 specific memory B-cell responses.

Secondary objectives are evaluation of HPV 16 and HPV 18 seropositivity and antibody GMC after one dose versus two or three doses at other timepoints up to 24 months post-vaccination; comparison of HPV 16 and HPV 18 antibody responses after two versus three doses; and evaluation of HPV 16 and HPV 18 antibody avidity.

The trial had a coprimary immunobridging objective to demonstrate non-inferiority of HPV 16 and HPV 18 antibody GMC after one dose of vaccine compared with historical cohorts of women aged 10–25 years who received a single dose of HPV vaccine and in whom efficacy had been demonstrated; these results are reported in a companion publication.[14]

Statistical analysis

With 155 participants in each arm, assuming a 20% loss-to-follow up over 36 months, we expected to have 130 girls in each arm 24 months after vaccination. If the true proportion seroconverting is the same in each arm, with 130 girls per arm, the study would have more than 90% power to demonstrate that the lower limit of the 95% CI for the difference (one-dose schedule–comparison schedule) is above –5%, indicating that seropositivity with the one-dose schedule was not decreased by more than 5·0%. This was the same non-inferiority margin that was used in the trials leading to licensure of the two-dose regimen in girls younger than 15 years.[15,16]

Our power calculations were also based on our coprimary objective of demonstrating non-inferiority of GMCs in the immunobridging analyses. If the true GMC ratio (one-dose schedule:comparison schedule) between arms is 1·0, with 130 subjects in each arm, we would have more than 90% power to demonstrate that the lower limit of the 95% CI for the ratio of GMCs is above 0·50, indicating that the one-dose schedule does not decrease HPV 16 or HPV 18 antibody GMC by more than 50%. This non-inferiority margin was based on pre-established standards from the US Food and Drug Administration that were used in other HPV vaccine bridging trials.[15,16] We assumed an SD of 0·50–0·60 $\log_{10}$ anti-HPV concentration and used a one-sided non-inferiority test at the 2·5% level.

In non-inferiority trials, intention-to-treat (ITT) analyses can increase the risk of falsely claiming non-inferiority, since these analyses often lead to smaller observed effects

than if all participants had adhered to the protocol.[17] Therefore, the primary immunogenicity analyses were done in the per-protocol population, ie, participants who received the allocated doses of HPV vaccine in the protocol-defined window and who were HPV antibody negative and DNA negative at enrolment for the specific genotype (HPV 16 or HPV 18) under analysis. As a sensitivity analysis, we repeated all analyses in participants who received at least one dose of HPV vaccine (total vaccinated cohort), based on the arm to which they were randomised (ie, ITT). The total vaccinated cohort was used for the safety analysis. The analysis plan was finalised before the trial ended and was approved by the independent Data and Safety Monitoring Board.

Baseline characteristics were presented by arm. We tabulated the number and proportion of girls in each arm who were HPV 16 or 18 seropositive at each timepoint. For each vaccine type and HPV genotype,

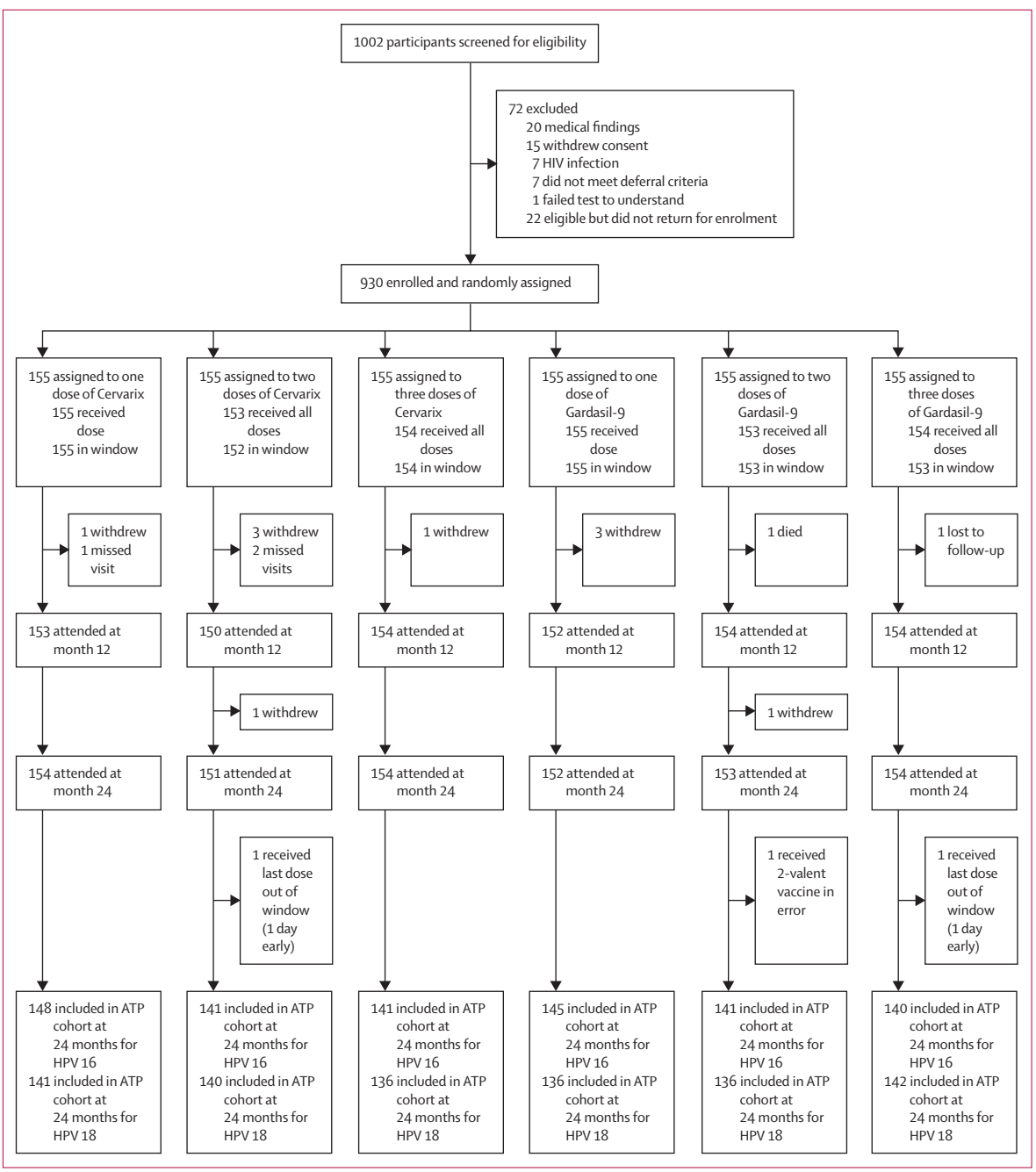

Figure 1: **Trial profile**
ATP=according-to-protocol.

	1 dose 2-valent (n=155)	2 doses 2-valent (n=155)	3 doses 2-valent (n=155)	1 dose 9-valent (n=155)	2 doses 9-valent (n=155)	3 doses 9-valent (n=155)	Total (n=930)
Age (years)	10 (9–12)	11 (10–12)	10 (9–12)	10 (9–12)	11 (10–13)	11 (9–13)	10 (9–12)
Age group							
9–10 years	85 (54·8%)	74 (47·7%)	85 (54·8%)	88 (56·8%)	70 (45·2%)	73 (47·1%)	475 (51·1%)
11–12 years	39 (25·2%)	45 (29·0%)	36 (23·2%)	41 (26·5%)	45 (29·0%)	41 (26·5%)	247 (26·6%)
13–14 years	31 (20·0%)	36 (23·2%)	34 (21·9%)	26 (16·8%)	40 (25·8%)	41 (26·5%)	208 (22·4%)
Years lived in Mwanza							
Entire life	116 (74·8%)	122 (78·7%)	121 (78·1%)	118 (76·1%)	121 (78·1%)	122 (78·7%)	720 (77·4%)
>5 years	20 (12·9%)	18 (11·6%)	17 (11·0%)	18 (11·6%)	21 (13·5%)	14 (9·0 %)	108 (11·6%)
≤5 years	19 (12·3%)	15 (9·7 %)	17 (11·0%)	19 (12·3%)	13 (8·4 %)	19 (12·3%)	102 (11·0%)
Living with							
Mother	33 (21·3%)	32 (20·6%)	29 (18·7%)	31 (20·0%)	32 (20·6%)	39 (25·2%)	196 (21·1%)
Father	6 (3·9 %)	5 (3·2 %)	4 (2·6 %)	6 (3·9 %)	6 (3·9 %)	2 (1·3 %)	29 (3·1 %)
Both parents	93 (60·0%)	95 (61·3%)	97 (62·6%)	93 (60·0%)	86 (55·5%)	91 (58·7%)	555 (59·7%)
Other	23 (14·8%)	23 (14·8%)	25 (16·1%)	25 (16·1%)	31 (20·0%)	23 (14·8%)	150 (16·1%)
Religion							
Catholic	57 (36·8%)	59 (38·1%)	74 (47·7%)	73 (47·1%)	63 (40·6%)	67 (43·2%)	393 (42·3%)
Other Christian	78 (50·3%)	77 (49·7%)	66 (42·6%)	68 (43·9%)	68 (43·9%)	73 (47·1%)	430 (46·2%)
Muslim	20 (12·9%)	19 (12·3%)	15 (9·7 %)	14 (9·0 %)	24 (15·5%)	15 (9·7 %)	107 (11·5%)
School type							
Primary	123 (79·4%)	122 (78·7%)	122 (78·7%)	127 (81·9%)	122 (78·7%)	119 (76·8%)	735 (79·0%)
Secondary	32 (20·6%)	33 (21·3%)	33 (21·3%)	28 (18·1%)	33 (21·3%)	36 (23·2%)	195 (21·0%)
Passed menarche							
Yes	20 (12·9%)	20 (12·9%)	19 (12·3%)	18 (11·6%)	20 (12·9%)	20 (12·9%)	117 (12·6%)
Ever cleansed vagina							
Yes	15 (9·7 %)	15 (9·7 %)	13 (8·4 %)	14 (9·0 %)	12 (7·7 %)	19 (12·3%)	88 (9·5 %)
Ever had sex							
Yes	1 (0·6 %)	2 (1·3 %)	5 (3·2 %)	1 (0·6 %)	4 (2·6 %)	5 (3·2 %)	18 (1·9 %)
Ever drank alcohol							
Yes	0	0	0	0	0	0	0
HPV 16 DNA positive	0	0	1 (0·6 %)	1 (0·6 %)	0	1 (0·6 %)	3 (0·3 %)
HPV 18 DNA positive	0	0	2 (1·3 %)	1 (0·6 %)	0	0	3 (0·3 %)
Any high risk HPV genotype DNA							
Yes	0	2 (1·3 %)	4 (2·6 %)	6 (3·9 %)	2 (1·3 %)	3 (1·9 %)	17 (1·8 %)
Any HPV genotype DNA							
Yes	0	2 (1·3 %)	4 (2·6 %)	7 (4·5 %)	2 (1·3 %)	5 (3·2 %)	20 (2·2 %)
HPV 16 seropositive	6 (3·9 %)	9 (5·8 %)	13 (8·4 %)	7 (4·5 %)	10 (6·5 %)	12 (7·7 %)	57 (6·1 %)
HPV 18 seropositive	13 (8·4 %)	10 (6·5 %)	16 (10·3%)	16 (10·3%)	16 (10·3%)	10 (6·5 %)	81 (8·7 %)

Data are median (IQR) or n (%).

Table 1: **Patient demographics**

we calculated the difference (one-dose schedule–comparison schedule) in the proportion of girls who were seropositive, and estimated the 95% CI for the difference using the exact method of Chan and Zhang.[18] Non-inferiority of seropositivity was concluded if the lower bound of the two-sided 95% CI for the difference was above –5%.

For each vaccine and HPV genotype, there were two primary hypothesis tests of non-inferiority of seropositivity 24 months after vaccination: one dose versus two doses, and one dose versus three doses (ie, a joint null hypothesis). Success was required for both tests to conclude non-inferiority; therefore, no adjustment for multiplicity was made to account for testing of multiple dose schedules. As a post-hoc sensitivity analysis, 97·5% CIs were calculated in accordance with the Bonferroni correction, to account for testing of multiple HPV genotypes.

For the analysis of antibody concentrations, we $\log_{10}$-transformed HPV genotype-specific antibody concentrations; those below the assay cutoff were given a value of half the cutoff before log transformation. The arithmetic mean $\log_{10}$ antibody concentration and 95% CIs for each arm were calculated, assuming a normal distribution.

	1 dose		2 doses		3 doses		Difference in seropositivity* (exact 95% CI)		
	n	Seropositive* (%)	n	Seropositive* (%)	n	Seropositive* (%)	1 dose–2 dose	1 dose–3 dose	2 dose–3 dose
2-valent									
HPV 16									
Month 7	148	147 (99·3%)	142	142 (100·0%)	141	140 (99·3%)	−0·7% (−3·8 to 2·0)	0·0% (−3·1 to 3·4)	0·7% (−2·0 to 4·0)
Month 12	147	146 (99·3%)	140	140 (100·0%)	141	141 (100·0%)	−0·7% (−3·8 to 2·0)	−0·7% (−3·8 to 2·0)	0†
Month 24	148	147 (99·3%)	141	141 (100·0%)	141	141 (100·0%)	−0·7% (−3·8 to 2·0)	−0·7% (−3·8 to 2·1)	0†
HPV 18									
Month 7	141	139 (98·6%)	141	141 (100·0%)	136	135 (99·3%)	−1·4% (−5·1 to 1·3)	−0·7% (−4·5 to 2·8)	0·7% (−2·0 to 4·1)
Month 12	140	139 (99·3%)	139	139 (100·0%)	136	136 (100·0%)	−0·7% (−4·0 to 2·1)	−0·7% (−4·0 to 2·1)	0†
Month 24	141	139 (98·6%)	140	140 (100·0%)	136	136 (100·0%)	−1·4% (−5·1 to 1·4)	−1·4% (−5·1 to 1·4)	0†
9-valent									
HPV 16									
Month 7	144	144 (100·0%)	142	142 (100·0%)	140	140 (100·0%)	0†	0†	0†
Month 12	145	145 (100·0%)	142	142 (100·0%)	140	140 (100·0%)	0†	0†	0†
Month 24	145	144 (99·3%)	141	141 (100·0%)	140	140 (100·0%)	−0·7% (−3·9 to 2·0)	−0·7% (−3·8 to 2·1)	0†
HPV 18									
Month 7	135	133 (98·5%)	137	137 (100·0%)	142	142 (100·0%)	−1·5% (−5·3 to 1·3)	−1·5% (−5·3 to 1·2)	0†
Month 12	136	131 (96·3%)	137	137 (100·0%)	142	142 (100·0%)	−3·7% (−8·4 to −0·7)	−3·7% (−8·4 to −0·7)	0†
Month 24	136	133 (97·8%)	136	136 (100·0%)	142	141 (99·3%)	−2·2% (−6·4 to 0·6)	−1·5% (−5·7 to 2·0)	0·7% (−2·1 to 4·0)

*Titres above the laboratory determined cutoff (HPV 16 1·309 IU/mL and HPV 18 1·109 IU/mL). †Exact 95% CIs for the difference by Chan and Zhang[18] method cannot be calculated because both proportions are 1·0, but there is still uncertainty around the point estimate.

Table 2: **Comparisons of antibody seropositivity after 1, 2 or 3 doses of human papillomavirus vaccine**

Log_{10} antibody concentrations were compared by use of a linear mixed effect model with log_{10} concentration as the response, dose group, timepoint, and a dose group time interaction term as fixed effects, and participant as a random effect to account for correlation of repeated measurements within participant. Separate models were used for each vaccine type and HPV genotype. The difference in log_{10} concentrations (reduced dose schedule–comparison schedule) and its 95% CI at each timepoint was estimated from the mixed effect model; the GMC ratio and 95% CIs were obtained by back-transformation. We used a similar analysis to compare antibody avidity between dose regimens, with separate models for each vaccine type and HPV genotype, and fixed effects for dose regimen, timepoint, and dose regimen–time interaction. For the secondary objectives, multiple comparisons were taken into account when interpreting the findings but no formal adjustments were made for multiplicity. This study was registered with ClinicalTrials.gov, NCT02834637.

Role of the funding source

The funders of this study did not have any role in the study design, data collection and analysis, data interpretation, or writing of this report.

Results

Between Feb 23, 2017, and Jan 6, 2018, we screened 1002 girls for eligibility. 50 girls were excluded at screening for medical findings (n=20), no consent (n=15), HIV infection (n=7), not meeting vaccine deferral criteria (n=7), or failing the TOU (n=1). 22 girls were eligible at screening but did not attend the enrolment visit. 930 (93%) of 1002 girls were enrolled and randomly assigned to receive one dose of Cervarix, two doses of Cervarix, three doses of Cervarix, one dose of Gardasil-9, two doses of Gardasil-9, or three doses of Gardasil (155 participants per group; figure 1). Of those enrolled, 922 (99%) received all scheduled doses within the protocol-defined window. Three girls withdrew, one was lost to follow-up and one died before completing her dose schedule. Two girls (one each in the two-dose 2-valent and three-dose 9-valent arms) received their 6-month dose one day early, and one girl in the two-dose 9-valent arm received the 2-valent vaccine in error. These eight girls were excluded from the per-protocol analyses but were included in the total vaccinated cohort analyses.

Baseline characteristics were similar between the six arms, with a median age of 10 years (IQR 9–12; table 1). 735 (79%) of 930 girls were in primary school, 555 (60%) lived with both parents, 117 (13%) had passed menarche, and 18 (2%) reported ever having had vaginal sex. Only 20 (2%) girls had evidence of any HPV infection on their vaginal swabs, of whom four were positive for HPV 16 or HPV 18 DNA. Overall, 57 girls (6%) were HPV 16 seropositive and 81 (9%) were HPV 18 seropositive at baseline.

Retention at 24 months was 918 (99%) of 930 participants. At 24 months, we included 856 (93%) of the 918 girls attending in the per-protocol analysis of anti-HPV 16

	1 dose		2 doses		3 doses		GMC ratio (95% CI)* 2 doses or 3 doses
	N	GMC, IU/mL	N	GMC, IU/mL	N	GMC, IU/mL	
2-valent							
HPV 16							
Day 0	149	<LLQ	145	<LLQ	141	<LLQ	..
Month 1	149	48 (42–56)	144	52 (46–59)	141	50 (43–59)	..
Month 7	148	16 (14–19)	142	1643 (1445–1868)	141	2658 (2221–3182)	0·62 (0·50–0·77)
Month 12	147	19 (17–23)	140	268 (232–309)	141	641 (539–762)	0·42 (0·33–0·52)
Month 24	148	23 (20–26)	141	163 (141–188)	141	412 (357–475)	0·39 (0·32–0·49)
HPV 18							
Day 0	142	<LLQ	144	<LLQ	137	<LLQ	..
Month 1	142	19 (16–22)	143	18 (15–21)	137	18 (16–21)	..
Month 7	141	8 (6–9)	141	582 (505–670)	136	727 (607–870)	0·80 (0·64–1·00)
Month 12	140	9 (7–10)	139	96 (83–111)	136	159 (132–190)	0·61 (0·48–0·76)
Month 24	141	10 (9–11)	140	50 (43–58)	136	107 (90–126)	0·47 (0·38–0·59)
9-valent							
HPV 16							
Day 0	148	<LLQ	143	<LLQ	141	<LLQ	..
Month 1	147	55 (48–63)	143	51 (43–59)	141	57 (50–64)	..
Month 7	144	16 (13–19)	142	1401 (1253–1566)	140	1025 (896–1174)	1·37 (1·11–1·68)
Month 12	145	13 (12–15)	142	253 (219–291)	140	218 (189–251)	1·16 (0·95–1·42)
Month 24	145	14 (12–16)	141	125 (107–146)	140	118 (102–137)	1·06 (0·87–1·30)
HPV 18							
Day 0	139	<LLQ	138	<LLQ	143	<LLQ	..
Month 1	138	20 (17–23)	138	17 (15–20)	143	19 (17–22)	..
Month 7	135	7 (6–8)	137	400 (352–454)	142	383 (334–440)	1·04 (0·83–1·30)
Month 12	136	5 (4–6)	137	59 (50–69)	142	67 (57–79)	0·87 (0·70–1·09)
Month 24	136	6 (5–7)	136	29 (25–35)	142	32 (27–38)	0·91 (0·73–1·14)

Data are ELISA serum antibody GMC (95% CI) unless otherwise specified. LLQ=lower limit of quantitation. GMC=geometric mean concentration. *Estimated with linear mixed effect model with log antibody titre as the response and dose group, timepoint, and a dose group-time interaction term as fixed effects, and participant as a random effect to account for correlation of repeated measurements within participant.

Table 3: **HPV 16 and HPV 18 antibody GMCs at all visits, by dose group and vaccine in the per-protocol cohort**

antibody responses, and 831 (91%) in the per-protocol analysis of anti-HPV 18. All but two participants were seropositive for HPV 16 IgG antibodies at 24 months (one participant in each of the one-dose arms was not HPV 16 seropositive). All but six participants were HPV 18 seropositive at 24 months (two in the one-dose 2-valent vaccine group, three in the one-dose 9-valent vaccine group, and one in the three-dose 9-valent vaccine group were not HPV 18 seropositive). Non-inferiority of seroconversion of anti-HPV 16 antibodies at 24 months was met for one dose compared with two doses or three doses for both vaccines (table 2). Non-inferiority of HPV 16 seroconversion was also met when using a more stringent 97·5% CI, with the lower limit of the 97·5% CI of at least –4·6 for all comparisons (appendix 2 p 1). Although non-inferiority was not met for seroconversion for anti-HPV 18 antibodies, at least 98% of girls in the one-dose arms of both vaccines were anti-HPV 18 antibody positive at 24 months.

Antibody GMCs at 24 months among girls in the per-protocol group who received one dose of the 2-valent vaccine were 23 IU/mL (95% CI 20–26) for HPV 16 and 10 IU/mL (95% CI 9–11) for HPV 18 (table 3). Among those receiving one dose of the 9-valent vaccine, GMCs were 14 IU/mL (95% CI 12–16) for HPV 16 and 6 IU/mL (95% CI 5–7) for HPV 18 at 24 months. As expected from previous studies, HPV 16 and HPV 18 antibody GMCs were higher among girls receiving two and three doses than among those receiving one dose (appendix 2 p 10) and were higher for HPV 16 than for HPV 18.[8,10] Among those receiving two doses of the 2-valent vaccine, HPV 16 antibody GMC was 163 IU/mL (95% CI 141–188) and HPV 18 antibody GMC was 50 IU/mL (95% CI 43–58) at 24 months. For those receiving two doses of the 9-valent vaccine, HPV 16 antibody GMC was 125 IU/mL (95% CI 107–146) and HPV 18 antibody GMC was 29 IU/mL (95% CI 25–35) at 24 months (table 3). For both HPV genotypes, antibody GMCs at 24 months were non-inferior when comparing two doses with three doses of the 9-valent vaccine (table 3). Antibody GMCs at 24 months among girls receiving three doses of the 2-valent vaccine were significantly higher than those

receiving two doses (412 IU/mL *vs* 163 IU/mL for HPV16 and 107 IU/mL *vs* 50 IU/ml for HPV18, respectively), and non-inferiority was not met for either HPV genotype. Our immunogenicity results were similar among the total vaccinated cohort for both vaccines and both HPV genotypes (appendix 2 pp 2–3).

Antibody GMCs among the two-dose and three-dose recipients peaked at 7 months and declined thereafter up until 24 months for both vaccines and both genotypes (figure 2). However, among one-dose recipients, HPV 16 and HPV 18 GMCs remained constant over time from 7 months to 24 months for both vaccines.

By contrast with antibody GMCs, there was no evidence of a difference between the one-dose, two-dose schedules and three-dose schedules in GM antibody avidity index for HPV 16 or HPV 18 of either vaccine (appendix 2 p 5; figure 3). GM avidity index ratios were around 1·0 for all comparisons, with the lower limit of the 95% CI more than 0·90 in all but one comparison (GM avidity index ratio comparing one dose with three doses of the 2-valent vaccine 0·93, 95% CI 0·88–0·97).

53 SAEs were experienced by 42 (4·5%) of 930 girls by 24 months (appendix 2 pp 6–7). Clinical malaria hospital admission was the most common SAE (50 events, 39 girls). A 10-year-old girl in the two-dose 9-valent vaccine arm died from severe malaria 4 months after vaccination. There was no evidence of a difference between arms in the number of SAEs and no SAE was related to the vaccine. We recorded 573 non-serious AEs over 24 months with no evidence of a difference between arms. The most common events were skin conditions (n=128, 22% of events), gastrointestinal conditions (n=63, 11%), and helminth infections or amoebiasis (n=63, 11%; appendix 2 p 8).

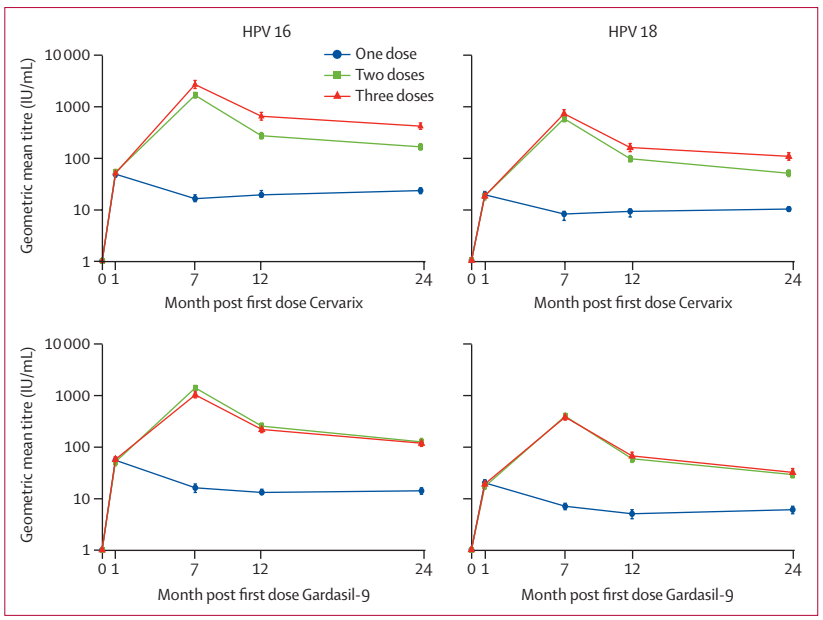

***Figure 2*: HPV 16 specific and HPV 18 specific antibody geometric means by number of HPV vaccine doses**

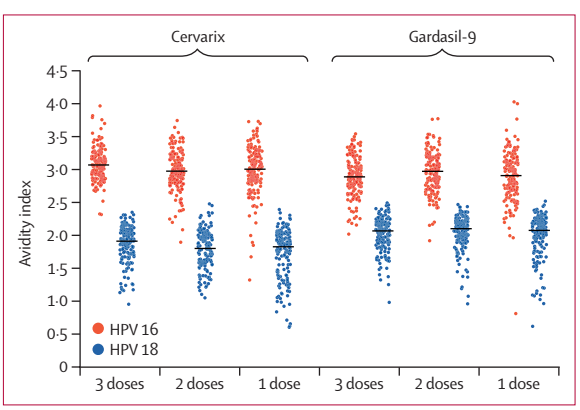

***Figure 3*: Distribution of HPV 16 and HPV 18 antibody avidity index at 24 months**

Each data point represents a single individual and the lines through the data points represents the median avidity index.

Discussion

This is the first randomised controlled trial to assess immune responses and safety of a single-dose HPV vaccine compared with two-dose or three-dose regimens among girls in the target age group for these vaccines. This is also the first trial in sub-Saharan Africa to examine immune responses to the currently recommended two-dose regimen compared with the originally recommended three-dose regimen. Our study is very timely since a randomised controlled trial in sexually active Kenyan women and girls aged 15–20 years (KEN SHE) has demonstrated excellent (>97%) efficacy with a single dose of either the 2-valent or the 9-valent vaccine against incident persistent HPV 16 or 18 infections at 18 months post-vaccination.[9] Vaccine efficacy against a broader range of oncogenic genotypes (HPV16, 18, 31, 33, 45, 52, or 58) in that trial was 88·9%. Efficacy data from randomised trials are crucial for providing evidence to support recommendations for changes to a vaccine dose regimen. The data from our study in the target age for vaccination complement these results by demonstrating a high rate of seroconversion following a single dose of HPV vaccine and robust immune responses at 2 years post-vaccination. The WHO's Strategic Advisory Group of Experts recommended updating the HPV vaccine dose schedule to allow countries to choose a one-dose or two-dose schedule for girls aged 9–14 years and young women aged 15–20 years.[19] Immunobridging of the DoRIS study results to KEN SHE immune responses is planned.

Consistent with previous studies, both vaccines in DoRIS were found to be well tolerated and no SAEs were considered related to vaccination. Malaria was the most common clinical event, which was not unexpected since malaria is endemic in the study area.

Our serology data support observations from non-randomised studies that a single dose of HPV VLP vaccines can induce strong and sustained IgG antibody

responses up to 2 years post-vaccination. HPV 16 and HPV 18 antibody GMCs reached a plateau after 7 months that was sustained to 24 months. Ongoing follow-up of this cohort will allow us to determine if these antibody concentrations remain stable over time, as observed following one dose of the 4-valent vaccine in India, where antibody levels have been stable for 4 years and efficacy has been demonstrated for 9 years, and the 2-valent vaccine in Costa Rica, where antibody levels have been stable for 11 years and efficacy has been demonstrated for 11 years.[7,8] In our study, antibody responses to two and three doses of the vaccines peaked 1 month after the last dose and then declined thereafter to 24 months. The post-vaccination antibody kinetics we have observed provide reassurance that immune responses to these vaccines in sub-Saharan African girls are similar to those seen in other geographical regions.

As has been shown in the CVT, PATRICIA, and India/IARC studies, antibody GMCs following one dose of HPV vaccine in the DoRIS trial were lower compared with GMCs after two or three doses.[6] However, these other studies have shown that protection provided by one dose against persistent HPV 16 and HPV 18 infection, the genotypes that cause 70% of cervical cancer cases, was no different than that offered by two and three doses.[6–8] Encouragingly, the first randomised trial assessing the efficacy of one dose (KEN SHE) has demonstrated that a single dose of the 2-valent or 9-valent HPV vaccine had 97·5% efficacy against persistent HPV 16 and HPV 18 infection at 18 months compared with the control vaccine.[9]

There is no known immune correlate of protection for L1 VLP HPV vaccines, but antibody responses are considered essential in the protection conferred by these vaccines. A single dose of a VLP HPV vaccine might be sufficient to protect against HPV infection and its sequelae for several reasons.[20] Passive transfer of serum or IgG from VLP-vaccinated animals to unvaccinated animals protects unvaccinated animals from papillomas associated with the cottontail rabbit papillomavirus.[21] Antibodies induced by the virus neutralise the virus in vitro and, in addition, within-trial cross-protection and in vitro cross-neutralisation are also mirrored. The recombinant, type-specific L1 capsid proteins comprising the VLPs in the current HPV vaccines are highly immunogenic with a large number of repetitive epitopes that self-assemble and mimic HPV virions.[22] Similar arrays are known to induce long-lasting and stable humoral responses and it seems that the structure of VLP vaccines allow these vaccines to induce durable immune responses more characteristically seen with other viruses and live vaccines that present high density epitopes.[20]

Although we did not meet the non-inferiority criterion for anti-HPV 18 seropositivity at 24 months after a single dose of either vaccine, more than 98% of girls were anti-HPV 18 seropositive at that timepoint, and non-inferiority was met for anti-HPV 16 seropositivity. In the primary analyses, we did not adjust for multiplicity of testing. However, there remained good evidence of non-inferiority for HPV 16 when using a more stringent 97·5% CI in accordance with the Bonferroni correction.

The two-dose vaccine schedule is being offered in many countries, including in sub-Saharan Africa, following the change in recommendation from three to two doses.[23] Several randomised trials previously reported non-inferior GMCs following two doses of the 2-valent and 4-valent HPV vaccines in young girls, compared with three-dose GMCs in women.[24] In our study, where immune responses following two-dose and three-dose schedules were compared in the same age groups, HPV 16 and HPV 18 GMCs for two doses were non-inferior to three doses for the 9-valent vaccine, but non-inferiority was not met for the 2-valent vaccine where a third dose of the vaccine led to a further rise in antibody concentrations. Since an immune correlate of protection is undefined, the significance of this finding is unclear.

This is the first study in sub-Saharan Africa to measure HPV antibody avidity to any dose of HPV vaccine. Avidity is believed to reflect the degree of antibody affinity maturation and reflects how strongly the antibody binds to its target antigen. The India/IARC trial examined the HPV 16 antibody avidity index generated by the 4-valent vaccine in a subset of plasma samples by use of a modified HPV-L1 genotype-specific binding antibody assay.[25] In that trial, the 18-month GM HPV 16 and HPV 18 antibody avidity index after one dose of the 4-valent vaccine was non-inferior to that after three doses. In the CVT, the avidity index increased with the number of doses of the 2-valent vaccine but, within each dose group, avidity index was stable up to 7 years.[26] In the Netherlands, no difference was seen in HPV 16 antibody avidity at 5 years following one, two, or three doses of the 2-valent vaccine, but HPV 18 avidity was higher for one dose than for two or three doses.[27] Some evidence suggests that antibody avidity might be affected by vaccine adjuvants. For hepatitis B vaccines, one study found that avidity maturation was more strongly promoted by the Adjuvant System (AS)01_B, $AS01_E$, AS03, and AS04 compared with the Alum adjuvant.[28] However, although the 2-valent (containing the ASO4 adjuvant) and 9-valent vaccines have different adjuvants, we found no evidence of a difference between the different dose schedules in GM antibody avidity index for HPV 16 or HPV 18, and the GM antibody avidity index at 24 months was similar between the vaccines when given at the same dose, suggesting that other factors might influence the antibody affinity maturation for these vaccines.

Our study found that vaccination with the 2-valent vaccine resulted in higher concentrations of HPV 16 and HPV 18 IgG antibodies compared with the 9-valent vaccine at any dose. These results are similar to other studies that compared the 2-valent and 4-valent vaccines.[29]

Despite this, both vaccines have extremely high efficacy against persistent HPV 16 and HPV 18 infection and related sequelae, such as grade 2 or higher cervical intraepithelial neoplasia. A study showed that, with a cost-effectiveness threshold of per-capita gross decimal product, a 2-valent vaccine (with cross-protection to other genotypes) would avert 17·2 million cervical cancer cases and the 9-valent vaccine would avert 18·5 million cervical cancer cases in Gavi, the vaccine alliance-eligible countries.[30] Costing data from our study and from the national HPV vaccination programme in Tanzania suggest that a one-dose schedule would be cost-saving and that delivery could be done at costs that would make HPV vaccination a very cost-effective intervention.[31]

Study limitations include the sample size that did not allow us to evaluate efficacy of single dose HPV vaccination in this population. However, as has been done for other HPV vaccination studies and following recommendations arising from a WHO–IARC workshop in 2013, we have immunobridged our results to cohorts in which efficacy of a one-dose schedule has been demonstrated, and results are presented in the companion paper.[14]

Our study has several strengths. We enrolled girls in Tanzania which, like many countries in sub-Saharan Africa, bears a high burden of cervical cancer and associated mortality. The representativeness of the trial setting and study population will allow the results to be generalisable to other parts of the continent. The study population is also in the target age group for vaccination, allowing us to evaluate antibody responses to one and two doses over time as girls pass through puberty. The study had excellent retention at 2 years and nearly all participants were vaccinated according to protocol. Immunogenicity analyses for HPV 16 and HPV 18 immune responses were done in a laboratory with significant expertise in HPV serology that has participated in numerous studies of single-dose HPV vaccine responses.[18] The inclusion of HPV 16 and HPV 18 antibody avidity is novel and encouragingly showed that a single dose of vaccine had similar avidity compared with two or three doses of the same vaccine.

Our findings show that, in healthy African girls living in a malaria-endemic region, a single dose of the 2-valent or 9-valent HPV vaccines was well tolerated and resulted in high seropositivity rates and induced stable vaccine responses that persisted to 2 years. Antibody kinetics were similar to other studies in older females in other countries. Follow-up of the DoRIS cohort is continuing to provide data on durability and stability of single dose immune responses. New vaccine efficacy results for single dose in sexually active women are encouraging and efficacy data from observational studies are available up to 9–11 years. A single-dose regimen could encourage countries that have not yet included HPV vaccines in their national vaccination programmes to now introduce these vaccines. A single dose might also allow countries to do one-off activities to reach girls who missed HPV vaccination, including during the COVID-19 pandemic, and to focus on achieving high one-dose coverage rates which could in turn provide faster herd immunity to unvaccinated individuals. All these steps will contribute to the WHO cervical cancer elimination strategy targets for 2030.

Contributors

DW-J and KB developed the initial idea and design of the study. DW-J, KB, RJH, CJL, JC, HW, PMa, SdS, and JD developed the protocol and were involved in the interpretation of the results. DW-J was responsible for funding acquisition. DW-J and KB were the joint principal investigators for the study and JC was the site principal investigator in Mwanza. DW-J, JC, HW, SK, PMu, TS, JI, BL, DM, BK, GM, and NC were responsible for the on-site conduct of the study and participated in monitoring and supervision of data collection. KB was responsible for the study statistical analysis and RH for data management of the study. JI, LP, TK, RW, CJL, SdS, MAP, and BL were responsible for laboratory aspects of the study. DM was the trial pharmacist and was responsible for study vaccine dispensing. DW-J drafted the manuscript and all authors commented and contributed to the final version. The corresponding author had full access to the data in the study and had responsibility for the final decision to submit the manuscript for publication.

Declaration of interests

DW-J reports a grant from GSK Biologicals in 2007 for a previous study on safety and immunogenicity of Cervarix in Tanzania unrelated to this submitted work. All other authors declare no competing interests.

Data sharing

De-identified participant data presented in this manuscript can be made available after publication following written request to the London School of Hygiene & Tropical Medicine and the Mwanza Intervention Trials Unit, Tanzania. Requests must be accompanied by a defined analysis plan addressed to the corresponding author which will be reviewed by the Mwanza Intervention Trials Unit Data Sharing Committee and senior investigators at the London School of Hygiene & Tropical Medicine. Requesting researchers will be required to sign a Data Access Agreement if approval is given.

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Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Watson-Jones D, Changalucha J, Whitworth H, et al. Immunogenicity and safety of one-dose human papillomavirus vaccine compared with two or three doses in Tanzanian girls (DoRIS): an open-label, randomised, non-inferiority trial. *Lancet Glob Health* 2022; **10:** e1473–84.

Kingamwili na usalama wa dozi moja ya chanjo ya virusi vya papilloma ikilinganishwa na dozi mbili au tatu kwa wasichana wa Tanzania (DoRIS): Utafiti wa wazi, unaotumia mbinu ya bahati nasibu na kutokuwa duni.

Utangulizi

Inakadiriwa asilimia 15 ya wasichana wenye umri wa miaka 9-14 duniani kote wamechanjwa dhidi ya virusi vya papilloma ya binadamu (VIPABI) kwa kutumia utaratibu uliopendekezwa wa dozi mbili au tatu. Utaratibu wa kutoa dozi moja ya chanjo ungekuwa rahisi na nafuu. Tunaripoti matokeo ya kinga ya mwili na usalama wa dozi tofauti za chanjo mbili za VIPABI kwa wasichana wa kitanzania.

Mbinu

Kwenye utafiti huu wa wazi, wa kutumia mbinu ya bahati nasibu, wa hatua ya tatu na kutokuwa duni, tulisajili wasichana wenye afya njema waliokuwa na umri wa miaka 9 -14 kutoka shule za serikali, Mwanza, Tanzania. Washiriki waliostahili kushiriki waliwekwa kwa njia ya bahati nasibu kupokea dozi moja, mbili au tatu ya ama chanjo ya valenti 2 (Cervarix, GSK Biologicals, Rixensart) au chanjo ya valenti 9(Gardasil-9, Sanofi Pasteur MSD, Lyon). Matokeo ya msingi ilikuwa ni kuwepo kwa kingamwili mahsusi kwa VIPABI 16 au 18 baada ya dozi moja ikilinganishwa na dozi mbili au tatu za chanjo hiyo hiyo ya VIPABI miezi 24 baada ya kuchanjwa. Usalama wa chanjo ulitathminiwa kwa madhara yalitajwa na mshiriki baada ya kuulizwa ndani ya siku 30 baada ya kila chanjo na madhara ya chanjo ambayo yalitajwa na mshiriki bila kuulizwa katika kipindi hadi miezi 24 baada ya kuchanjwa au mpaka hudhurio la mwisho. Matokeo ya msingi yalihusisha washiriki waliofuata protokali na usalama wa chanjo uliangaliwa kwa washiriki wote waliochanjwa. Utafiti umesajiliwa kwenye clinicaltrials.gov, NCT02834637

Matokeo

Kati ya Februari 23, 2017 na Januari 6, 2018, wasichana wapatao 1002 walifanyiwa tathmini kuona kama wanastahili kushiriki kwenye utafiti. Wasichana 72 hawakuwa na sifa za kushiriki na 22 walikuwa na sifa za kushiriki lakini hawakuhudhuria hudhurio la uandikishaji. Wasichana 930 waliandikishwa na kwa kutumia njia ya bahati nasibu walipangwa kupokea dozi moja ya Cervarix(washiriki 155), dozi mbili za Cervarix(washiriki 155) na dozi tatu za Cervarix(washiriki 155), dozi moja ya Gardasil-9(washiriki 155), dozi mbili za Gardasil-9(washiriki 155) au dozi tatu za Gardasil-9(washiriki 155). Washiriki 922 walipokea dozi zote kwa kufuata utaratibu uliopangwa na ndani ya kipindi maalum kilichowekwa (watatu walijitoa, mmoja alipotea kwenye ufuatiliaji, na mmoja alifariki kabla ya kumaliza; wawili walipokea dozi zao za miezi sita kabla ya kipindi maalumu kilichopangwa na mmoja alipokea chanjo isiyo ya valenti sahihi kimakosa; washiriki wote 930 waliingizwa kwenye kundi la washiriki waliochanjwa. Washiriki 918 (99%) katika ya 930 waliweza kuhudhuria hadi mwezi wa 24. Katika kundi la washiriki waliofanya mahudhurio yao kwa kufuata protokali(ATP), mpaka mwezi wa 24, asilimia 99 ya washiriki waliopokea dozi moja ya chanjo yoyote ya VIPABI walikuwa na kingamwili (IgG) mahususi dhidi ya VIPABI 16, ikilinganishwa na asilimia 100 ya washiriki waliopokea dozi mbili na asilimia 100 ya washiriki waliopokea dozi tatu. Hii iliweze kutimiza kigezo kilichowekwa awali ya kutokuwa duni. Kingamwili mahususi dhidi ya VIPABI18 katika mwezi wa 24 haikukidhi kigezo cha kutokuwa duni kwa dozi moja ikilinganishwa na dozi mbili au dozi tatu kwa chanjo zote, ingawa zaidi ya asilimia 98 ya wasichana kwenye makundi yote ya chanjo walikuwa na kingamwili dhidi ya VIPABI 18. Matukio 53 ya madhara makubwa (SAE) yalitokea kwa washiriki 42 (4.5%) kati ya 930, tukio lililotokea mara nyingi kuliko yote lilikuwa kulazwa hospitali kwa ajili ya malaria. Msichana mmoja alifariki kwa ajili ya malaria. Idadi ya matukio ilikuwa sawa kwa makundi yote ya chanjo na hakuna matukio ya madhara makubwa ambayo yalihusishwa na kuchanjwa.

Tafsiri

Dozi moja ya valenti 2 au valenti 9 ya chanjo ya VIPABI kwa wasichana wa miaka 9- 14 ilitoa mwitikio thabiti wa kinga ya mwili hadi miezi 24, ikionyesha kuwa utaratibu wa kutoa dozi pungufu unaweza kufaa katika kinga dhidi ya maambukiz ya VIPABI kwa wasichana walio kwenye kundi la umri uliolengwa kwa ajili ya kuchanjwa.

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Supplementary appendix 2

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Supplementary Table 1. Comparisons of antibody seropositivity after 1, 2 or 3 doses of HPV vaccine at M24, with 97.5% confidence intervals to account for multiple comparisons

	1 dose		2 doses		3 doses		Difference in seropositivity[1] (exact 97.5% CI)		
	N	Seropositive[1] (%)	N	Seropositive[1] (%)	N	Seropositive[1] (%)	1 dose – 2 dose	1 dose – 3 dose	2 dose – 3 dose
According to protocol (ATP) cohort[2]									
2-valent									
HPV16	148	147 (99.3%)	141	141 (100.0%)	141	141 (100.0%)	-0.7% (-4.5- 2.9)	-0.7% (-4.5- 2.9)	0[3]
HPV18	141	139 (98.6%)	140	140 (100.0%)	136	136 (100.0%)	-1.4% (-5.9- 2.1)	-1.4% (-5.9- 2.2)	0[3]
9-valent									
HPV16	145	144 (99.3%)	141	141 (100.0%)	140	140 (100.0%)	-0.7% (-4.6- 2.8)	-0.7% (-4.7- 2.9)	0[3]
HPV18	136	133 (97.8%)	136	136 (100.0%)	142	141 (99.3%)	-2.2% (-7.3- 1.4)	-1.5% (-6.7- 2.7)	-0.7% (-4.7- 3.0)
Total vaccinated cohort (TVC)[4]									
2-valent									
HPV16	154	153 (99.4%)	151	151 (100.0%)	154	154 (100.0%)	-0.6% (-4.3- 2.6)	-0.6% (-4.4- 2.7)	0[3]
HPV18	154	152 (98.7%)	151	151 (100.0%)	154	154 (100.0%)	-1.3% (-5.5- 2.0)	-1.3% (-5.4- 2.0)	0[3]
9-valent									
HPV16	152	151 (99.3%)	153	153 (100.0%)	154	154 (100.0%)	-0.7% (-4.4- 2.6)	-0.7% (-4.5- 2.6)	0[3]
HPV18	152	149 (98.0%)	153	153 (100.0%)	154	153 (99.4%)	-2.0% (-6.5- 1.3)	-1.3% (-6.0- 2.7)	-0.6% (-4.4- 2.6)

[1]Seropositivity defined as titres above the laboratory determined cut-off (HPV16 = 1.309 IU/mL; HPV18 = 1.109 IU/mL). [2]DoRIS participants who were ELISA antibody negative and DNA negative at baseline (pre-vaccination) for the HPV genotype under analysis. [3]Exact 95% confidence intervals for the difference using method of Chang and Zhang cannot be calculated because both proportions are 1.0, but there is still uncertainty around the point estimate. [4]DoRIS participants who received at least one dose of vaccine, analysed in their randomised arm irrespective of doses or vaccine received, or their HPV DNA or serostatus at baseline.

Supplementary Table 2. Comparisons of antibody seropositivity post HPV vaccination with 1, 2 or 3 doses of 2-valent or 9-valent (total vaccinated cohort[1])

	1 dose		2 doses		3 doses		Difference in seropositivity[2] (exact 95% CI)		
	N	Seropositive[2] (%)	N	Seropositive[2] (%)	N	Seropositive[2] (%)	1 dose – 2 dose	1 dose – 3 dose	2 dose – 3 dose
2-valent									
HPV-16									
Month 7	154	153 (99.4%)	152	152 (100.0%)	154	153 (99.4%)	-0.6% (-3.7- 1.9)	0.0% (-3.0- 3.0)	0.6% (-1.9- 3.7)
Month 12	153	152 (99.3%)	150	150 (100.0%)	154	154 (100.0%)	-0.7% (-3.7- 2.0)	-0.7% (-3.7- 1.9)	0[3]
Month 24	154	153 (99.4%)	151	151 (100.0%)	154	154 (100.0%)	-0.6% (-3.6- 1.9)	-0.6% (-3.7- 1.8)	0[3]
HPV-18									
Month 7	154	152 (98.7%)	152	152 (100.0%)	154	153 (99.4%)	-1.3% (-4.7- 1.2)	-0.6% (-4.1- 2.4)	0.6% (-1.9- 3.7)
Month 12	153	152 (99.3%)	150	150 (100.0%)	154	154 (100.0%)	-0.7% (-3.7- 2.0)	-0.7% (-3.7- 1.9)	0[3]
Month 24	154	152 (98.7%)	151	151 (100.0%)	154	154 (100.0%)	-1.3% (-4.7- 1.3)	-1.3% (-4.7- 1.2)	0[3]
9-valent									
HPV-16									
Month 7	151	151 (100.0%)	154	154 (100.0%)	154	154 (100.0%)	0[3]	0[3]	0[3]
Month 12	152	152 (100.0%)	154	154 (100.0%)	154	154 (100.0%)	0[3]	0[3]	0[3]
Month 24	152	151 (99.3%)	153	153 (100.0%)	154	154 (100.0%)	-0.7% (-3.7- 1.9)	-0.7% (-3.7- 1.9)	0[3]
HPV-18									
Month 7	151	149 (98.7%)	154	154 (100.0%)	154	154 (100.0%)	-1.3% (-4.7- 1.2)	-1.3% (-4.7- 1.2)	0[3]
Month 12	152	147 (96.7%)	154	154 (100.0%)	154	154 (100.0%)	-3.3% (-7.6- -0.6)	-3.3% (-7.6- -0.6)	0[3]
Month 24	152	149 (98.0%)	153	153 (100.0%)	154	153 (99.4%)	-2.0% (-5.7- 0.6)	-1.3% (-5.1- 1.8)	0.6% (-1.9- 3.6)

[1]DoRIS participants who received at least one dose of vaccine, analysed in their randomised arm irrespective of doses or vaccine received, or their HPV DNA or serostatus at baseline. [2]Titres above the laboratory determined cut-off (HPV16 = 1.309 IU/mL; HPV18 = 1.109 IU/mL). [3]Exact 95% confidence intervals for the difference using method of Chang and Zhang cannot be calculated because both proportions are 1.0, but there is still uncertainty around the point estimate.

Supplementary Table 3. HPV16 and HPV18 antibody geometric mean titres (GMT) at all visits, by dose group and vaccine (total vaccinated cohort[1])

	1 dose		2 doses		3 doses		GMT ratio (95% CI)[3] 2 dose/ 3 doses
	N[1]	GMT[2] (95% CI) (IU/mL)	N[1]	GMT[2] (95% CI) (IU/mL)	N[1]	GMT[2] (95% CI) (IU/mL)	
2-valent							
HPV 16							
Day 0	155	<LLQ[4]	155	<LLQ[4]	155	<LLQ[4]	–
Month 1	155	50 (43 -57)	154	52 (46 -59)	155	51 (44 -59)	–
Month 7	154	16 (14 -19)	152	1641 (1453 -1854)	154	2501 (2084 -3002)	0.66 (0.53 -0.81)
Month 12	153	19 (17 -23)	150	267 (233 -307)	154	623 (530 -733)	0.43 (0.35 -0.53)
Month 24	154	23 (20 -26)	151	163 (142 -187)	154	402 (351 -461)	0.40 (0.33 -0.50)
HPV 18							
Day 0	155	<LLQ[4]	155	<LLQ[4]	155	<LLQ[4]	–
Month 1	155	19 (16 -22)	154	18 (16 -21)	155	20 (17 -23)	–
Month 7	154	8 (6 -9)	152	593 (517 -679)	154	708 (594 -845)	0.84 (0.68 -1.04)
Month 12	153	8 (7 -10)	150	93 (80 -107)	154	158 (134 -187)	0.59 (0.47 -0.72)
Month 24	154	10 (8 -11)	151	51 (44 -58)	154	106 (91 -124)	0.48 (0.39 -0.59)
9-valent							
HPV 16							
Day 0	155	<LLQ[4]	155	<LLQ[4]	155	<LLQ[4]	–
Month 1	154	56 (48 -65)	155	51 (44 -59)	155	59 (53 -66)	–
Month 7	151	16 (14 -20)	154	1376 (1237 -1531)	154	1017 (897 -1154)	1.35 (1.11 -1.65)
Month 12	152	14 (12 -15)	154	249 (217 -285)	154	218 (191 -249)	1.14 (0.94 -1.39)
Month 24	152	14 (12 -16)	153	123 (106 -142)	154	117 (102 -135)	1.05 (0.87 -1.28)
HPV 18							
Day 0	155	<LLQ[4]	155	<LLQ[4]	155	<LLQ[4]	–
Month 1	154	20 (17 -23)	155	17 (15 -20)	155	20 (17 -22)	–
Month 7	151	7 (6 -8)	154	398 (354 -449)	154	386 (338 -440)	1.03 (0.84 -1.27)
Month 12	152	5 (5 -6)	154	58 (50 -68)	154	68 (58 -79)	0.86 (0.69 -1.06)

[1]DoRIS participants who received at least one dose of vaccine, irrespective of their HPV DNA or serostatus at baseline. [2]ELISA serum antibody geometric mean titre (GMT). [3]Estimated with linear mixed effect model with log antibody titre as the response and dose group, time point, and a dose group-time interaction term as fixed effects, and participant as a random effect to account for correlation of repeated measurements within participant. [4]Lower limit of quantitation.

Supplementary Table 4. Comparisons of geometric mean (GM) antibody avidity index (AI) after 1, 2 or 3 doses of HPV vaccine in DoRIS trial (ATP cohort[1])

	1 dose		2 doses		3 doses		Geometric mean AI ratio[3] (95% CI)		
	N	GM avidity index[2]	N	GM avidity index[2]	N	GM avidity index[2]	1 dose / 2 dose	1 dose / 3 dose	2 dose / 3 dose
2-valent									
HPV-16									
Month 12	147	2.73 (2.66 -2.81)	140	2.82 (2.77 -2.88)	141	2.96 (2.92 -3.01)	0.97 (0.94 -0.99)	0.92 (0.90 -0.95)	0.95 (0.93 -0.98)
Month 24	148	2.95 (2.89 -3.02)	141	2.97 (2.91 -3.02)	141	3.08 (3.04 -3.12)	1.00 (0.97 -1.02)	0.96 (0.93 -0.99)	0.96 (0.94 -0.99)
HPV-18									
Month 12	140	1.57 (1.51 -1.64)	139	1.73 (1.68 -1.78)	136	1.79 (1.73 -1.84)	0.91 (0.87 -0.96)	0.88 (0.84 -0.93)	0.97 (0.92 -1.02)
Month 24	141	1.69 (1.62 -1.76)	140	1.76 (1.70 -1.81)	136	1.82 (1.77 -1.88)	0.96 (0.92 -1.01)	0.93 (0.88 -0.97)	0.96 (0.92 -1.01)
9-valent									
HPV-16									
Month 12	145	2.59 (2.51 -2.67)	142	2.86 (2.81 -2.92)	140	2.74 (2.68 -2.79)	0.90 (0.88 -0.93)	0.95 (0.92 -0.98)	1.05 (1.01 -1.08)
Month 24	145	2.86 (2.79 -2.94)	141	2.94 (2.89 -3.00)	140	2.88 (2.82 -2.93)	0.97 (0.94 -1.00)	1.00 (0.96 -1.03)	1.02 (0.99 -1.06)
HPV-18									
Month 12	136	1.92 (1.86 -1.98)	137	2.03 (1.98 -2.08)	142	1.95 (1.90 -2.00)	0.95 (0.91 -0.98)	0.98 (0.95 -1.02)	1.04 (1.00 -1.08)
Month 24	136	1.98 (1.92 -2.05)	136	2.05 (2.01 -2.10)	142	1.99 (1.95 -2.04)	0.97 (0.93 -1.00)	1.00 (0.96 -1.03)	1.03 (0.99 -1.07)

[1]According to protocol: DoRIS participants who were ELISA antibody negative and DNA negative at baseline (pre-vaccination) for the HPV genotype under analysis.
[2]Geometric mean avidity index. [3]Estimated with linear mixed effect model with log avidity index as the response and dose group, time point, and a dose group-time interaction term as fixed effects, and participant as a random effect to account for correlation of repeated measurements within participant.

Supplementary Table 5. Number of participants with at least one serious adverse event, and number of events, by trial arm from enrolment to Month 24 visit (total vaccinated cohort)

		1D 2-valent (N=155)	2D 2-valent (N=155)	3D 2-valent (N=155)	1D 9-valent (N=155)	2D 9-valent (N=155)	3D 9-valent (N=155)	Total (N=930)
All SAEs	Number of girls (%)	8 (5.2 %)	4 (2.6 %)	6 (3.9 %)	8 (5.2 %)	8 (5.2 %)	8 (5.2 %)	42 (4.5 %)
	(Number of events)	*(15)*	*(4)*	*(7)*	*(8)*	*(9)*	*(10)*	*(53)*
Components of SAEs								
Death	Number of girls (%)	0 (–)	0 (–)	0 (–)	0 (–)	1 (0.6 %)	0 (–)	1 (0.1 %)
	(Number of events)	*(0)*	*(0)*	*(0)*	*(0)*	*(1)*	*(0)*	*(1)*
Hospitalisation	Number of girls (%)	8 (5.2 %)	3 (1.9 %)	6 (3.9 %)	7 (4.5 %)	7 (4.5 %)	8 (5.2 %)	39 (4.2 %)
	(Number of events)	*(15)*	*(3)*	*(7)*	*(7)*	*(8)*	*(10)*	*(50)*
Life-threatening condition	Number of girls (%)	0 (–)	0 (–)	0 (–)	0 (–)	0 (–)	0 (–)	0 (–)
Persistent disability	Number of girls (%)	0 (–)	0 (–)	0 (–)	0 (–)	0 (–)	0 (–)	0 (–)
Congenital abnormality	Number of girls (%)	0 (–)	1 (0.6 %)	0 (–)	0 (–)	0 (–)	0 (–)	1 (0.1 %)
	(Number of events)	*(0)*	*(1)*	*(0)*	*(0)*	*(0)*	*(0)*	*(1)*
Other medically important event	Number of girls (%)	0 (–)	0 (–)	0 (–)	1 (0.6 %)	0 (–)	0 (–)	1 (0.1 %)
	(Number of events)	*(0)*	*(0)*	*(0)*	*(1)*	*(0)*	*(0)*	*(1)*

Supplementary Table 6. Serious adverse event by diagnosis and trial arm, from enrolment to Month 24 visit (total vaccinated cohort)

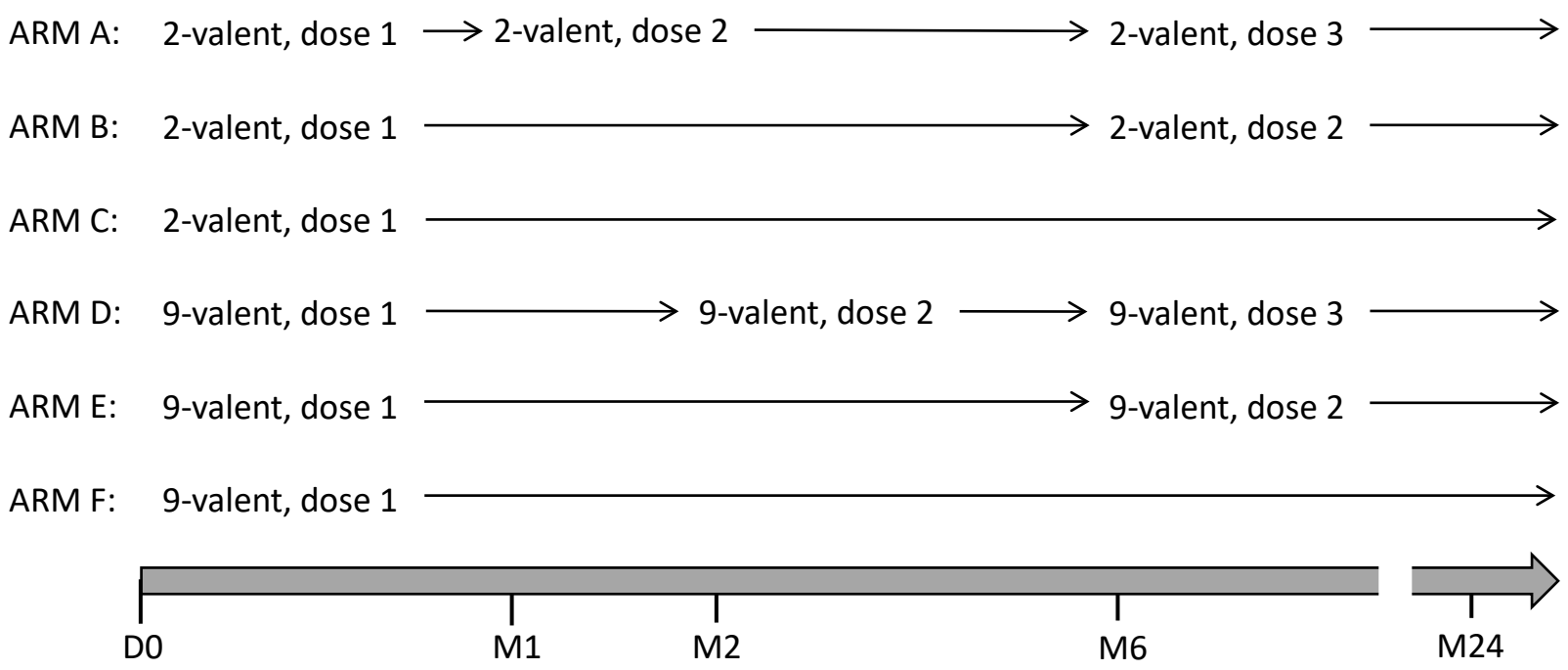
Number of events	1D 2-valent	2D 2-valent	3D 2-valent	1D 9-valent	2D 9-valent	3D 9-valent	Total
Severe malaria	14	3	3	6	9	9	44
Urinary tract infection	0	0	1	0	0	1	2
Gastroenteritis	0	0	0	1	0	0	1
Dehydration due to fever	1	0	0	0	0	0	1
Vasovagal syncope	0	0	2	0	0	0	2
Snake bite	0	0	1	0	0	0	1
Spontaneous abortion	0	0	0	1	0	0	1
Congenital anomaly	0	1	0	0	0	0	1
Total events	**15**	**4**	**7**	**8**	**9**	**10**	**53**

Supplementary Table 7. Number of non-serious adverse events[1], by trial arm, from enrolment to M24 visit (total vaccinated cohort)

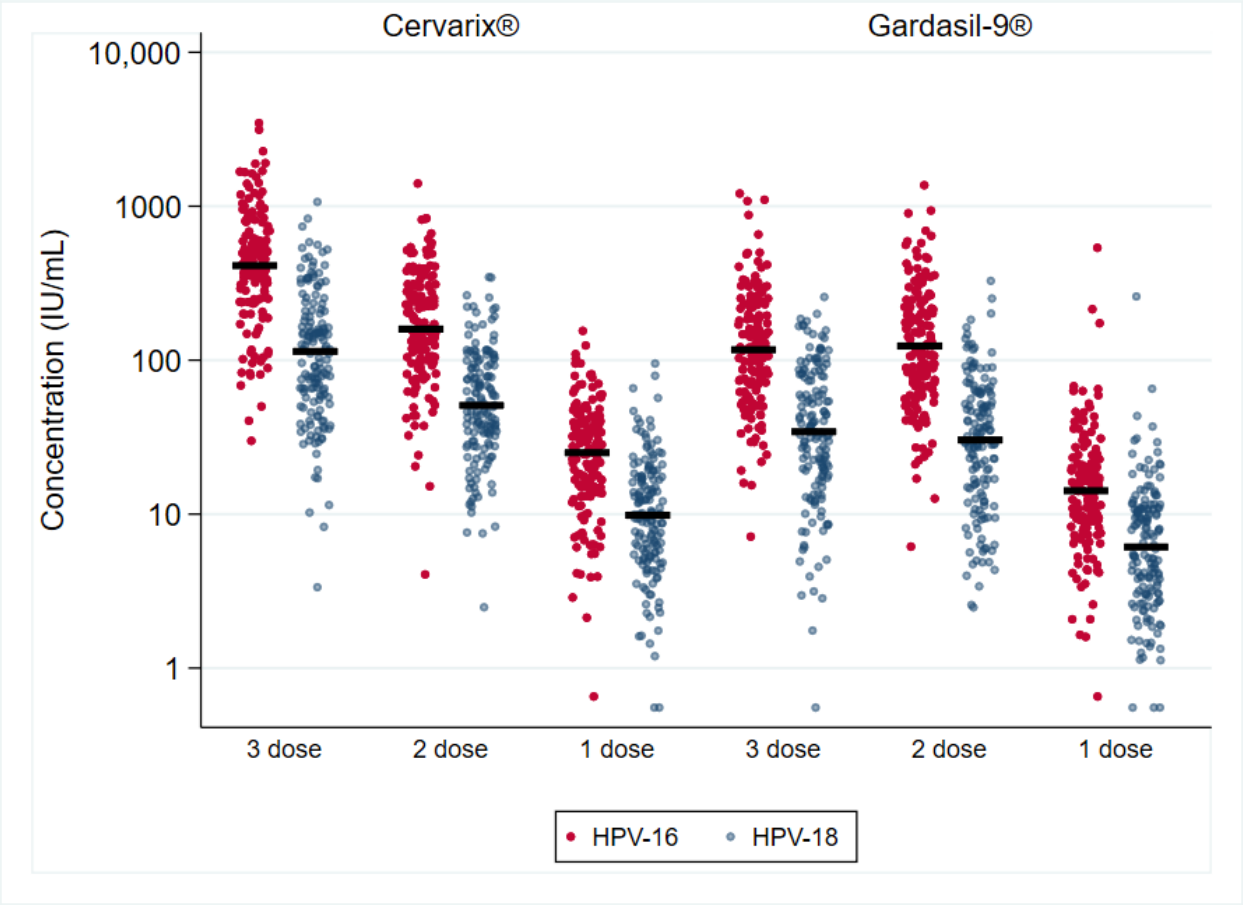
Adverse event	1D 2-valent N events (% of all events)	2D 2-valent N events (% of all events)	3D 2-valent N events (% of all events)	1D 9-valent N events (% of all events)	2D 9-valent N events (% of all events)	3D 9-valent N events (% of all events)	Total N events (% of all events)
Malaria[2]	5 (6.5 %)	11 (10.9%)	5 (4.9 %)	2 (2.3 %)	7 (6.9 %)	13 (12.4%)	43 (7.5 %)
Fever / headache[3]	3 (3.9 %)	2 (2.0 %)	1 (1.0 %)	2 (2.3 %)	0 (–)	4 (3.8 %)	12 (2.1 %)
Skin / dermatological problem	21 (27.3%)	19 (18.8%)	25 (24.5%)	31 (35.6%)	12 (11.9%)	20 (19.0%)	128 (22.3%)
Gastrointestinal disorder[4]	5 (6.5 %)	8 (7.9 %)	9 (8.8 %)	12 (13.8%)	20 (19.8%)	9 (8.6 %)	63 (11.0%)
Respiratory disorder	7 (9.1 %)	9 (8.9 %)	8 (7.8 %)	3 (3.4 %)	13 (12.9%)	9 (8.6 %)	49 (8.6 %)
Urinary tract / renal disorder	5 (6.5 %)	5 (5.0 %)	8 (7.8 %)	3 (3.4 %)	3 (3.0 %)	7 (6.7 %)	31 (5.4 %)
Orthopaedic disorder	0 (–)	0 (–)	0 (–)	0 (–)	0 (–)	1 (1.0 %)	1 (0.2 %)
Helminth infection / amoebiasis /schistosomiasis	17 (22.1%)	9 (8.9 %)	10 (9.8 %)	10 (11.5%)	4 (4.0 %)	13 (12.4%)	63 (11.0%)
Accidental injury	3 (3.9 %)	4 (4.0 %)	5 (4.9 %)	0 (–)	1 (1.0 %)	2 (1.9 %)	15 (2.6 %)
Minor surgery/dental disorders	1 (1.3 %)	3 (3.0 %)	5 (4.9 %)	3 (3.4 %)	4 (4.0 %)	1 (1.0 %)	17 (3.0 %)
Eye disorder	2 (2.6 %)	7 (6.9 %)	9 (8.8 %)	5 (5.7 %)	18 (17.8%)	7 (6.7 %)	48 (8.4 %)
ENT disorder	2 (2.6 %)	10 (9.9 %)	8 (7.8 %)	7 (8.0 %)	7 (6.9 %)	11 (10.5%)	45 (7.9 %)
Haematological disorder	1 (1.3 %)	5 (5.0 %)	1 (1.0 %)	0 (–)	4 (4.0 %)	0 (–)	11 (1.9 %)
Sexually transmitted infection	0 (–)	0 (–)	1 (1.0 %)	0 (–)	0 (–)	1 (1.0 %)	2 (0.3 %)
Neurological disorder	2 (2.6 %)	3 (3.0 %)	2 (2.0 %)	2 (2.3 %)	2 (2.0 %)	0 (–)	11 (1.9 %)
Cardiovascular disorder	1 (1.3 %)	2 (2.0 %)	1 (1.0 %)	0 (–)	0 (–)	2 (1.9 %)	6 (1.0 %)
Gynaecological disorder	1 (1.3 %)	1 (1.0 %)	2 (2.0 %)	0 (–)	1 (1.0 %)	0 (–)	5 (0.9 %)
Musculoskeletal disorder	1 (1.3 %)	3 (3.0 %)	1 (1.0 %)	6 (6.9 %)	5 (5.0 %)	4 (3.8 %)	20 (3.5 %)
Other	0 (–)	0 (–)	1 (1.0 %)	1 (1.1 %)	0 (–)	1 (1.0 %)	3 (0.5 %)
All non-serious AEs	**77 (100%)**	**101 (100%)**	**102 (100%)**	**87 (100%)**	**101 (100%)**	**105 (100%)**	**573 (100%)**

[1]Note: more than one adverse event may be recorded for a participant on the same date, if symptoms are judged to be result of more than one condition. [2]Confirmed or suspected malaria. [3]Fever/headache without associated malaria. [4]Gastrointestinal disorder without associated malaria.

Supplementary Figure 1 Vaccination schedule by arm



Supplementary Figure 2. Distribution of HPV-16 and HPV-18 antibody concentrations (IU/mL) at 24 months by arm. Each data point represents a single individual and the line through the data points represents the median concentration



Paper 5 Comparing one dose of HPV vaccine in girls aged 9-14 years in Tanzania (DoRIS) with one dose of HPV vaccine in historical cohorts: an immunobridging analysis of a randomised controlled trial

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Thesis Title	HPV epidemiology and HPV vaccination in East Africa		
Primary Supervisor	Deborah Watson-Jones		

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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)	This paper presents the results from the primary immunobridging objective of the DoRIS trial. I am the joint Principal Investigator (PI), along with Deborah Watson-Jones, on the DoRIS trial. In collaboration Deborah Watson-Jones, I co-developed the initial ideas, designed the study, secured the funding and developed the study protocol and questionnaires. I am also the DoRIS trial statistician, and had overall responsibility for the trial data management and statistical analyses. I wrote the protocol and analysis plan for the immunobriding component of the trial presented in this paper, analysed the data and interpreted the results. I am the first and corresponding author, wrote the first draft of the manuscript, and was responsible for replying to reviewers'comments during the peer-review process.

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Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose of HPV vaccine in historical cohorts: an immunobridging analysis of a randomised controlled trial

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Summary

Background Human papillomavirus (HPV) vaccines are given as a two-dose schedule in children aged 9–14 years, or as three doses in older individuals. We compared antibody responses after one dose of HPV vaccine in the Dose Reduction Immunobridging and Safety Study (DoRIS), a randomised trial of different HPV vaccine schedules in Tanzania, to those from two observational HPV vaccine trials that found high efficacy of one dose up to 11 years against HPV16 and HPV18 (Costa Rica Vaccine Trial [CVT] and Institutional Agency for Research on Cancer [IARC] India trial).

Methods In this immunobridging analysis of an open-label randomised controlled trial, girls were recruited from 54 government schools in Mwanza, Tanzania, into the DoRIS trial. Girls were eligible if they were aged 9–14 years, healthy, and HIV negative. Participants were randomly assigned (1:1:1:1:1:1), using permutated block sizes of 12, 18, and 24, to one, two, or three doses of the 2-valent vaccine (Cervarix, GSK Biologicals, Rixensart, Belgium) or the 9-valent vaccine (Gardasil 9, Sanofi Pasteur MSD, Lyon, France). For this immunobridging analysis, the primary objective was to compare geometric mean concentrations (GMCs) at 24 months after one dose in the per-protocol population compared with in historical cohorts: the one-dose 2-valent vaccine group in DoRIS was compared with recipients of the 2-valent vaccine Cervarix from CVT and the one-dose 9-valent vaccine group in DoRIS was compared with recipients of the 4-valent vaccine Gardasil (Merck Sharp & Dohme, Whitehouse Station, NJ, USA) from the IARC India trial. Samples were tested together with virus-like particle ELISA for HPV16 and HPV18 IgG antibodies. Non-inferiority of GMC ratios (DoRIS trial *vs* historical cohort) was predefined as when the lower bound of the 95% CI was greater than 0·50. This study is registered with ClinicalTrials.gov, NCT02834637.

Findings Between Feb 23, 2017, and Jan 6, 2018, we screened 1002 girls for eligibility, of whom 930 were enrolled into DoRIS and 155 each were assigned to one dose, two doses, or three doses of 2-valent vaccine, or one dose, two doses, or three doses of 9-valent vaccine. 154 (99%) participants in the one-dose 2-valent vaccine group (median age 10 years [IQR 9–12]) and 152 (98%) in the one-dose 9-valent vaccine group (median age 10 years [IQR 9–12]) were vaccinated and attended the 24 month visit, and so were included in the analysis. 115 one-dose recipients from the CVT (median age 21 years [19–23]) and 139 one-dose recipients from the IARC India trial (median age 14 years [13–16]) were included in the analysis. At 24 months after vaccination, GMCs for HPV16 IgG antibodies were 22·9 international units (IU) per mL (95% CI 19·9–26·4; n=148) for the DoRIS 2-valent vaccine group versus 17·7 IU/mL (13·9–22·5; n=97) for the CVT (GMC ratio 1·30 [95% CI 1·00–1·68]) and 13·7 IU/mL (11·9–15·8; n=145) for the DoRIS 9-valent vaccine group versus 6·7 IU/mL (5·5–8·2; n=131) for the IARC India trial (GMC ratio 2·05 [1·61–2·61]). GMCs for HPV18 IgG antibodies were 9·9 IU/mL (95% CI 8·5–11·5: n=141) for the DoRIS 2-valent vaccine group versus 8·0 IU/mL (6·4–10·0; n=97) for the CVT trial (GMC ratio 1·23 [95% CI 0·95–1·60]) and 5·7 IU/mL (4·9–6·8; n=136) for the DoRIS 9-valent vaccine group versus 2·2 IU/mL (1·9–2·7; n=129) for the IARC India trial (GMC ratio 2·12 [1·59–2·83]). Non-inferiority of antibody GMCs was met for each vaccine for both HPV16 and HPV18.

Interpretation One dose of HPV vaccine in young girls might provide sufficient protection against persistent HPV infection. A one-dose schedule would reduce costs, simplify vaccine delivery, and expand access to the vaccine.

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See **Comment** page e1373

See **Articles** page e1473

For the KiSwahili translation of the abstract see **Online** for appendix 1

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Research in context

Evidence before this study

We identified a 2019 review of published reports of the efficacy of single dose HPV vaccination. All studies in the review were observational studies of participants in three large HPV vaccine trials who did not complete their allocated schedules. These included the International Agency for Research on Cancer (IARC) HPV vaccine trial in India, the Costa Rica Vaccine Trial (CVT), and the PATRICIA multicentre trial conducted in 14 countries. HPV16 and HPV18 infection was rare in all vaccinated participants up to 7 years after the first dose and all studies reported comparable efficacy of one, two, and three doses of HPV vaccine against HPV16 and HPV18 infection despite differences in antibody levels between the dose groups. We updated this review by searching the Medline, EMBASE, Global Health Database, and Cochrane Central Register of Controlled Trials databases for publications between Aug 1, 2018, and Dec 10, 2021, using the terms "human papillomavirus" AND "vaccines" AND ("immunogenicity" OR "efficacy/effectiveness") AND "dosage". We identified two additional studies that extended the CVT and IARC India studies, which found that vaccine efficacy against HPV16 and HPV18 infection endpoints was similar between participants who received one, two, or three doses, and antibody responses remained stable over 11 years for CVT and 9 years for IARC India. Additionally, we identified the first randomised controlled trial of single dose HPV vaccine efficacy, the KEN SHE trial, in girls and women aged 15–20 years in Kenya, which found 97·5% vaccine efficacy for one dose of HPV vaccine compared with a control vaccine at 18 months. However, there is still a paucity of efficacy data from girls in the target age for vaccination (9–14 years).

Added value of this study

The Dose Reduction Immunobridging and Safety Study (DoRIS) trial in Tanzanian girls is the first randomised clinical trial to our knowledge to assess the safety and immune responses of a single dose of HPV vaccine compared with two and three doses in girls in the target age for vaccination (9–14 years). Here we present an immunobridging study comparing single-dose vaccine immunogenicity data from the DoRIS trial with historical immunogenicity and efficacy against persistent HPV16 and HPV18 infection data derived from single-dose recipients from two previous, large HPV vaccine clinical trials (CVT and IARC India). We found that HPV16 and HPV18 antibody concentrations and seropositivity at 24 months after one dose in young girls in Tanzania were non-inferior to those in adult women (aged 18–25 years) who received one dose in the CVT or girls (aged 10–18 years) who received one dose in the IARC India trial.

Implications of all the available evidence

One dose of HPV vaccine induces antibody responses that are comparable in different geographies and contexts, and a single dose is likely to be effective against persistent HPV16 and HPV18 infection and associated disease. A single dose HPV vaccine schedule could substantially reduce the costs of vaccine purchase and delivery, alleviate vaccine supply constraints, and expand access to the vaccine in the countries that need it most.

Introduction

The elimination of cervical cancer, caused by human papillomavirus (HPV) infection, is high on the public health agenda following WHO's 2020 global call for action.[1] Sub-Saharan Africa has the highest cervical cancer incidence and mortality rates globally, and access to screening is often restricted or absent.[2] Prophylactic HPV virus-like particle (VLP) vaccines are safe and effective in preventing cervical HPV infection and its sequelae. However, estimated global HPV vaccine coverage among girls aged 9–14 years in 2019 was only 15% for full vaccination and 7% in Gavi, the Vaccine Alliance, eligible countries.[3]

Four licensed HPV vaccines are available: the two 2-valent vaccines (Cervarix [GSK Biologicals, Rixensart, Belgium] and Cecolin [Xiamen Innovax Biotech, Xiamen, China]) that target HPV16 and HPV18; the 4-valent vaccine (Gardasil [Merck Sharp & Dohme, Whitehouse Station, NJ, USA]) that targets HPV 6, HPV11, HPV16, and HPV18; and the 9-valent vaccine (Gardasil-9 [Sanofi Pasteur MSD, Lyon, France]) that targets nine genotypes (HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV45, HPV52, and HPV58).

The vaccines were originally licensed as a three-dose schedule, but a two-dose schedule was approved in girls younger than 15 years in 2016.[4] However, the costs of setting up and sustaining a multi-dose HPV vaccine programme that targets young girls remain a barrier to HPV vaccine introduction.[5] By the end of 2019, only 24% of low-income and middle-income countries (LMICs) had included HPV vaccination in their national immunisation schedules and complete series coverage is often low.[6] Therefore, new vaccination approaches are needed if the WHO goal of cervical cancer elimination is to be met. A one-dose vaccine schedule, if effective, could simplify and reduce the costs of vaccine purchase and delivery, facilitate the sustainability of national programmes, and potentially increase uptake of vaccination.

Because of the challenges in accruing virological or disease endpoints for efficacy trials when HPV vaccination is given to girls before sexual debut, efficacy of the two-dose schedule of HPV vaccination in young girls has been assessed through immunobridging trials, and the schedule was approved on the basis of antibody data.[7–9] In immunobridging trials, anti-HPV antibody concentrations for specific HPV genotypes in a new population group are compared with those in a population group where efficacy has been shown, with the aim of showing that immune responses in the new population are non-inferior to those seen in the original

population. If immune responses are shown to be non-inferior, then efficacy is also assumed to be comparable.

Data from observational studies suggest that one dose of HPV vaccine might confer durable protection against HPV infection and cervical cancer precursors up to 11 years after vaccination.[10,11] Recently, the first randomised trial of single dose efficacy, the KEN SHE trial, in sexually active women aged 15–20 years, found 97·5% efficacy against incident persistent HPV16 and HPV18 infection at 18 months compared with a control vaccine.[12]

We did a randomised trial of reduced dose schedules of two HPV vaccines in girls aged 9–14 years in Tanzania to establish whether a single dose of HPV vaccine produces immune responses that are likely to be effective in preventing cervical cancer in sub-Saharan Africa.[13] Here we report immunobridging results at 24 months after vaccination, one of the trial's primary objectives, comparing immune responses after one dose in girls aged 9–14 years in Tanzania with those in historical cohorts of girls and young women aged 10–25 years who received one dose and in whom efficacy has been reported.[14,15]

Methods

Study design and population

In this open-label, randomised controlled trial (Dose Reduction Immunobridging and Safety Study [DoRIS]), we assessed the immunogenicity of two HPV vaccines, the 2-valent HPV vaccine Cervarix and 9-valent vaccine Gardasil-9, in Mwanza, in northwestern Tanzania. Trial procedures have been published previously.[16] Briefly, girls aged 9–14 years were recruited from 54 government schools. Girls were eligible if they were healthy (as determined by a physician on the basis of medical history and a physical examination) and HIV negative. Full eligibility criteria have been published elsewhere.[13]

The trial was approved by the Tanzanian Medical Research Coordinating Committee (NIMR/HQ/R.8A/Vol.IX/2236) and the ethics committee of the London School of Hygiene & Tropical Medicine (11568). Written or thumbprint informed consent was obtained from parents or guardians of participants, with written or thumbprint assent from participants.

For our immunobridging analysis, we chose two historical cohorts that received one dose of HPV vaccine. These cohorts came from two HPV vaccine trials: the Costa Rica Vaccine trial (CVT)[14] and the Institutional Agency for Research on Cancer (IARC) India trial.[15] We chose these studies because they are the only two large-scale studies of one dose of HPV vaccine to our knowledge that have data on long-term efficacy (11 years for CVT and 9 years for the IARC India trial). Although the IARC India trial used the 4-valent vaccine Gardasil rather than the 9-valent vaccine, both vaccines have the same manufacturer (Merck), and have similar immunogenicity and efficacy against HPV16 and HPV18.[17] The 9-valent vaccine contains a higher dose of antigen and adjuvant than the 4-valent vaccine: 60 µg of HPV16 and 40 µg of HPV18 L1 antigens and 500 µg aluminium hydroxyl-phosphate sulfate adjuvant compared with 40 µg of HPV16 and 20 µg of HPV18 L1 antigens and 250 µg of adjuvant, respectively.

Randomisation and masking

Girls in DoRIS were randomly assigned (1:1:1:1:1:1), using random permuted block sizes of 12, 18, and 24, to one of six groups comprising three different dose schedules of the 2-valent HPV vaccine Cervarix or 9-valent vaccine Gardasil-9: a three-dose schedule given over 6 months; two doses given over 6 months; or a single dose. The randomisation list was computer-generated by an independent statistician and trial participant identification numbers assigned sequentially in the order of treatment allocation and put into opaque sealed envelopes. Due to the nature of the intervention, once assigned treatment allocation was open label.

Procedures

In DoRIS, girls were asked to collect a vaginal swab before vaccination, which was used to detect HPV DNA. We collected blood samples for HPV immune responses including IgG antibodies to HPV16 and HPV18 VLPs and antibody avidity at baseline, and month 1, 7, 12, 24, and 36. Girls in the one-dose and two-dose groups have been enrolled in a trial extension and samples will also be taken at month 60. Here we report data from the 24-month follow-up visit for the one-dose groups.

The CVT was a community-based, double-blind, randomised, controlled trial of the 2-valent vaccine Cervarix in women aged 18–25 years in Costa Rica.[14,18] Between June 28, 2004, and Dec 21, 2005, 7466 women were enrolled and randomly assigned (1:1) to receive three doses of the 2-valent vaccine or a control vaccine (hepatitis A vaccine), given at baseline, and at 1 and 6 months. Women who did not attend the study clinic within the specified vaccination window did not receive the scheduled dose; therefore, 1480 (765 in the HPV vaccine group) women received only one or two doses of vaccine, mainly because of pregnancy and referral to colposcopy.[19] Initial follow-up was for 4 years; blood samples for immunogenicity and cervical samples for HPV DNA testing were collected annually during that period. At the end of the trial, women in the HPV vaccine group were invited to participate in a long-term follow-up study and a new unvaccinated control group was recruited; participants were followed up twice a year until August, 2017. Vaccine efficacy against prevalent HPV16 and HPV18 infections at 11 years after HPV vaccination was 82·1% in the one-dose group (with two infections among 112 women), 83·8% in the two-dose group (with one infection among 62 women), and 80·2% in the three-dose group (with 27 infections among 1365 women) compared with the unvaccinated group (with 178 infections among 1783 women). There

was no evidence of differences in vaccine efficacy or HPV infection rates across dose groups.[10] HPV16 and HPV18 geometric mean concentrations (GMCs) in the one-dose group reached a plateau at 6 months after vaccination and remained stable over 11 years.[10,20]

The IARC India trial was a large, multicentre, cluster-randomised controlled trial comparing the efficacy of two doses versus three doses of the 4-valent vaccine in girls and young women aged 10–18 years.[11,15] Overall, 17729 individuals were recruited between Sept 1, 2009, and April 8, 2010, at which point trial enrolment and vaccination was suspended by the Indian Government for reasons unrelated to the study. Therefore, some participants received fewer than their allocated number of doses, and 4950 individuals received only one dose. After suspension, the trial was converted to a longitudinal cohort study by default and a group of age-matched and site-matched unvaccinated controls were recruited. Participants have been followed up annually with blood sample collection for immunogenicity from a sample of participants representing all ages of the vaccinated population and cervical sample collection for HPV DNA testing, starting 18 months after participants got married or 6 months after their first child. Follow-up is planned until 2026. Compared with the unvaccinated group (32 infections among 1260 women), vaccine efficacy against persistent HPV16 and HPV18 infection at 10 years after vaccination was 95·4% in the one-dose group (with one infection among 2135 women) and was not significantly different from vaccine efficacy in the two-dose group (93·1%; with one infection among 1452 women) and three-dose group (93·3%; with one infection among 1460 women).[11]

In this immunobridging study, we used blood samples from all girls in the one-dose groups in DoRIS who attended the 24 month visit within a window of 22–28 months after vaccination. For the CVT and IARC India trial, we took a random sample of up to 140 participants from the one dose groups in each trial; participants were eligible for the immunobridging study if they attended the 24 month visit within the same window as in DoRIS, had efficacy data available, and had sufficient serum samples from the day 0 and month 24 visits remaining for re-testing. The one-dose 2-valent vaccine group in DoRIS was compared with one-dose group of the same 2-valent vaccine in the CVT, and the one-dose 9-valent vaccine group in DoRIS was compared with one-dose group of the 4-valent vaccine in the India trial.

We measured antibodies to HPV16 and HPV18 by type-specific VLP ELISA at the Frederick National Laboratory for Cancer Research HPV Immunology Laboratory (Frederick, MD, USA).[21] Samples for the immunobridging analyses (ie, from day 0 and month 24) from the three trials were batched (ie, processed and analysed at the same time by the same analyst) and tested together to minimise variability. Antibody concentrations greater than or equal to the lower limit of detection were prespecified to indicate seropositivity (for HPV16, ≥1·309 international units [IU] per mL; for HPV18, ≥1·109 IU/mL).

In DoRIS, we did HPV DNA genotyping at enrolment (day 0) using the Anyplex HPV28 detection assay (Seegene, Seoul, South Korea) at the Catalan Institute of Oncology (Barcelona, Spain). In the CVT, PCR-based HPV DNA testing at enrolment was done at the Delft Diagnostic Laboratory (Delft, Netherlands) with amplification and probe hybridisation using the SPF10 HPV DNA enzyme immunoassay system, followed by typing with the LiPA25 version 1 line detection system.[22] HPV DNA testing at enrolment was not done in the IARC India study.

Outcomes

The primary outcome of the DoRIS trial was to compare HPV16-specific and HPV18-specific seropositivity in participants who received one dose of vaccine with those who received two or three doses of the same vaccine, 24 months after vaccination.[13] For this immunobridging analysis, the overall aim was to compare vaccine-induced HPV genotype-specific immune responses in DoRIS participants who received one dose of HPV vaccine with those in two historical cohorts of girls and young women who received only one dose of HPV vaccine, in whom efficacy has been reported.

The primary immunobridging objective of the DoRIS trial was to determine whether HPV16 and HPV18 antibody GMCs at 24 months in girls who received one dose in DoRIS were non-inferior to those of one-dose historical cohorts in the CVT and IARC India studies. The secondary immunobridging objective was to determine whether HPV16 and HPV18 seropositivity was non-inferior at 24 months. The 24 month timepoint was chosen for the immunobridging objectives because one dose antibody concentrations are expected to have reached plateau levels by that timepoint.[20]

Statistical analysis

With 155 participants in each HPV-dose schedule group in DoRIS, assuming a loss to follow up of 20% over 36 months, we expected to have 130 girls in each group at the 24 month visit for the primary non-inferiority analyses. If the true GMC ratio (DoRIS *vs* comparison cohort) between groups is 1·0, with 130 participants at 24 months in each group, we had more than 90% power to show that the lower limit of the 95% CI for the GMC ratio was greater than 0·50, indicating that the one-dose schedule in girls in Tanzania did not lead to HPV16 and HPV18 antibody GMCs of 50% or lower than those of the comparison cohort in which efficacy was observed. We assumed an SD of 0·50–0·60 log10 anti-HPV concentration,[23] and used a one-sided non-inferiority test at the 2·5% level. If the true proportion of participants who seroconvert is the same in each group, with 130 girls per group, we had more than 90% power to show that the

lower limit of the 95% CI for the difference (DoRIS minus comparison cohort) was greater than –5%, indicating that seropositivity with the one-dose schedule in Tanzania was at least more than 95% of the seropositivity in the historical cohort.

The primary immunobridging analysis was in the per-protocol cohort, which included participants who received only one dose of HPV vaccine and who were HPV antibody negative (for the DoRIS *vs* CVT and the DoRIS *vs* IARC India comparisons), and HPV DNA negative (DoRIS *vs* CVT comparison) at enrolment for the specific genotype under analysis. Secondary analyses included all participants who received one dose of HPV vaccine, irrespective of baseline antibody or HPV DNA status (ie, total vaccinated cohort).

We did separate analyses to compare immune responses after one dose of the 2-valent vaccine in DoRIS with one dose of the 2-valent vaccine in the CVT, and responses after one dose of the 9-valent vaccine in DoRIS with one dose of the 4-valent vaccine in the IARC India trial. We log10-transformed HPV genotype-specific antibody concentrations for analysis. We gave antibody concentrations below the assay cutoff a value of half the cutoff before log transformation. We calculated arithmetic mean log10 antibody concentrations and 95% CIs for each group, assuming a normal distribution.

We calculated the difference in HPV genotype-specific log10 concentrations at 24 months between the two groups (DoRIS minus comparison cohort) and its 95% CI; we obtained the GMC ratio and its 95% CI by back-transformation. The antibody response was determined to be non-inferior if the lower bound for the two-sided 95% CI for the GMC ratio was above 0·50; this margin was defined a priori on the basis of that used in several previous HPV vaccine trials.[24,25]

We calculated the number and proportion of girls in each group who were seropositive for HPV16-specific and HPV18-specific antibodies at 24 months. For each vaccine type and HPV genotype, we calculated the difference (DoRIS minus comparison cohort) in the proportion who were seropositive and estimated the 95% CI for the difference using the exact method of Chan and Zhang.[26] Non-inferiority of seropositivity was concluded if the lower bound of the two-sided 95% CI for the difference was above –5%.

In a prespecified secondary analysis, we used linear regression to compare log10 concentrations between one dose of 9-valent vaccine in DoRIS and one dose of 4-valent vaccine in the IARC India trial, adjusting for age as a categorical variable. We back-transformed regression coefficients and 95% CIs to express the estimates as GMC ratios. Because there was no overlap in the age ranges between DoRIS and CVT, we did no adjustments for age. We also did a post-hoc subgroup analysis restricted to girls who were younger than 15 years at the time of vaccination for the 9-valent vaccine group in DoRIS and the 4-valent group in IARC.

We used linear regression models with a term for study group to obtain p values; p values of less than 0·05 were considered to be statistically significant.

We used SAS (version 9.1) and Stata (version 17) for all analyses. This study is registered with ClinicalTrials.gov, NCT02834637.

Role of the funding source

The funders of this study did not have any role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Feb 23, 2017, and Jan 6, 2018, 1002 girls were screened for eligibility, and 930 were enrolled in DoRIS and assigned to either one dose, two doses, or three doses of 2-valent vaccine, or one dose, two doses, or three doses of 9-valent vaccine (n=155 per group; full details of enrolment and randomisation have been published elsewhere[13]). 154 (99%) of 155 participants in the one-dose 2-valent vaccine group and 152 (98%) of 155 in the one-dose 9-valent vaccine group attended the 24 month

	DoRIS (2-valent vaccine; n=154)	CVT (2-valent vaccine; n=115)	DoRIS (9-valent vaccine; n=152)	IARC India (4-valent vaccine; n=139)
Age, years				
Median	10 (9–12)	21 (19–23)	10 (9–12)	14 (13–16)
9–14	154 (100%)	0	152 (100%)	74 (53%)
15–19	0	115 (100%)	0	65 (47%)
HPV16 seropositive at baseline				
Yes	6 (4%)	16 (14%)	7 (5%)	8 (6%)
No	148 (96%)	99 (86%)	145 (95%)	131 (94%)
HPV18 seropositive at baseline				
Yes	13 (8%)	16 (14%)	16 (11%)	9 (6%)
No	141 (92%)	99 (86%)	136 (89%)	130 (94%)
HPV16 DNA positive at baseline				
Yes	0	3 (3%)	1 (1%)	NA*
No	154 (100%)	112 (97%)	151 (99%)	NA*
HPV18 DNA positive at baseline				
Yes	0	4 (3%)	1 (1%)	NA*
No	154 (100%)	111 (97%)	151 (99%)	NA*
HPV16 seropositive or DNA positive at baseline				
Yes	6 (4%)	18 (16%)	7 (5%)	NA*
No	148 (96%)	97 (84%)	145 (95%)	NA*
HPV18 seropositive or DNA positive at baseline				
Yes	13 (8%)	18 (16%)	16 (11%)	NA*
No	141 (92%)	97 (84%)	136 (89%)	NA*
Included in per-protocol analysis				
HPV16	148 (96%)	97 (84%)	145 (95%)	131 (94%)
HPV18	141 (92%)	97 (84%)	136 (89%)	129 (93%)

Data are median (IQR) or n (%). CVT=Costa Rica Vaccine trial. DoRIS=Dose Reduction Immunobridging and Safety Study. HPV=human papillomavirus. IARC=Institutional Agency for Research on Cancer. NA=not applicable. *Baseline DNA status was not measured in IARC India trial.

Table 1: **Demographic characteristics at baseline among one dose recipients in DoRIS included in immunobridging analyses, by vaccine received, and one dose recipients in historical cohorts**

	Participants*	GMC (IU/mL)†	Seroconversion‡
HPV 16 IgG antibody			
DoRIS (2-valent vaccine)	148	22·9 (19·9–26·4; 14·7–40·0)	147 (99%)
CVT (2-valent vaccine)	97	17·7 (13·9–22·5; 7·3–38·7)	96 (99%)
DoRIS (9-valent vaccine)	145	13·7 (11·9–15·8; 8·9–21·4)	144 (99%)
Aged <15 years (post hoc)	145	13·7 (11·9–15·8; 8·9–21·4)	144 (99%)
India (4-valent vaccine)	131	6·7 (5·5–8·2; 3·3–16·1)	121 (92%)
Aged <15 years (post hoc)	68	9·7 (7·7–12·1; 5·0–21·1)	68 (100%)
HPV 18 IgG antibody			
DoRIS (2-valent vaccine)	141	9·9 (8·5–11·5; 5·7–17·7)	139 (99%)
CVT (2-valent vaccine)	97	8·0 (6·4–10·0; 3·7–15·5)	96 (99%)
DoRIS (9-valent vaccine)	136	5·7 (4·9–6·8; 3·0–10·8)	133 (98%)
Ages 15 years (post hoc)	136	5·7 (4·9–6·8; 3·0–10·8)	133 (98%)
India (4-valent vaccine)	129	2·2 (1·9–2·7; 1·2–4·1)	99 (77%)
Ages <15 years (post hoc)	69	2·7 (2·1–3·4; 1·4–4·5)	57 (83%)

Data are n, GMC (95% CI; IQR), or n (%), unless otherwise stated. CVT=Costa Rica Vaccine trial. DoRIS=Dose Reduction Immunobridging and Safety Study. HPV=human papillomavirus. IARC=Institutional Agency for Research on Cancer. GMC=geometric mean concentration. *Includes DoRIS and CVT participants who were ELISA antibody negative and HPV DNA negative, and IARC India participants who were ELISA antibody negative, at baseline (before vaccination) for the HPV genotype under analysis. †ELISA serum antibody GMC. ‡Seroconversion was defined as concentrations greater than or equal to the laboratory determined cutoff (HPV16=1·309 IU/mL; HPV18=1·109 IU/mL) among girls who were seronegative for the HPV genotype at baseline.

Table 2: **GMCs and seroconversion rates at 24 months after a single dose HPV vaccination between DoRIS and historical cohorts (per-protocol population*)**

	GMC ratio (DoRIS/ historical cohort)	Adjusted GMC ratio†	Difference in seroconversion (DoRIS – historical control)
HPV16 IgG antibody			
DoRIS *vs* CVT	1·30 (1·00 to 1·68)	··‡	0·4% (–3·1 to 5·1)
DoRIS *vs* IARC India	2·05 (1·61 to 2·61)	1·29 (0·91 to 1·82)	6·9% (2·4 to 13·1)
Aged <15 years (post hoc)	1·42 (1·10 to 1·83)	1·29 (0·94 to 1·76)	–0·7% (–4·0 to 5·0)
HPV18 IgG antibody			
DoRIS *vs* CVT	1·23 (0·95 to 1·60)	··‡	–0·4% (–4·4 to 4·4)
DoRIS *vs* IARC India	2·57 (2·02 to 3·27)	1·75 (1·22 to 2·50)	21·0% (13·5 to 29·5)
Aged <15 years (post hoc)	2·12 (1·59 to 2·83)	1·75 (1·23 to 2·49)	15·2% (6·1 to 26·3)

Data in parentheses are 95% CIs. CVT=Costa Rica Vaccine trial. DoRIS=Dose Reduction Immunobridging and Safety Study. HPV=human papillomavirus. IARC=Institutional Agency for Research on Cancer. GMC=geometric mean concentration. *Includes DoRIS and CVT participants who were ELISA antibody negative and HPV DNA negative, and IARC India participants who were ELISA antibody negative, at baseline (before vaccination) for the HPV genotype under analysis. †Adjusted for age. ‡Adjustment not done for comparisons between DoRIS and CVT because there is no overlap in the age range.

Table 3: **Comparison of GMCs and seroconversion rates at 24 months after a single dose HPV vaccination between DoRIS and historical cohorts (per-protocol population*)**

See Online for appendix 2

visit within the 22–28 month window and so were eligible for the total vaccinated cohort for the immunobridging analysis. In the CVT, 115 (42%) of 277 one dose recipients were eligible, and all were included in the immunobridging analysis. In the IARC India trial, 139 (93%) of 150 eligible one dose recipients were randomly selected for this analysis.

Baseline characteristics were similar between the two one-dose groups in DoRIS but, because of the design of the different trials, DoRIS participants were younger than the one-dose recipients in the CVT and IARC India trial (table 1). Baseline HPV16 and HPV18 seropositivity was similar between the DoRIS and IARC India trial participants, and lower in the DoRIS trial than in the CVT, consistent with the older age range of the CVT.

In the per-protocol comparison of the 2-valent vaccine, 147 (99%) of 148 participants in DoRIS and 96 (99%) of 97 participants in the CVT were seropositive for IgG antibodies to HPV16 at 24 months, and 139 (99%) of 141 in DoRIS and 96 (99%) of 97 in the CVT were seropositive for IgG antibodies for HPV18 (table 2). HPV16 and HPV18 antibody GMCs were higher after one dose of the 2-valent vaccine in DoRIS than in CVT, although the difference was not significant (table 2). Non-inferiority of antibody concentrations for the 2-valent vaccine was met for both HPV genotypes, with GMC ratios (DoRIS *vs* CVT) of 1·30 (95% CI 1·00 to 1·68) for HPV16 and 1·23 (0·95 to 1·60) for HPV18. Non-inferiority was also met for seropositivity, with a difference in seroconversion (DoRIS minus CVT) of 0·4% (95% CI –3·1 to 5·1) for HPV16 and –0·4% (–4·4 to 4·4) for HPV18 (table 3).

In the per-protocol comparison of the 9-valent vaccine with the 4-valent vaccine, 144 (99%) of 145 participants in DoRIS and 121 (92%) of 131 in the IARC India trial were seropositive for IgG antibodies to HPV16 at 24 months, and 133 (98%) of 136 in DoRIS and 99 (77%) of 129 in the IARC India trial were seropositive for IgG antibodies to HPV18 (table 2). For both HPV genotypes, antibody GMCs were higher after one dose of the 9-valent vaccine in DoRIS than after one dose of the 4-valent vaccine in the IARC India trial (HPV16 and HPV18: p<0·0001). Non-inferiority of antibody concentrations was met for the 9-valent versus 4-valent vaccine for both HPV genotypes, with GMC ratios (DoRIS *vs* IARC India trial) of 2·05 (95% CI 1·61–2·61) for HPV16 and 2·57 (2·02–3·27) for HPV18. After adjusting for age, the GMC ratios were 1·29 (95% CI 0·91–1·82) for HPV16 and 1·75 (1·22–2·50) for HPV18. Non-inferiority of seropositivity at 24 months was also met, with a difference (DoRIS minus IARC India trial) of 6·9% (95% CI 2·4–13·1) for HPV16 and 21·0% (13·5–29·5) for HPV18.

In secondary analyses in the total vaccinated cohort, we found non-inferiority of antibody GMCs and seropositivity for the 2-valent vaccine (DoRIS *vs* CVT) and 9-valent versus 4-valent vaccine (DoRIS *vs* IARC India trial) comparisons for both HPV genotypes (figure; appendix 2 p 1). In the post-hoc subgroup analysis comparing one dose of the 9-valent vaccine in girls in DoRIS with the 4-valent vaccine restricted to girls younger than 15 years in the IARC India trial, we found non-inferiority of antibody GMCs and seropositivity (tables 2, 3).

Discussion

In this immunobridging study, including the first randomised trial of a single dose of HPV vaccine in girls

aged 9–14 years, we found that immune responses at 24 months in girls in Tanzania were non-inferior to those in study populations aged 18–25 years in Costa Rica and 10–18 years in India who received one dose and in whom one-dose efficacy against persistent HPV infection has been reported.[14,15] These encouraging results show that a single dose of HPV vaccine induces immune responses that are comparable in different populations and geographical contexts, and add to the evidence that a single dose is likely to be effective against persistent HPV16 and HPV18 infection and associated disease.

Recently, the first randomised controlled trial of single-dose efficacy (KEN SHE), in Kenyan girls and women aged 15–20 years, found that efficacy of both the 2-valent vaccine Cervarix and the 9-valent vaccine Gardasil-9 against persistent HPV16 and HPV18 infection at 18 months after vaccination was 97·5% compared with the meningococcal vaccine control group.[12] We are planning to do an immunobridging analysis of the DoRIS results and the KEN SHE results in the future.

In April, 2022, WHO's Strategic Advisory Group of Experts on Immunization met to assess the evidence on the efficacy of the single-dose HPV vaccination schedule, including the results from DoRIS. The committee recommended that the HPV vaccine dose schedule be updated to allow countries to choose a one-dose or two-dose schedule for girls aged 9–14 years and for young women aged 15–20 years.[27]

Because HPV-related disease (cervical intraepithelial neoplasia grade 2 or worse) and virological endpoints (persistent infection) might take a long time to accrue and require costly studies, gynaecological examinations, and sampling that might be considered unacceptable in girls in some settings, WHO recommends that immunobridging trials are appropriate for licensure of new dose schedules of HPV vaccines in young adolescents.[8,28] Although there is no defined immune correlate of protection to inform licensure, non-inferiority of antibody concentrations is recommended as the main trial endpoint. This recommendation aligns with the large body of evidence that protection after HPV L1 VLP vaccination is mediated via systemic induction of neutralising antibodies, which are effective at very low concentrations.[29] Antibody concentrations after one dose are known to be inferior to two or three doses, despite similar efficacy. Therefore, licensure of a single dose schedule requires efficacy trials with virological endpoints, along with well-designed immunobridging studies comparing antibody concentrations after one dose in different population groups to antibody concentrations in populations in which virological efficacy of one dose has been reported. If antibody concentrations in the new population are shown to be non-inferior to those in populations in which efficacy has been found, then protection is also expected to be the same.

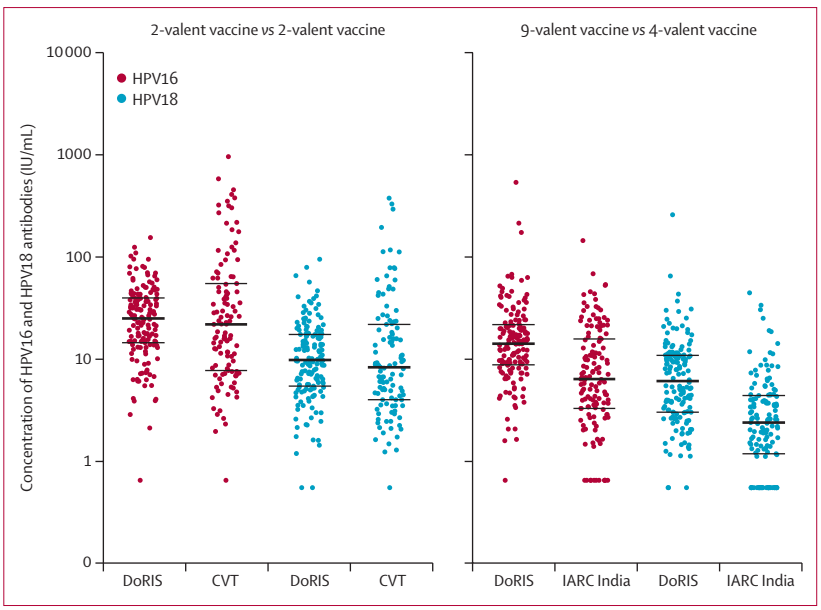

Figure: **Distribution of HPV16 and HPV18 antibody concentrations at 24 months after a single dose of HPV vaccine, by study group (total vaccinated cohort)**
Each datapoint represents a single individual and the line through the datapoints indicates the median concentration, with IQR shown by error bars. CVT=Costa Rica Vaccine trial. DoRIS=Dose Reduction Immunobridging and Safety Study. HPV=human papillomavirus. IARC=Institutional Agency for Research on Cancer.

When comparing antibody GMCs, we used a non-inferiority margin of 0·50, which was met for all comparisons. If we had used a more stringent margin of 0·67, indicating that antibody GMCs in DoRIS were not reduced by more than 33%, it would also have been met in both the per-protocol and total vaccinated cohort analyses for each trial, and the post-hoc comparison of antibody responses among girls younger than 15 years in the IARC India trial. In the total vaccinated cohort, antibody concentrations in participants in DoRIS remained non-inferior to those of the historical cohorts; although 16% of participants in CVT were HPV16 or HPV18 DNA or seropositive at enrolment and so vaccination might have acted as a booster of their response to natural infection. Interestingly, GMCs in DoRIS were not significantly higher than those in the CVT, despite the older age of participants in the CVT than in DoRIS. The higher GMCs and seroconversion rates observed in DoRIS than in the IARC India trial might in part be due to the higher dose of antigen and adjuvant in the 9-valent vaccine than in the 4-valent vaccine, particularly for HPV18, for which the antigen dose has been doubled. This finding might also be explained in part by the age difference, because participants in DoRIS were younger on average than those in the IARC India trial.

Data from the CVT have shown that one dose of the 2-valent vaccine provides sustained HPV16 and HPV18 antibody levels for at least 11 years and that vaccine efficacy among women who received one dose was not significantly different from those who received

three doses.[10] Similarly, the IARC India trial has shown sustained antibody levels after one dose of the 4-valent vaccine with no difference in protection against persistent HPV16 and HPV18 infection compared with three doses for up to 9 years.[11]

Although participants in DoRIS were on average younger than those in the CVT and IARC India trials, restricting to the same age group in the IARC India study in a post-hoc analysis made no difference to the results. Because vaccinating girls in preadolescence produces higher GMCs than when vaccinated later in life,[23,24] the age difference is unlikely to affect results at later timepoints.

Strengths of our study include the immunobridging analysis of results for two HPV vaccines in two population groups among whom long-term efficacy has been found, allowing us to investigate the reproducibility of the one-dose results across three different geographical regions and different vaccines. DoRIS was run in a region with an extremely high burden of cervical cancer and where vaccination is most needed. We tested the samples from DoRIS, CVT, and the IARC India trial in the same batch, using a well validated assay,[21] to minimise potential variability and allow robust comparisons between the studies.

Our study also had several limitations. One limitation of our study is that, although the vaccines used in DoRIS and the IARC India trial are similar (9-valent and 4-valent vaccines), they are not identical. However, a randomised trial of the two vaccines has shown that, despite their differences, they have similar efficacy and immunogenicity for HPV genotypes in common.[30] Other limitations include a follow-up period of only 24 months. Immunogenicity data will also be collected from DoRIS participants at 5 years after vaccination and immunobridging analyses to later timepoints from CVT and the IARC India trial are planned. Additionally, a trial in Tanzania of one-dose HPV vaccination in boys is underway (NCT04953130).

For more on the **CVT** see https://dceg.cancer.gov/research/who-we-study/cohorts/costa-rica-vaccine-trial

In summary, our findings contribute to the evidence that one dose of HPV vaccine might provide strong protection against cervical cancer and be a promising strategy towards achieving cervical cancer elimination in sub-Saharan Africa and elsewhere. A single dose HPV vaccine schedule could substantially reduce the costs of vaccine purchase and delivery, alleviate vaccine supply constraints, and expand access in the countries that need it most.

Contributors

KB, DW-J, LAP, ARK, AH, and PB developed the initial idea and design of the immunobridging study. KB, ARK, PB, LAP, and DW-J developed the protocol. DW-J, KB, and JC were joint principal investigators and CJL, RJH, JD, JI, PM, and HW were coinvestigators of the DoRIS trial. KB and DW-J accessed and verified the DoRIS data that were used in this study. AH and RH were joint principal investigators and CP and ARK were coinvestigators of the CVT. ARK and JTS accessed and verified the CVT data that were used in this study. PB was the principal investigator for the IARC India trial; EL and RM contributed to the conduct of the trial, and accessed and verified the data that were used in this study. JI, LAP, and TJK were responsible for laboratory aspects of the study. KB analysed the data, with input from ARK, RM, RJH, and DW-J. KB, DWJ, JC, LAP, TJK, ARK, RJH, CJL, JTS, and PB interpreted the results. KB, ARK, PB, JC, AH, CP, HW, RH, CJL, JTS, JD, LAP, and DW-J critically reviewed all material for important intellectual content. KB drafted the manuscript and all authors commented and contributed to the final version. All authors had full access to the data in the study and had final responsibility for the decision to submit the manuscript for publication.

Declaration of interests

KB, HW, and DW-J report a grant from Merck for a new study of single-dose HPV vaccination in males in Tanzania, unrelated to this submitted work. PB, ARK, JTS, HW, and DW-J are members of the Single Dose HPV Vaccine Evaluation Consortium, coordinated by PATH and funded by the Bill & Melinda Gates Foundation. PB reports a grant from GSK Biologicals for a previous study on safety and immunogenicity of Cervarix in India unrelated to this submitted work during his previous position at Chittaranjan National Cancer Institute, Kolkata, India. DW-J reports a grant from GSK Biologicals in 2007 for a previous on safety and immunogenicity of Cervarix in Tanzania, unrelated to this submitted work. JTS reports that he was a named inventor on US Government-owned HPV vaccine patents that were licensed to GlaxoSmithKline and Merck and for which the US National Cancer Institute (NCI) previously received licensing fees. NCI's licenses have now expired but JTS was previously entitled to royalties to a specified amount, as determined by federal law governing technological transfer activities by US Government employees. All other authors declare no competing interests.

Data sharing

Deidentified participant data presented in this Article can be made available after publication following written request to the London School of Hygiene & Tropical Medicine (LSHTM) and the Mwanza Intervention Trials Unit (MITU), Tanzania. Requests must be accompanied by an analysis plan, which will be reviewed by the MITU Data Sharing Committee and lead investigators for each trial. Requesting researchers will be required to sign a Data Access Agreement if approval is given. De-identified participant data from the blinded phase of the CVT can be shared with outside collaborators for research to understand more about the performance of the HPV vaccine, immune response to the vaccine, and broader study factors associated with the natural history of HPV infection and risk factors for infection and disease. Outside collaborators can apply to access the protocols and data online; to request an application and information pack, email CVTDataSharing@westat.com. The trial summary, current publications, and contact information for the CVT are available online.

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THE LANCET
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Supplementary appendix 1

This translation in KiSwahili was submitted by the authors and we reproduce it as supplied. It has not been peer reviewed. *The Lancet*'s editorial processes have only been applied to the original in English, which should serve as reference for this manuscript.

Tafsiri hii katika Kiswahili iliwasilishwa na waandishi wa makala na tunaitoa kama ilivyowasilishwa. Haijahakikiwa na wataalam wenzao. Mchakato wa kuhariri wa Lancet umetumika kwenye nakala ya awali ya kiingereza tu, ambayo inapaswa kutumika kama rejea kwa makala hii.

Supplement to: Baisley K, Kemp TJ, Kreimer AR, et al. Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose of HPV vaccine in historical cohorts: an immunobridging analysis of a randomised controlled trial. *Lancet Glob Health* 2022; **10:** e1485–93.

Kulinganisha dozi moja ya chanjo ya VIPABI kwa wasichana wenye umri wa miaka 9-14 nchini Tanzania (DoRIS) na dozi moja ya chanjo ya VIPABI kwa makundi ya kihistoria ya washiriki wa tafiti: Uchambuzi wa kulinganisha kingamwili katika utafiti uliotumia mbinu ya bahati nasibu.

Utangulizi

Chanjo dhidi ya virusi vya papilloma ya binadamu(VIPABI) zinatolewa kwa utaratibu wa dozi mbili kwa watoto wenye umri wa miaka 9- 14 au utaratibu wa dozi tatu kwa watu wazima. Tulilinganisha mwitikio wa kingamwili baada ya dozi moja ya chanjo ya VIPABI kwenye Utafiti wa Kupunguza Dozi, na kulinganisha kingamwili na Usalama wa chanjo(DoRIS), utafiti unaotumia mbinu ya bahati nasibu na taratibu tofauti za kutoa chanjo ya VIPABI nchini Tanzania, na ule kutoka tafiti mbili za kiuchunguzi za chanjo ya VIPABI ambazo zilionyesha ufanisi mkubwa wa dozi moja katika kipindi cha hadi miaka 11 dhidi ya VIPABI16 na VIPABI18 (Utafiti wa Chanjo wa Costa Rica[CVT] na Utafiti uliofanyika India, na Taasisi ya kimataifa ya Utafiti wa Saratani[IARC]).

Mbinu

Katika uchambuzi huu wa kulinganisha kingamwili kwenye utafiti wa wazi unaotumia njia ya bahati nasibu na uliodhibitiwa ("open-label randomised controlled trial"), wasichana walioshirikishwa katika utafiti wa DoRIS walitoka katika shule 54 za serikali ndani ya Mwanza, Tanzania,. Wasichana walikuwa na sifa za kushiriki kama walikuwa na umri wa miaka 9- 14, wenye afya njema na wasiokuwa na maambukizi ya VVU. Washiriki waliwekwa kwa kutumia njia ya bahati nasibu katika mpangilio wa (1:1:1:1:1:1) kwa kutumia bloku zilizoruhusiwa za ukubwa ("permutated block sizes") wa 12, 18 na 24 kwa dozi moja, mbili au tatu za chanjo ya valenti 2(Cervarix, GSK Biologicals, Rixensart, Belgium) au za chanjo ya valenti 9 (Gardasil 9, Sanofi Pasteur MSD, Lyon, France). Kwa uchambuzi huu wa kulinganisha kingamwili, lengo la msingi lilikuwa ni kulinganisha wastani wa wingi wa kinga mwili kijiometriki (GMC) katika kipindi cha miezi 24 baada ya dozi moja kwa washiriki waliofuata muongozo wa utafiti ikilinganishwa na washiriki wa tafiti za kihistoria: kundi la dozi moja ya chanjo ya valenti 2 kwenye utafiti wa DoRIS lililinganishwa na waliopokea chanjo ya valenti 2 ya Cervarix kutoka CVT na kundi la dozi moja ya chanjo ya valent 9 kwenye utafiti wa DoRIS lililinganishwa na waliopokea chanjo ya valenti 4 ya Gardasil(Merck Sharp & Dohme, Whitehouse Station, NJ, USA) kutoka kwenye utafiti wa India IARC. Sampuli zilichunguzwa pamoja kwa kutumia njia ya ELISA iliyotumia chembechembe zinazofanana na virusi("virus-like particle") za VIPABI16 na VIPABI18 katika kugundua kingamwili ya IgG. Kutokuwa duni kwa uwiano wa GMC (Utafiti wa DoRIS dhidi ya washiriki wa kihistoria wa utafiti) ulifafanuliwa awali kama kiwango cha chini cha asilimia 95 CI kilikuwa juu zaidi ya 0.50. Utafiti huu umesajiliwa kwenye ClinicalTrials.gov, NCT02834637.

Matokeo

Kati ya Februari 23, 2017, na Januari 6, 2018, wasichana wasichana wapatao 1002 walifanyiwa tathmini kuona kama wanastahili kushiriki i, kati yao 930 walisajiliwa katika uatafiti wa DoRIS na kila 155 walipangwa kwenye dozi moja, mbili au tatu za chanjo ya valenti 2 au dozi moja, mbili au tatu za chanjo ya valent 9. Washiriki 154(99%) kwenye kundi la dozi moja ya chanjo ya valenti 2 (umri wa kati wa washiriki miaka 10 [IQR 9- 12]) na 152(98%) kwenye kundi la dozi moja ya chanjo ya valenti 9(umri wa kati wa washiriki miaka 10 [IQR 9- 12]) walichanjwa na wakahudhuria hudhurio la miezi 24, na hivyo waliingizwa kwenye uchambuzi. Washiriki 115 waliopokea dozi moja kutoka CVT (umri wa kati wa washiriki miaka 21[19-23]) na 139 waliopokea dozi moja kutoka utafiti wa India IARC (umri wa kati wa washiriki miaka 14[13- 16]) waliingizwa kwenye uchambuzi. Miezi 24 baada ya kuchanjwa, wingi wa kinga mwili kijiometriki dhidi ya VIPABI16 ilikuwa 22.9 "international units"(IU) kwa mL(95% CI 19·9–26·4; n=148) kwa kundi la chanjo ya valenti 2 la DoRIS ikilinganishwa na 17.7 IU/mL(13·9–22·5;

n=97) kwa CVT (wastani wa wingi wa kinga mwili 1.30[95% CI 1.00-1.68]) na 13.7 IU/ mL(11·9–15·8; n=145) kwa kundi la chanjo ya valenti 9 la DoRIS ikilinganishwa na 6.7 IU/mL (5·5–8·2; n=131) kwenye utafiti wa India IARC (wastani wa kinga mwili kijometriki 2·05 [1·61–2·61]). Wastani wa kijiometriki wa kinga mwili dhidi ya VIPABI18 ilikuwa 9·9 IU/mL (95% CI 8·5–11·5: n=141) kwa kundi la chanjo ya valenti 2 la DoRIS dhidi ya 8·0 IU/mL (6·4–10·0; n=97) kwenye utafiti wa CVT (wastani wa kiojiometriki wa kinga mwili 1·23 [95% CI 0·95–1·60]) na 5·7 IU/mL (4·9–6·8; n=136) kwa kundi la chanjo ya valenti 9 la DoRIS dhidi ya 2·2 IU/mL (1·9–2·7; n=129) ya kwenye utafiti wa India IARC ((wastani wa kijiometriki wa kinga mwili 2·12 [1·59–2·83]); Kutokuwa duni kwa wingi wa kinga mwili kijiometriki kulifikiwa kwa kila chanjo dhidi ya VIPABI16 na VIPABI18.

Tafsiri

Dozi moja ya chanjo ya VIPABI kwa wasichana wadogo inaweza kutoa kinga ya kutosha dhidi ya maambukizi endelevu ya VIPABI. Utaratibu wa kutoa dozi moja unaweza kupunguza gharama, kurahisisha utoaji wa chanjo, na kuongeza upatikanaji wa chanjo.

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Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Baisley K, Kemp TJ, Kreimer AR, et al. Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose of HPV vaccine in historical cohorts: an immunobridging analysis of a randomised controlled trial. *Lancet Glob Health* 2022; **10:** e1485–93.

Supplementary Table 1. Comparisons of geometric mean concentrations (GMC) and seroconversion rates at Month 24 post-single dose HPV vaccination between DoRIS and historical cohorts in whom efficacy has been shown (vaccinated population[1])

		N[1]	GMC[2] (95% CI) (IU/mL)	IQR[3]	Seropositive[4] N (%)	Seroconverted[5] N (%)
Month 24						
HPV 16 IgG antibody						
1 dose	DoRIS (2-valent)	154	22.7 (19.8 - 26.1)	14.5 - 39.8	153 (99.4%)	147 (95.5%)
1 dose	CVT (2-valent)	115	24.0 (18.5 - 31.2)	7.8 - 55.0	114 (99.1%)	98 (85.2%)
	GMC ratio (DoRIS/CVT) (95% CI)			0.95 (0.72 -1.24)		
	Adjusted GMC ratio[6] (95% CI)			-		
	Difference in seroconversion (DoRIS – CVT) (95.0% CI)			10.2% (2.4-18.5)		
1 dose	DoRIS (9-valent)	152	14.1 (12.2 - 16.3)	8.8 - 21.8	151 (99.3%)	144 (94.7%)
1 dose	India (4-valent)	139	6.6 (5.4 - 8.0)	3.3 - 15.8	128 (92.1%)	121 (87.1%)
	GMC ratio (DoRIS/India) (95% CI)			2.14 (1.68 -2.71)		
	Adjusted GMC ratio[6] (95% CI)			1.43 (1.01 -2.00)		
	Difference in seroconversion (DoRIS – India) (95% CI)			7.7% (0.9-15.0)		
HPV 18 IgG antibody						
1 dose	DoRIS (2-valent)	154	9.6 (8.3 - 11.1)	5.5 - 17.5	152 (98.7%)	139 (90.3%)
1 dose	CVT (2-valent)	115	10.0 (7.9 - 12.7)	4.0 - 21.9	114 (99.1%)	98 (85.2%)
	GMC ratio (DoRIS/CVT) (95% CI)			0.96 (0.74 -1.25)		
	Adjusted GMC ratio[6] (95% CI)			-		
	Difference in seroconversion (DoRIS – CVT) (95.0% CI)			5.0% (-3.2-13.7)		
1 dose	DoRIS (9-valent)	152	6.0 (5.2 - 7.0)	3.0 - 10.9	149 (98.0%)	133 (87.5%)
1 dose	India (4-valent)	139	2.3 (1.9 - 2.7)	1.2 - 4.4	108 (77.7%)	99 (71.2%)
	GMC ratio (DoRIS/India) (95% CI)			2.60 (2.07 -3.28)		
	Adjusted GMC ratio[6] (95% CI)			1.87 (1.34 -2.62)		
	Difference in seroconversion (DoRIS – India) (95% CI)			16.3% (6.1-25.8)		

[1]All participants (irrespective of ELISA antibody or HPV DNA status at baseline). [2]ELISA serum antibody geometric mean concentration (GMC). [3]Interquartile range, a measure of the variability, or spread, of the data. The lower and upper values represent the 25th and 75th percentile of the distribution, respectively (i.e. 50% of the data lie between these two values). [4]Positivity defined by the laboratory determined cut-off (HPV16 = 1.309 IU/mL; HPV18 = 1.109 IU/mL). [5]Seroconversion defined as concentrations greater than or equal to the laboratory determined cut-off among girls who were seronegative for the HPV genotype at baseline. [6]Adjusted for age. Adjustment not done for comparisons between DoRIS and CVT, because there is no overlap in the age range.

APPENDIX 1. Summary of key pre-licensure randomised controlled efficacy trials of 3-dose HPV vaccine schedule in women [current licensed HPV vaccines only]

Trial; main report(s)	Registration; Year started; Location	Vaccine	Age range (years)	Length of follow-up (main trial)	N enrolled	Primary endpoint	Cohort for primary endpoint	Vaccine efficacy (primary endpoint)
V501-007; Villa et al. Lancet Oncol 2005;[157] Villa et al. Br J Cancer 2006[158]	NCT00365716; 2000; Brazil, Europe, USA (5 countries)	Gardasil	16-23	3 years (extension to 5 years)	551 (241 in extension)	Combined incidence of HPV6/11/16/18-related 6-month persistent infection, CIN1+[a] and external genital disease	HPV 6/11/16/18 seronegative on D0 and DNA negative through Month (M)7; received all 3 doses; protocol compliant	89.5 (95%CI=70.7–97.3) at 3 years 95.8 (95%CI=83.8 – 99.5) at 5 years
FUTURE I; Garland et al NEJM 2007[159]	NCT00092521; 2002; Asia, Pacific, Europe, Americas (16 countries)	Gardasil	16-24	4 years	5455	HPV6/11/16/18 related anogenital, vulvar or vaginal warts or lesions HPV6/11/16/18 related CIN1+	HPV 6/11/16/18 DNA and seronegative through M7; received all 3 doses; protocol compliant	100% (95%CI=94–100) for anogenital, vulvar or vaginal warts or lesions 100% (95%CI=94–100) for CIN1+
FUTURE II; FUTURE II Study Group. NEJM 2007[160]	NCT00092534; 2002; Asia, Europe, Americas (13 countries)	Gardasil	15-26	4 years	12,167	HPV16/18 related CIN2+[b]	HPV 16/18 seronegative at Day (D)0 and DNA negative through M7; received all 3 doses; protocol compliant	98% (95.89%CI=86 – 100)
HPV-001, HPV-007 Harper et al. Lancet 2004;[161] Harper et al. Lancet 2006[162]	NCT00689741, NCT00120848; 2001; Canada, US, Brazil	Cervarix	15-25	18 months (phase 1); 27 months (phase 2); 4.5 years (extension)	1113 (776 in extension)	Incident HPV16/18 infection (phase 1) 6-month persistent HPV16/18 infection (phase 2) Incident HPV16/18 infection (extension)	HPV 16/18 seronegative and DNA negative at baseline; received all 3 doses; protocol compliant	Incident HPV16/18 (phase 1): 91·6% (95%CI=64·5–98·0) Persistent HPV16/18: 100% (95%CI=47·0–100) Incident HPV16/18 (extension): 96·9 (95%CI=81·3–99·9)
FUTURE III Munoz et al. Lancet 2009[163]	NCT00090220; 2004; Asia, Europe, Colombia, USA (7 countries)	Gardasil	24-45	4 years	3819	Combined incidence of HPV6/11/16/18-related 6-month persistent infection, CIN1+ and external genital disease	HPV 6/1116/18 seronegative at D0 and DNA negative through M7; received all 3 doses; protocol compliant	90·5% (95% CI 73·7–97·5)
PATRICIA Paavonen et al. Lancet 2009[164]	NCT00122681; 2004; Asia, Pacific, Europe, Americas (14 countries)	Cervarix	15-25	4 years	18,644	HPV16/18 associated CIN2+	HPV 16/18 seronegative at D0 and DNA negative though M6; received all 3 doses; protocol compliant	92·9% (95%CI=79·9–98·3)

Trial; main report(s)	Registration; Year started; Location	Vaccine	Age range (years)	Length of follow-up (main trial)	N enrolled	Primary endpoint	Cohort for primary endpoint	Vaccine efficacy (primary endpoint)
CVT Hildesheim et al. Vaccine 2014[165]	NCT00128661; 2004; Costa Rica	Cervarix	18-25	4 years	7466	HPV16/18 associated CIN2+	HPV 16/18 DNA negative though M6; received 3 doses; protocol compliant	89.8% (95%CI=39.5–99.5)
VIVIANE Skinner et al. Lancet 2014;[166] Wheeler et al. Lancet Infect Dis 2016[167]	NCT00294047; 2006; Australia, Europe, Americas, Asia (12 countries)	Cervarix	26-35	7 years (interim analysis at 4 years)	5752	Combined endpoint of HPV16/18 associated 6-month persistent infection or CIN1+	HPV16/18 seronegative at D0 and DNA negative through M6; received all 3 doses; protocol compliant	81·1% (97·7% CI=52·1–94·0) at 4 years 90·5% (96.2% CI=78·6 –96·5) at 7 years
V503-001 Huh et al. Lancet 2017[168]	NCT00543543; 2007; New Zealand, Asia, Europe, Americas (18 countries)	Gardasil9 (vs Gardasil)	16-26	54 months	14,215	Combined incidence of HPV31/33/45/52/58 -related CIN2+, VIN2+[c] or VaIN2+[d]	Seronegative on D0 for HPV types being analysed and DNA negative through M7; received all 3 doses; protocol compliant	97·4% (95%CI=85·0–99·9)
HPV-039 Zhu et al. Int J Cancer 2014[169]	NCT00779766; 2008; China	Cervarix	18-25	24 months	6053	6-month persistent infection or CIN1+ associated with HPV16/18	HPV16/18 seronegative at D0 and DNA negative through M6; received all 3 doses;	94.2% (62.7–99.9)
V501-027 Yoshikawa et al. Cancer Sci 2013[170]	NCT00378560; 2006; Japan	Gardasil	18-26	30 months	1021	Combined incidence of HPV6/11/16/18-related 6-month persistent infection, CIN1+ and external genital lesions	HPV 6/11/16/18 seronegative on D0 and DNA negative through M7; received all 3 doses; protocol compliant	87.6 (95%CI=59.2–97.6)
V501-041 Wei et al. Vaccine 2019[171]	NCT00834106; 2009; China	Gardasil	20-45	30 months (base study); 78 months (extension)	3006	Combined incidence of HPV6/11/16/18-related 6-month persistent infection, CIN1+ and external genital lesions (base study) HPV16/18 related CIN2+ (extension)	HPV 6/11/16/18 seronegative on D0 and DNA negative through M7; received all 3 doses; ≥1 follow-up visit after M7; protocol compliant	76.0 (95%CI=43.7–91.1) (base study) 100% (95% CI=32.3–100) (extension)
Konno et al. Int J Gynecol Cancer 2010[172]	NCT00316693; 2006; Japan	Cervarix	20-25	24 months	1040	6-month persistent HPV16/18 infection	HPV 16/18 seronegative at D0 and DNA negative through M6; received all 3 doses	100% (95.5% CI=71.3–100)
HPV-PRO-003 Zhao et al. Lancet Infect Dis 2022[173]	NCT01735006; 2012; China	Cecolin	18-45	66 months	7372	(1) HPV16/18-related CIN2+, VIN2+ or VaIN2+	HPV 16/18 seronegative at D0 and DNA negative through M7; received all 3 doses; protocol compliant	(1) 100% (95%CI=67·2– 100·0) (2) 97·3% (95%CI=89·9– 99·7)

Trial; main report(s)	Registration; Year started; Location	Vaccine	Age range (years)	Length of follow-up (main trial)	N enrolled	Primary endpoint	Cohort for primary endpoint	Vaccine efficacy (primary endpoint)
						(2) HPV16/18-related 6-month persistent infection		
311-HPV-1003 Unpublished; see package insert	NCT02733068; 2014; China	Walrinvax	18-30	60 months	12,000	HPV16/18 related CIN2+	HPV 16/18 seronegative and DNA negative at D0 and M6; received all 3 doses; protocol compliant	78.6% (95% CI: 23.3–96.1)

[a]Cervical intraepithelial neoplasia (CIN) any grade, adenocarcinoma in situ (AIS) or invasive cervical carcinoma. [b]Cervical intraepithelial neoplasia grade 2/3, adenocarcinoma in situ or invasive cervical carcinoma; [c]Vulvar intraepithelial neoplasia (VIN) grade 2/3, vulvar cancer; [d]Vaginal intraepithelial neoplasia (VaIN) grade 2/3, vaginal cancer.

APPENDIX 2. Paper: A dose-reduction HPV vaccine immunobridging trial of two HPV vaccines among adolescent girls in Tanzania (the DoRIS trial) – Study protocol for a randomised controlled trial

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Primary Supervisor	Deborah Watson-Jones		

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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)	This paper describes the study design and protocol of the DoRIS trial. I am the joint Principal Investigator (PI), along with Deborah Watson-Jones, on the DoRIS trial. In collaboration Deborah Watson-Jones, I co-developed the initial ideas, designed the study, secured the funding and developed the study protocol and questionnaires. I am also the DoRIS trial statistician, and had overall responsibility for the trial data management and statistical analyses. I am the first and corresponding author on this paper, wrote the first draft of the manuscript, and was responsible for replying to reviewers'comments during the peer-review process.

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A dose-reduction HPV vaccine immunobridging trial of two HPV vaccines among adolescent girls in Tanzania (the DoRIS trial) – Study protocol for a randomised controlled trial

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ABSTRACT

Background: Human papillomavirus (HPV) infection is the primary cause of cervical cancer. In 2018, the World Health Organization (WHO) Director General announced his commitment to eliminate cervical cancer, with HPV vaccination as a priority. However, the costs of setting up a multi-dose HPV vaccination programme remain a barrier to its introduction.

Methods/Design: We are conducting a randomised-controlled trial of reduced dose schedules of HPV vaccine in Tanzania to establish whether a single dose produces immune responses that will be effective in preventing cervical cancer. 930 girls aged 9–14 years in Mwanza, Tanzania, were randomised to one of 6 arms, comprising 3 different dose schedules of the 2-valent (Cervarix) and 9-valent (Gardasil-9) HPV vaccines: 3 doses; 2 doses given 6 months apart; or a single dose. All participants will be followed for 36 months; those in the 1 and 2 dose arms will be followed for 60 months. Trial outcomes focus on vaccine immune responses including HPV 16/18-specific antibody levels, antibody avidity, and memory B cell responses. Results will be immunobridged to historical cohorts of girls and young women in whom efficacy has been demonstrated.

Discussion: This is the first randomised trial of the single dose HPV vaccine schedule in the target age group. The trial will allow us to examine the quality and durability of immune responses of reduced dose schedules in a population with high burden of malaria and other infections that may affect vaccine immune responses. Initial results (24 months) are expected to be published in early 2021.

1. Background

Human papillomavirus (HPV) infection, the primary cause of cervical cancer, is a major public health problem in sub-Saharan Africa (SSA). East Africa has an estimated cervical cancer incidence of around 40/100,000 [1], among the highest in the world. In many countries in SSA, screening is absent or limited, and treatment is often sub-optimal.

In 2018, the Director-General of the World Health Organization (WHO) announced his commitment to eliminate cervical cancer [2]. Prophylactic HPV vaccines, critical for this elimination goal, are safe and highly effective at preventing HPV infection and associated disease. Three HPV vaccines are licensed; the bivalent vaccine protects against HPV 16/18 (Cervarix®), the 4-valent vaccine against HPV 6/11/16/18 (Gardasil®), and the 9-valent vaccine against 9 genotypes (HPV 6/11/

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16/18/31/33/45/52/58) (Gardasil-9®).

The vaccine was originally given as a 3-dose schedule. However, a 2-dose schedule was approved in 2016 for girls aged <15 years [3]. Of 127 countries that had included HPV vaccines in their national programmes by May 2020, only 22 are low- or middle-income countries (LMIC) [4]. The costs of setting up and sustaining a multi-dose HPV vaccine programme remain a barrier to its introduction [5,6]. Cost and logistics have also limited the implementation of extended age range 'catch-up' campaigns in existing programmes [7]. New vaccination strategies are therefore needed to enable cervical cancer elimination. A 1-dose schedule could reduce costs and simplify vaccine delivery, facilitate rollout of national programmes and catch-up campaigns, and dramatically reduce the cervical cancer burden globally.

Data suggest that 1 dose of HPV vaccine may confer sufficient protection against HPV infection and cervical cancer precursors. Women who received 1 or 2 doses of Cervarix® in the Costa Rica Vaccine (CVT) and PATRICIA trials (due to non-completion of the 3-dose schedule) had similar efficacy against HPV infection over 4 years of follow-up compared with those who received 3 doses [8]. Women who received fewer than 3 doses in the CVT are being followed long-term, and the 11-year efficacy and immunogenicity data support durable protection from 1 dose [9]. Furthermore, 1 dose provided antibody levels well above those found following natural infection. A trial of Gardasil® in India found that participants who received only 1 dose had similar incident and persistent HPV infections over 7 years as those receiving 3 doses [10]. Whilst these results challenge the established belief that protein-based subunit vaccines require a prime-boost regimen, they provide insufficient evidence to change vaccine recommendations because of their non-randomised design and post-hoc character.

The 2-dose schedule in girls aged <15 years was approved based on immunogenicity studies in high and upper middle-income countries. However, it is conceivable that the efficacy of reduced-dose schedules could be affected by intercurrent infections such as helminths or malaria [11]. We are conducting a randomised-controlled trial of reduced dose schedules of 2 HPV vaccines in Tanzania, to establish whether 1 dose produces immune responses that are likely to be effective in preventing cervical cancer in SSA. This is the first randomised trial of the single dose schedule in 9 to 14 year-old girls, the primary target group for this vaccine globally.

1.1. Trial objectives and outcomes

The overall objective of this trial is to determine whether a single dose of the bivalent vaccine (Cervarix®) or 9-valent vaccine (Gardasil-9®) produces immune responses that are non-inferior to those following 2 and 3 doses when given to HIV negative girls aged 9 to 14 years in a malaria-endemic region of Tanzania, and whether these immune responses are affected by malaria infection. We will also compare immune responses after 1 dose in young girls in Tanzania with those in historical cohorts of girls and young women who received 1, 2 or 3 doses of HPV vaccine, in whom efficacy has been demonstrated.

The trial outcomes focus on vaccine immune responses as measured by: (1) the proportion of participants seroconverting to HPV types 16/18; (2) geometric mean titre (GMT) of HPV 16/18-specific antibodies; (3) HPV 16/18-specific antibody avidity; and (4) HPV 16/18-specific memory B cell responses.

The trial has two co-primary objectives: 1) to demonstrate non-inferiority of HPV 16/18-specific seropositivity following 1 dose of HPV vaccine compared with 2 or 3 doses of the same vaccine at month (M)24; and 2) to demonstrate non-inferiority of antibody GMT at M24, when comparing the 1 dose regimen of either vaccine with historical cohorts of women aged 10–25 years who received 1 dose, in whom efficacy has been demonstrated. Secondary immunogenicity objectives include evaluation of HPV 16/18 antibody GMT and seropositivity at other timepoints, evaluation of antibody avidity and memory B cell responses, comparison of immune responses after 2 versus 3 doses, comparisons of the same dose regimen between vaccine types, and comparisons between girls who had malaria at the time of vaccination and those who did not. The primary focus is on HPV16/18; however, the antibody response to the other HPV genotypes covered by the 9-valent vaccine will also be explored. Other secondary objectives are evaluating cost effectiveness and acceptability of the 1 dose schedule.

Girls in the 1- and 2-dose arms will be invited to enrol in a trial extension, to examine the durability and stability of immune responses up to 60 months. The primary objective of the trial extension is to demonstrate non-inferiority of HPV 16/18-specific seropositivity when comparing 1 dose with 2 doses of the same vaccine at M60.

2. Methods

2.1. Study design and population

This is an open-label, individually-randomised controlled trial of two HPV vaccines being conducted at the Mwanza Intervention Trials Unit (MITU), in the lake zone region of north-western Tanzania [Dose Reduction Immunobridging and Safety Study of two HPV vaccines in Tanzanian girls (DoRIS); NCT02834637].

The trial has 6 arms comprising 3 different dose schedules of the bivalent or 9-valent HPV vaccines: the originally recommended 3 dose schedule; 2 doses given 6 months apart; or a single dose (Table 1). All girls will be followed for 36 months; those who consent to the extension will be followed for 60 months.

The trial has enrolled 930 HIV-negative schoolgirls living in Mwanza. Enrolment began in March 2017 and ended in January 2018; follow-up is expected to end in May/June 2021 for the main trial (owing to SARS-CoV-2 outbreak and postponement of some M36 visits) or January/February 2023 for the extension. Girls were eligible for inclusion in the main trial if aged 9–14 years, planning to be resident in Mwanza for 36 months and willing and able to give informed assent, with informed consent from parent/guardian. Girls were excluded if they had previously received any dose of HPV vaccination, had a past history of cervical lesions or genital warts, had received treatment for positive cervical screening, were pregnant at screening, were immunocompromised, including HIV infection, or were unwell on the basis of medical history, clinical examination or laboratory tests. At the M36 visit, all girls in the 1 and 2 dose arms will be invited to participate in the trial extension.

2.2. Randomisation

Eligible participants were randomised to one of the 6 study arms in a 1:1:1:1:1:1 allocation, using random permuted block sizes of 12, 18 and 24. The randomisation list was computer-generated by an independent statistician, with the treatment allocation order defined by the blocks and sequence within blocks. Trial participant identification numbers were generated within the computer program, and sequentially assigned in the order of the treatment allocations.

Table 1
Design of the trial Dose Reduction Immunobridging and Safety Study of two HPV vaccines in Tanzanian girls (DoRIS).

Arm	2-valent HPV vaccine (Cervarix®)			9-valent HPV vaccine (Gardasil-9®)			Total
	3 doses[a]	2 doses[b]	1 dose	3 doses[c]	2 doses[b]	1 dose	
	A	B	C	D	E	F	
Number of girls	155	155	155	155	155	155	930

[a] Given at Day(D)0, Month(M) 1 and M6.
[b] Given at D0 and M6.
[c] Given at D0, M2 and M6.

A set of sequentially-numbered opaque sealed envelopes, each containing a unique participant identification number with its allocation, were prepared by the independent statistician in advance of the enrolment visit and sent to the research clinic. At the enrolment visit, after eligibility was confirmed, the study clinician responsible for enrolment opened the next available envelope in the numbered sequence in order to find the participant's identification number and assigned allocation. The identification number and allocation were recorded on the participant's case report form (CRF).

2.3. Sample size

With the 2- and 3-dose schedules of either HPV vaccine, it is estimated that 99% will be seropositive for HPV16/18 at M24 [12]. With 155 in each HPV-dose schedule arm, assuming <5% have HPV 16/18 antibodies or are HPV 16/18 DNA positive at enrolment (based on our previous studies in Tanzania), [13–15] and a projected 20% loss to follow up (LTFU) over 36 months, we expect to have around 130 girls in each arm at the M24 visit for the primary outcome analyses, 120 girls at M36, and 100 at M60.

If the true proportion seroconverting is the same in each arm, with 130 girls per arm, we will have >90% power to conclude that seropositivity with the reduced dose schedule is not decreased by more than 5.0%, using a one-sided non-inferiority test at the 2.5% level (Table 2). This non-inferiority margin is the same that was used in the trials leading to licensure of the 2-dose regimen in girls aged <15 years [16]. If the true GMT ratio (reduced dose arm: comparison cohort) between groups is 1.0, with 130 girls in each group, we will have >90% power to conclude that the reduced dose schedule does not decrease anti-HPV 16/18 GMT by more than 50%, corresponding with a reduction of 0.30 in log titre. The non-inferiority margin was based on pre-established standards from the US Food and Drug Administration (FDA) that have been used in other HPV vaccine bridging trials [16,17]. We have assumed an SD of 0.50–0.60 log10 anti-HPV titre [12], and used a one-sided non-inferiority test at the 2.5% level.

2.4. Study interventions

Both vaccines used in this trial are licensed by the US Food and Drug Administration (FDA). The bivalent HPV vaccine (Cervarix®), produced by GSK Biologicals, contains HPV 16/18 virus-like-particles (VLP). The 9 valent vaccine is produced by Merck (Gardasil-9®) and contains HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 VLPs. The bivalent vaccine has an adjuvant consisting of monophosphoryl lipid A (MPL) and aluminium hydroxide. MPL is a detoxified bacterial lipopolysaccharide which is a TLR-4 agonist which causes activation of both innate and adaptive immune responses [18] The 9-valent vaccine uses a more traditional aluminium adjuvant (aluminium hydroxyl-phosphate sulphate), similar to that of the 4-valent vaccine, but in a higher dose. Antibody levels produced by the bivalent vaccine are significantly higher than those produced by the 4-valent vaccine both for HPV 16 and 18 and for cross-protected types [19].

Table 2
Non-inferiority margins that can be demonstrated with 90% power, for different assumptions of number of subjects evaluable in each arm.

Outcome	Number evaluable[a]	True value in population	Non-inferiority margin[b]	Power
HPV 16/18 proportion seroconverting	130	99%	4.0%	90%
	100	99%	4.6%	90%
	85	99%	5.0%	90%
HPV 16/18 antibody GMT ratio[c]	130	1.0	0.57	90%
	100	1.0	0.53	90%
	85	1.0	0.50	90%

[a] Evaluable subjects are those attending at M24, M36 or M60 who are HPV 16/18 DNA and antibody negative at enrolment.
[b] Non-inferiority defined as lower bound for 95% 2-sided CI for difference in proportions/ratio of GMT being above this margin.
[c] Assuming an SD of 0.60 log10 anti-HPV titre.

3. Study procedures

3.1. Preparatory activities

Girls were enrolled from 36 primary and 18 secondary government day schools in Ilemela municipality, Mwanza city. In the month before the screening visit, study mobilisers held meetings with community and religious leaders and heads of schools to explain the trial. Parents/guardians of potentially eligible girls attending the selected schools were invited to a meeting at the school where the trial and informed consent and assent procedures were explained. Parents/guardians were then approached individually and invited to attend the research clinic with their daughters for screening.

3.2. Screening and enrolment

A summary of the study procedures is shown in Table 3. At the screening visit, girls had the trial aims, eligibility criteria and procedures explained. Parents/guardians and girls were asked for their informed written/witnessed consent and assent, respectively. All girls aged ≥12 years were required to pass a Test of Understanding (TOU) in order to be eligible for enrolment; for younger girls, the parent/guardian was required to pass the TOU. Parents/guardians and girls were allowed to retake the test twice if they failed to reach the pass score of >90%. If the TOU was passed, girls were screened for eligibility, including a medical history with a physical examination if indicated, HIV counselling and testing and a urine test was performed for pregnancy. Girls who were HIV positive were not eligible for enrolment, but were encouraged to share the test result with their parent/guardian, and were referred for CD4 count assessment and HIV care. Girls who were found to be pregnant at the screening or enrolment visit were considered to be a screening failure and were also ineligible.

The enrolment visit was within 30 days after screening. A brief interview was conducted, another urine pregnancy test was done, and eligibility criteria were re-confirmed by the study clinician. If deemed eligible, the participant was enrolled and randomised to receive the first dose of vaccine. Girls who were ineligible because of medical history and/or physical examination were referred to a doctor for appropriate medical management according to local treatment guidelines.

Digital fingerprints were taken in order to confirm a participant's identity throughout the study. The fingerprint record was stored electronically and linked only to the participant identification number, not to the participant's name or any personal identifiers. Each participant was also given a study photo identification (ID) card. Before the first dose was given, a venous blood sample was collected for immunogenicity assays, HSV-2 serology, and a dried blood spot (DBS) for storage for malaria testing by polymerase chain reaction (PCR). Two nurse-assisted, self-administered vaginal swabs were collected for HPV DNA testing and HPV genotyping. This method of sample collection has been successfully used in other studies of HPV in girls in Mwanza [14,15].

3.3. Vaccination phase

Vaccination was conducted at enrolment (Day(D) 0; all arms), M1 or M2 (3-dose arms; Cervarix and Gardasil-9, respectively) and M6 (2- and 3-dose arms). A short medical history and repeat urine pregnancy test was done at each vaccination visit prior to vaccination. Vaccination was postponed if the girl was deemed to have an acute illness that precluded vaccination. Girls who were pregnant did not receive any further doses of vaccine but continued with the study follow-up visits.

Vaccines were administered via intramuscular injection into the

Table 3
Summary of study procedures.

Study procedure	Screen < 30 d	D0	M1	M2	M3	M6	M7	M12	M18	M24	M30	M36	M42[g]	M48[g]	M54[g]	M60[g]
Informed consent/assent	X															
Informed consent/assent for trial extension												X[g]				
Demographics & tracing info	X											X[g]				
Medical history[a]	X															
Test of Understanding	X															
Blood sampling for HIV	X															
Pregnancy test	X	X	X[b]	X[b]		X[b]										
Check LMP & pregnancy test if indicated			X[c]	X[c]	X	X[c]	X	X		X		X		X		X
Eligibility check	X	X														
Clinic visit	X	X	X	X[d]	X[d]	X	X	X		X		X		X		X
Home, clinic or school visit									X		X		X		X	
Blood sampling for immunogenicity		X	X				X	X		X		X				X
Vaginal swabs for HPV genotyping		X														
Blood sampling for malaria		X	X[b]	X[b]		X										
Blood sampling for HSV-2		X					X[e]	X[e]		X[e]		X[e]				X
Review of medical history[a]		X	X[b]	X[b]		X[b]										
Check deferral criteria and contraindications		X	X[b]	X[b]		X[b]										
Vaccine administration		X	X[b]	X[b]		X[b]										
Recording of AEs in 30 days post vaccination			X	X[f]	X[f]		X[f]									
Recording of unsolicited AEs/SAE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Timing of laboratory assays																
HPV antibody ELISA		X	X				X	X		X		X				X
HPV antibody avidity								X		X		X				
Memory B cells		X	X				X	X		X		X				
PSV Luminex assay		X	X				X	X		X		X				X
Malaria		X	X[b]	X[b]		X										
HSV-2		X					X[e]	X[e]		X[e]		X[e]				X
HIV	X															

[a] Examination if warranted.
[b] Only for those randomised to vaccine at that visit.
[c] For those not randomised to receive vaccine at that visit.
[d] M2 visit attended by 3 dose arms only; M3 visit attended by 3 dose Gardasil-9 arm only.
[e] Storage of serum sample for HSV-2 serology at last visit attended.
[f] Questions about AEs that occurred in the 30 days since the last dose, only for those participants who received vaccine at the previous visit.
[g] Extension activities will be conducted for girls in 1 and 2 dose arms only.

deltoid region of the upper arm. After vaccination, participants were observed for at least 30 min, with appropriate medical treatment and equipment available in case of any anaphylactic reaction. The study staff monitored the participant's vital signs and looked for injection site reactions and other adverse events, which were recorded on the CRF. At each vaccination visit, a blood sample was collected for a DBS, which was stored for malaria PCR testing.

Participants attended the clinic one month after each vaccination visit for questioning about adverse events in the 30 days after vaccination, and for blood sampling for immunogenicity outcomes. The windows for vaccination and blood sampling are in Table 4.

3.4. Follow-up

All participants were asked to attend the clinic at M6 and M7 to collect a blood sample for a DBS for malaria PCR (M6) and for vaccine immunogenicity assays (M7). Scheduled follow-up visits are at M12, M24 and M36, and a blood sample is collected for immunogenicity. Participants in the trial extension will also be followed up at M48 and M60, and an immunogenicity blood sample will be collected at M60.

At M18 and M30 (and M42 and M54 in the trial extension), participants are visited at home or at school to ensure that they are still living in Mwanza and to update contact details if needed. Participants are questioned about AEs at all study visits. In addition, to help ensure a high rate of retention between visits, participants are sent an SMS reminder or telephoned about the trial every 3 months.

In April 2020, the trial was temporarily suspended owing to the SARS-CoV-2 outbreak; a protocol amendment was submitted to increase the window for the M36 blood sample (Table 4). The Tanzanian National Health Research Ethics Sub-Committee (*NatHREC*) gave permission for studies to resume activities as per protocol on 18 May 2020 provided that training is done in small groups and COVID-19 preventative measures, such as mask-wearing, physical distancing and hand hygiene, are implemented for research activities. The trial team resumed month 36 visits on 3 August 2020.

3.5. Laboratory assays

Whole blood samples of up to 20 mL (depending on girl's weight) are collected for immunological assays, in order to provide 10 mL of serum and 10 mL for peripheral blood mononuclear cells (PBMC). All samples are processed and stored initially at the laboratory at the National Institute of Medical Research (NIMR) in Mwanza, before being shipped to the relevant laboratory for analysis. HPV 16/18 antibodies will be measured by a qualified anti-VLP ELISA assay at the Frederick National Laboratory for Cancer Research HPV Immunology Laboratory in Maryland, USA [20]. The primary analyses will be based on antibody GMT as measured in this VLP ELISA. The HPV 16/18-specific antibody avidity index (AI) will be determined in the ELISA by the ratio of antibody concentrations in serum samples treated or not treated with Guanidine-HCl [21]. HPV 16/18-specific memory B cell responses will be measured in PBMCs by a B cell ELISPOT assay at the Centre for Immunology and

Table 4
Window periods for vaccination and follow-up visits.

Procedure	Arms[a]	Visit	Recommended date	Minimum date	Maximum date
Vaccination visits					
Dose 1	All	D0	Date of first vaccination (DFV)	N/A	N/A
Dose 2	A	M1	DFV +30 days	DFV +30 days	DFV +60 days
Dose 2	D	M2	DFV +60 days	DFV +30 days	DFV +90 days
Dose 2	B and E	M6	DFV +181 days	DFV +181 days	DFV +271 days[b]
Dose 3	A and D	M6	DFV +181 days	DFV +181 days	DFV +271 days[b]
Follow-up visits					
D0 blood sample	All	D0	DFV	N/A	N/A
M1 blood sample and AE recording[c]	All	M1	DFV +30 days	DFV +30 days	DFV +60 days
M2 AE recording[c]	A	M2	Date of second vaccination +30 days	Date of second vaccination +30 days	Date of second vaccination +60 days
M2 blood sample for malaria	D	M2	Date of second vaccination	N/A	N/A
M3 AE recording[c]	D	M3	Date of second vaccination +30 days	Date of second vaccination +30 days	Date of second vaccination +60 days
M6 blood sample for malaria	A, B, D and E	M6	Date of last vaccination	Date of last vaccination	Date of last vaccination
M6 blood sample for malaria	C and F	M6	DFV +181 days	DFV +181 days	DFV +211 days
M7 blood sample	A, B, D and E	M7	Date of last vaccination +30 days	Date of last vaccination +30 days	Date of last vaccination +60 days
M7 blood sample	C and F	M7	DFV +211 days	DFV +211 days	DFV +241 days
M12 blood sample	All	M12	DFV +361 days	DFV +361 days	DFV +391 days
M24 blood sample	All	M24	DFV +723 days	DFV +723 days	DFV +753 days
M36 blood sample	All	M36	DFV +1085 days	DFV +1055 days	DFV +1265 days[d]
M60 blood sample	B, C, E, F	M60	DFV +1809 days	DFV +1779 days	DFV +1839 days

[a] Arms A, B and C receive 3 doses, 2 doses and 1 doses of Cervarix®, respectively. Arms D, E and F receive 3 doses, 2 doses and 1 doses of Gardasil-9®, respectively.
[b] For ethical reasons, girls may receive the last dose up to 360 days after DFV; however, they may be excluded from the immunogenicity analysis.
[c] Solicited signs and symptoms in the 30 days after each vaccine dose.
[d] Window extended because of SARS-CoV-2 outbreak and postponement of some M36 visits.

Infection, York, UK; detectable HPV type-specific memory B-cells will be defined as >1 antigen-specific memory B cell/million memory B cells [22]. Serum HPV antibody titres to the HPV genotypes in the 9-valent vaccine are being measured by a pseudovirion (PsV)-based antibody Luminex assay at the Karolinska Institute, Sweden; the assay has shown high correlations with VLP-ELISA and neutralisation assays, and with natural infection [23–25]. HPV DNA genotyping at D0 was done using the Anyplex HPV28 (Seegene, South Korea) detection assay at the Catalan Institute of Oncology, Barcelona.

3.6. Data management

All completed CRFs and laboratory forms are submitted to the MITU data section. Data are double-entered into a study-specific database by trained data entry staff, using the OpenClinica open source software. Data checks and data cleaning are done by trained data managers at MITU under the supervision of a senior data manager. Submitted CRFs and forms are stored securely in locked filing cabinets in the MITU data department. At the conclusion of the study, the database will be archived in accordance with internal procedures.

3.7. Statistical analysis

In non-inferiority trials, intention-to-treat (ITT) analyses may increase the risk of falsely claiming non-inferiority, since these analyses often lead to smaller observed effects than if all participants had adhered to the protocol [26]. Therefore, for the non-inferiority objectives, we will conduct the primary analyses in the per-protocol (PP) population, and repeat all analyses in the intention-to-treat (ITT) population as a sensitivity analysis. The PP population will be girls who receive the allocated doses of vaccine within the specified windows in Table 4 and are HPV antibody and DNA negative at enrolment for the specific genotype under analysis.

Seropositivity for a particular HPV vaccine genotype will be defined as antibody level above the assay cut-off; the cut-off value will be defined by the laboratory before the analysis begins. We will measure the proportion of girls in each arm who are seropositive for each HPV vaccine genotype, and calculate the difference (reduced dose group minus comparison group) between arms. We will estimate the 95% CI for the difference using the Farrington and Manning approach [27]. Non-inferiority will be concluded if the lower 95% CI for the difference is above −5%.

The ratio of HPV genotype-specific GMTs will be obtained from an analysis of variance (ANOVA) of log10 antibody titres as the response variable. Separate analyses will be done for each vaccine genotype and time point. The ANOVA model will include trial group as a fixed effect. For each vaccine type (Cervarix and Gardasil-9), a contrast will be derived from the ANOVA model comparing the mean of log10 titre in the reduced dose group with that in the comparison group, using the residual error from the ANOVA. The GMT ratio and its 95% CI will be derived from back-transformation of the mean and 95% CI from this contrast. Non-inferiority will be concluded if the lower bound for the 95% two-sided CI for the GMT ratio (reduced dose group divided by comparison group) is above 0.50.

The primary analyses will exclude girls with missing immunogenicity results (complete case), but a sensitivity analysis using multiple imputation of missing data may be done at month 36, and/or month 60.

For the primary objectives, non-inferiority for each vaccine type will be concluded if the lower limit for the 95% CI for the seroconversion difference between 1 dose vs 2 doses, and between 1 dose vs 3 doses, is above −5% for both HPV16 and HPV18. For comparisons with historical cohorts, non-inferiority for each vaccine type will be concluded if the lower limit of the 95% CI is for the GMT ratio (1D/historical cohort) is above 0.50 for both HPV16 and HPV18. For the secondary objectives, a hierarchy of testing for the non-inferiority objectives will be prespecified in a statistical analysis plan.

A subgroup analysis will be done to compare immune responses between girls who were positive and negative for malaria at vaccination. Since malaria is measured at different timepoints relative to enrolment depending on arm, these will primarily focus on comparisons within arm, or between the same dose regimens (e.g. 1 dose of bivalent vs. 1 dose of 9-valent).

Full details of the statistical methods will be covered in a formal Statistical Analysis Plan that will incorporate a formal plan for the

immunobridging analyses. For the secondary objectives, a hierarchy of testing for the non-inferiority objectives will be pre-specified. The analysis plan will also include pre-specified criteria for non-inferiority, plans for adjustment for multiplicity, and other statistical considerations for non-inferiority trials with immunogenicity endpoints, as outlined by Liu et al. [28] The final analysis plan will be approved by the Independent Data Safety and Monitoring Board (IDSMB), the Trial Steering Committee (TSC), and the Principal Investigators before the randomisation code is released and the data are analysed.

3.8. Immunobridging

We will bridge our results to historical cohorts of girls and young women aged 10–25 years who received 1, 2 or 3 doses, in whom efficacy has been demonstrated. These include the previous trial of the bivalent vaccine in Costa Rica, the CVT (NCT00128661), which vaccinated young women aged 18–25 years [29], and the International Agency for Research on Cancer (IARC) trial of the 4-valent vaccine in India (NCT00923702), which vaccinated girls and young women aged 10–18 years [10]. In addition, we will bridge our immunogenicity results to those of the National Cancer Institute's (NCI) large randomised controlled trial to evaluate the efficacy of the bivalent and 9-valent vaccines given as 1 or 2 doses to girls aged 12–16 years in Costa Rica (the ESCUDDO trial; NCT03180034) and with which our trial protocol has been harmonised. Bridging with a recently started efficacy trial in Kenya in young women aged 15–20 (KEN-SHE; NCT03675256) is also planned. Results from these trials are expected in 2023 (KEN-SHE) and 2025 (ESCUDDO).

3.9. Ethics and oversight

The trial protocol was approved by the ethical committee of the Medical Research Coordinating Committee, Tanzania (NIMR/HQ/R.8A/Vol.IX/2236), and the London School of Hygiene and Tropical Medicine (11568). Written informed consent is obtained from parents/guardians, with written assent from participants. A TSC and IDSMB were established to monitor trial progress. A community advisory board (CAB) comprising parents, teachers and other community members was established to advise the research team. Trial monitoring is being done by independent trial monitors from Kenya Medical Research Institute (KEMRI) in Kenya.

4. Discussion

This is the first randomised trial of the single dose schedule of HPV vaccine in 9–14 year old girls, the primary target group for HPV vaccine globally, and the first randomised trial of HPV vaccine dose reduction in SSA. Final results from the main trial are expected in early 2022, with interim results submitted for publication in early 2021. Other trials evaluating single dose protection have recently begun in Costa Rica, The Gambia and Kenya, with results available at the end of 2022 or later. These trials are all complementary, examining single-dose HPV vaccination for girls, adolescents, and young women aged 4–20 years, and address different scientific and programmatic questions.

In addition to comparisons between trial arms, our trial will compare vaccine-induced HPV-specific immune responses in young girls in Tanzania with those in historical cohorts of girls and young women who received 1, 2 or 3 doses of HPV vaccine, in whom efficacy has been demonstrated. We will also bridge our results with those from ongoing efficacy trials in Costa Rica and Kenya; our trial protocol is also harmonised with that of the ongoing trial in Costa Rica to maximise comparability between the 2 trials. Since it is difficult to evaluate HPV vaccine efficacy in young girls because of the time needed to accrue endpoints, immunobridging studies are used to infer protection when efficacy has been demonstrated in another population [30].

The true immunological correlates of protection for HPV vaccines have not yet been established. Age is a key determinant of antibody responses following HPV vaccination, with young girls having significantly higher antibody GMTs than young women [12,17]. Although the 2 dose regimen in girls aged <15 years has been approved based on vaccine-specific antibody levels, there is increasing recognition that vaccine efficacy depends on both quantity and quality of antibodies induced by the vaccine. Quality, measured by avidity of antibodies for the antigen, depends on priming of B cells which produce antibodies with different affinities for antigen. It is not known whether antibody affinity, memory B cell responses and durability of protection with fewer doses of HPV vaccine may be affected by intercurrent infections such as malaria or helminths. Our previous trial of 3 doses of the bivalent HPV vaccine in Tanzania found that girls who had malaria at the time of vaccination had significantly higher HPV 16/18 antibody levels one month after the last dose compared with girls who did not have malaria [31]. Malaria induces polyclonal antibodies which may enhance vaccine-induced anti-VLP antibodies, but the quality of these antibodies related to vaccine-induced protection is not known [32]. Ours, and the other ongoing one-dose trials, will help provide definitive answers to questions about non-inferiority of 1 dose of HPV vaccine compared with 2 doses, in terms of immunogenicity and HPV infection, and the feasibility of dose reduction.

Following the call from the WHO Director General in 2018, a Global Strategy for elimination of cervical cancer as a public health problem was drafted [33]. This calls for a comprehensive approach that includes prevention, screening and treatment, with a proposed global target that 90% of girls aged ≤15 years have been vaccinated for HPV by 2030. In a meeting of the WHO Strategic Advisory Group of Experts (SAGE) in October 2018, HPV vaccination was declared to be the most critical intervention for eliminating cervical cancer [34]. More recently, the World Medical Association announced its commitment to cervical cancer elimination, emphasising the need to improve HPV vaccination coverage [35]. However, the commitment to eliminate cervical cancer will be difficult to achieve without novel vaccination strategies to reduce HPV infection. A single dose schedule could help achieve this goal by reducing the cost and complexity of delivery.

In 2019, estimated global coverage among girls in the target range for vaccination (9–14 years) was 40%, and only 8–9% of 10-20-year-old girls have been vaccinated [36]. An estimated 30% of girls aged 9–14 years globally live in countries that have introduced the HPV vaccine, which means that many girls in the target age range for the vaccine are likely to remain unvaccinated [37]. The Tanzanian national HPV vaccination programme was rolled out in 2018, and is delivering 2 doses of the 4-valent vaccine (Gardasil®) to girls aged 14 years. However, coverage in 2019 was only 49% [unpublished data from the Tanzanian Ministry of Health provided to MITU/NIMR]. Furthermore, HPV vaccine supply has been constrained since 2018, which has affected HPV vaccination programmes worldwide, and supply is predicted to remain constrained for the next 3–5 years [37]. In their 2018 meeting, SAGE called for a comprehensive evaluation of options for the best use and allocation of the limited vaccine supply [35]. Given the large number of countries that have yet to adopt an HPV vaccination program, the lower cost and greater flexibility of a 1 dose HPV vaccination schedule has the potential to increase HPV vaccine introductions globally. The 1 dose schedule would also facilitate the introduction of the HPV-FASTER scheme, which proposes to combine HPV vaccination in women aged up to 30 years with at least one HPV-screening test, as a means to accelerate cervical cancer elimination [7].

Strengths of our trial are the comparison of two vaccine types, and 3 dosing schedules, allowing us to compare between/within vaccine types and dose schedules. Our outcomes focus on a full range of immune responses, including anti-VLP antibody levels, neutralising antibodies, antibody avidity, and memory B cell responses and the impact of malaria on these responses. There are no data on HPV vaccine antibody avidity or B cell memory from SSA, and no data on these functional aspects of the immune response for the 9-valent vaccine, so this will be the first

trial to examine and compare these. We are also including a component to evaluate cost effectiveness and acceptability of the 1 dose schedule.

A limitation of our trial is that we are not collecting efficacy data because of the long duration of follow-up and large sample size that would be required, and because this is being done in the trial in Costa Rica with which we are harmonised (the ESCUDDO trial; NCT03180034). We are immunobridging to that trial and other earlier large efficacy studies in a variety of populations and settings, which will allow us to infer reproducibility of efficacy across different regions.

In summary, our trial will contribute robust evidence of the effect of the 1 dose schedule on a range of immune responses among young girls in SSA, and whether these may provide sufficient protection against HPV infection. The combined evidence from this and other ongoing 1 dose trials will provide critical information for policy-makers on the efficacy of this HPV vaccination strategy, which could alleviate vaccine supply constraints and expand access to the vaccine in the countries that need it most.

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APPENDIX 3. Laboratory assays used in DoRIS trial

Assay	Marker	Timepoints	Sample type	Laboratory
L1 VLP ELISA	HPV-16/18 IgG antibody concentrations	M0, 1, 7, 12, 24, 36, 60, 84 & 108	Serum	Frederick National Laboratory for Cancer Research, USA
ELISA-based avidity	HPV 16/18 IgG antibody avidity index	M0, 12, 24 & 36, 84 & 108	Serum	Frederick National Laboratory for Cancer Research, USA
HPV Multiplex immunoassay	HPV-6, 11, 16, 18, 31, 33, 45, 52 & 58 IgG antibody concentrations	M0, 12, 24, 36, 60, 84 & 108	Serum	Frederick National Laboratory for Cancer Research, USA
Pseudovirion (PsV) Luminex	HPV-6, 11, 16, 18, 31, 33, 45, 52 & 58 IgG antibody concentrations	M0, 1, 7, 12, 24, 36, 60, 84 & 108	Serum	Karolinska Institute, Sweden
Memory B cell ELISPOT	HPV 16/18-specific memory B cell response	M0, 1, 7, 12, 24 & 36	PBMC[1]	Centre for Immunology and Infection, York, UK
qPCR[2]	Malaria	M0, 1, 2 & 6	Dried blood spot	LSHTM, UK
Roche linear array	HPV DNA	M0	Vaginal swab	Catalan Institute of Oncology, Spain
HIV rapid test[3]	HIV serostatus	M0	Serum	NIMR Mwanza, Tanzania

[1]Peripheral blood mononuclear cells. [2]Quantitative PCR. [3]Two rapid tests (serial testing), with second test done only if first test is reactive; rapid tests repeated if discordant. Participants with persistently discordant results tested by ELISA

APPENDIX 4. Paper: Rapid acquisition of HPV around the time of sexual debut in adolescent girls in Tanzania

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Surname/Family Name	Baisley		
Thesis Title	HPV epidemiology and HPV vaccination in East Africa		
Primary Supervisor	Deborah Watson-Jones		

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SECTION E

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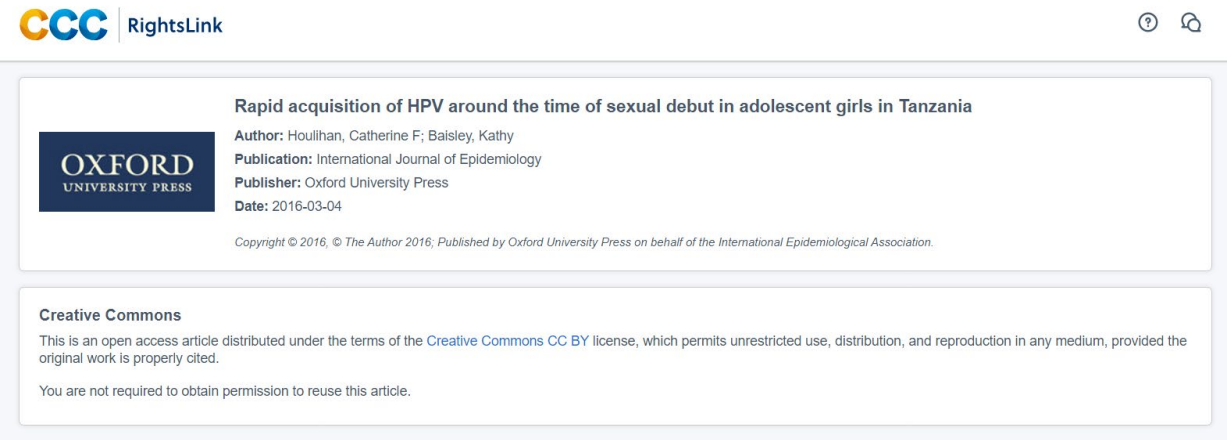

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Infection and Cancer

Rapid acquisition of HPV around the time of sexual debut in adolescent girls in Tanzania

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

Results: A total of 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 remaining 105 girls contributed 323 adequate specimens. Incidence of any new HPV genotype was 225/100 person-years (pys), and incidence of vaccine types HPV-6, -11, -16 and -18 were 12, 2, 2 and 7/100 pys, respectively. 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 HPVinfection was 5 months, and infection duration was 6 months.

Conclusion: This is the first description of HPV acquisition after first sex in sub-Saharan Africa where the incidence of cervical cancer is amongst the highest in the world. HPV incidence was very high after first sex, including some vaccine genotypes, and infection duration was short. This very high HPV incidence may help explain high cervical cancer rates, and supports recommendations that the HPV vaccine should be given to girls before first sex.

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

Key Messages

- This is the first description of HPV acquisition after first sex in sub-Saharan Africa where the incidence of cervical cancer is amongst the highest in the world.
- HPV incidence was very high after first sex, including of vaccine genotypes.
- Duration of HPV infection was short in these adolescent girls.

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 'higHRisk' (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-standardized 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 have shown that the highest prevalence is in women under 25 years old.[6] From limited studies which have tested girls for HPV before and after first 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 15- and 16-year-old unvaccinated girls and followed them 3-monthly 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 three 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; able to attend appointments; and willing to self-administer a vaginal swab. Since the enrolment procedures involved parental consent followed by participant assent and assessment for eligibility, we elected to additionally include some girls who reported sex in order to prevent stigmatization of girls, since their virginity could potentially be inferred by parents/others. We therefore 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.

Study procedures

The London School of Hygiene and Tropical Medicine Ethics Committee and the Medical Research Coordinating Committee, Tanzania, approved the study protocol in 2011. Consent procedures have been previously described.[11] Girls were enrolled between January and August 2012, and followed 3-monthly for 18 months. At each visit, girls had a face-to-face interview in Swahili with a female study nurse using a structured paper questionnaire,[11] and one nurse-assisted, self-administered vaginal Dacron swab was obtained, irrespective of reported sex. Girls who reported previous sex were offered a pregnancy test and asked about symptoms of reproductive tract infections at every visit. 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 passing sexual debut before or during the study.

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°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, USA). We used the updated HPV types from the International Human Papillomavirus (HPV) Reference Center (www.hpvcenter.se), and therefore reclassified HPV55 as HPV44, HPV64 as HPV34, and CP6108 as HPV89, and merged HPV-IS39 with HPV82. Therefore, 36 HPV genotypes were detected (HPV6, -11, -16, -18, -26, -31, -33, -34, -35, -39, -40, -42, -44, -45, -51, -52, -53, -54, -56, -58, -59, -61, -62, -66, -67, -68, -69, -70, -71, -72, -73, -81, -82, -83, -84, and -89). For this study, we classified HPV genotypes in IARC groups I (termed carcinogenic) and IIA (termed probably carcinogenic) as HR; HPV -16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -68. All remaining genotypes were classified 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, MA, USA), and analysed using STATA V13.0 (StataCorp LP, TX, 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. Further detail on statistical methods can be found in the Supplementary material (available as Supplementary data at *IJE* online).

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 that is no longer detected) was 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 date was pre-enrolment) or date of sexual debut (among girls who reported sexual debut during follow-up). 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 incidences of all new HPV, new HR-HPV and new LR-HPV infections were calculated among: (i) all sexually active girls; (ii) girls who reported sexual debut during follow-up; and (iii) HPV-naïve girls who reported sexual debut during follow-up. 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 the incidence of new HPV infections among all sexually active girls were estimated using random effects Poisson regression.

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). Kaplan-Meier methods were used to estimate the median and mean duration of genotype-specific infections and the proportion of infections cleared at 12 months. Cox regression with robust standard errors was used to examine risk factors for clearance.

Results

Cohort screening, enrolment and follow-up

We located 1177 (75.7%) of 1555 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 (Supplementary Figure 1, available as Supplementary data at *IJE* online). Of those screened, 503 (80.1%) were eligible and enrolled. Overall, 106 (21.1%) participants reported first sex: 29 at enrolment, 77 during follow-up. 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). The median [interquartile range (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 and 1 (1.1%) was positive. 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%);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 only 1 girl reported being circumcised.

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

HPV type	Prevalent (%)[a]	New infections/ pys (rate/100 pys)[b]	New infections that were cleared (%)[c]	*N* cleared/pys (rate/100 pys)	Mean (median) months duration (Kaplan-Meier)[d]
HigHRisk 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[e]	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)	–	–	–
HPV34	0	1/45.4 (2.2)	0 (0%)	0/0.1 (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)
HPV44	0	4/43.8 (9.1)	1 (25%)	1/1.3 (78.4)	6.2* (4.9)
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* (†)
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)
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)
HPV89	0	7/43.6 (16.1)	1 (14%)	1/1.8 (55.6)	7.0* (4.9)
All LR infections[e]	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)**

[a]Positive for that genotype at the enrolment visit, among 29 girls who were sexually active at enrolment.

[b]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 most recent available HPV result before the gap.

[c]Clearance defined as ≥ 2 consecutive samples negative for the specific genotype; denominator is total genotype-specific new infections.

[d]Mean duration of new infections estimated using Kaplan-Meier methods restricted by the longest follow-up time (i.e. duration).

[e]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.

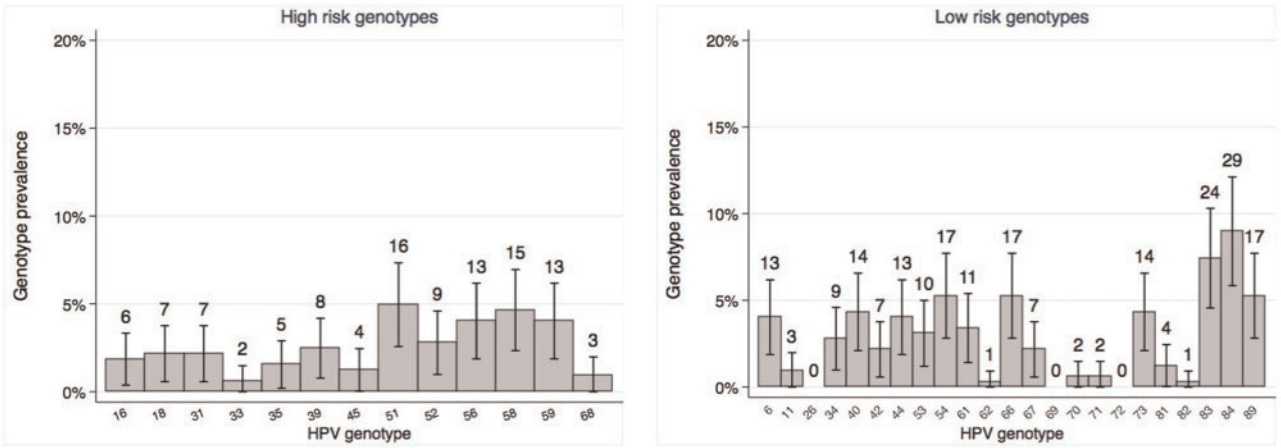

Figure 1. HPV genotype point prevalence (95% confidence interval), and number of infections at all visits after reported first sex in 105 adolescent girls.

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.

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. A total of 28 new HR infections and 57 new LR infections were detected during follow-up (Table 1). The most common HR genotypes were HPV51 (5.0% of visits), HPV58 (4.6%), HPV56 (4.0%) and HPV59 (4.0%) (Figure 1).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 1). The highest incidence rates (per 100 pys) of HR types were for HPV58 (14.2), HPV51 (9.6) and HPV18 (6.7).

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 2), and to first HR-HPV was 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 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, these were 193 (95% CI: 118-316) and 72 (95% CI: 42-122), respectively. Overall, HPV was detected in 46% of 'sexually active visits' in all girls (Table 2).

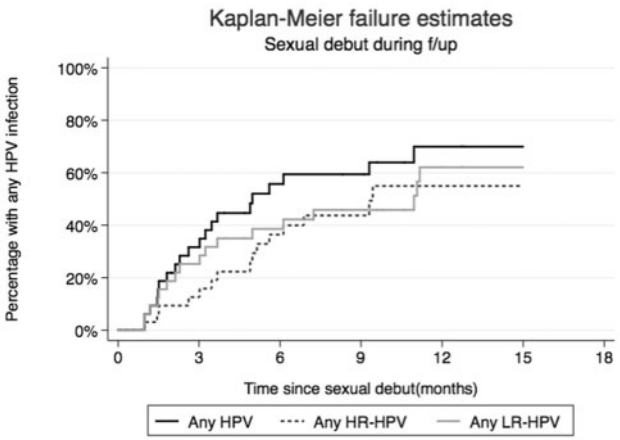

Figure 2. Time from sexual debut to first infection with any HPV, any HR HPV or any LR HPV, among 41 girls who reported sexual debut during follow-up and were HPV-naïve at time of reported sexual debut.

Kaplan Meier curves are calculated separately for each HPV group.

Risk factors for incidence of new HPV infection

In the adjusted analysis (Table 3) there was evidence of an association with: not being in a regular job or training as compared with those with an occupation [adjusted (a) RR = 1.95, 95% CI: 1.1-3.42], with the reporting of recent sex (aRR 2.48, 95% CI: 1.40-4.37) and having known the most recent partner for longer (aRR 3.15, 95% CI: 1.32-7.50). There was weak evidence of a higher rate of new HPV infections among girls reporting three or more partners compared with only one partner, and weak evidence of a lower rate among girls who reported vaginal cleansing (aRR 0.69, 95% CI: 0.43-1.10).

Table 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 ($N = 76$)[a]	Girls who reported sexual debut during study and were HPV-naïve ($N = 41$)[b]	Reported sexual debut prior to enrolment ($N = 29$)
Incidence				
	New infections/person-years (rate/100 person-years, 95% CI)[c]			
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)
Prevalence				
	Total infections (number of visits with at least one infection/sexually active visits; % of all visits)[d]			
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%)

[a]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).

[b]HPV incidence among 41 girls who reported passing sexual debut during the study and no HPV was detected before reported sexual debut.

[c]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 contributes to the analysis, therefore total number of infections is different from that in Table 1.

[d]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.

HPV duration and clearance

During follow-up, 33 girls acquired at least one new HPV genotype and contributed 85 new infections to the genotype-specific duration and clearance analysis. 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. This was 6.0 and 6.1 months for new HR and new LR-HPV genotypes, respectively. Overall rate of clearance (per 100 pys) was 90.4 for any HPV genotype. After adjustment for age, there were no significant associations with any examined factors (Table 4).

Discussion

In this study, we demonstrate 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 these first 6 months. 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 re-infection 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 re-activations. 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 100 pys for each of the HPV types covered by the quadrivalent vaccine (HPV6, -11, -16 and -18); and between 1.3 and 13.6 per 100 pys 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 (2.3/100 pys) and HPV-18 (6.7/100 pys) 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 (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 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,

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

	Number of infections[b]/ person-years (rate/100 pys)	Crude RR (95% CI)	Adjusted RR (95% CI)[c]
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)
Socioeconomic 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 3 months		$P = 0.005$	**$P = 0.008$**
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[d]	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[d]	53/21.7 (287)	–	–
Age difference of most recent partner		$P = 0.11$	$P = 0.10$
$\leq$ 2 years	8/8.9 (82)	1	1
3–5 years	26/11.0 (215)	2.65 (1.03-6.83)	1. (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[d]	49/22.6 (316)	–	–
Used condom at most recent 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)

(Continued)

Table 3. Continued

	Number of infections[b]/ person-years (rate/100 pys)	Crude RR (95% CI)	Adjusted RR (95% CI)[c]
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[d]	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**
< 1 month	26/16.1 (143)	1	**1**
1-6 months	64/28.0 (252)	1.76 (0.89-3.47)	**1.76 (0.90-3.46)**
6+ months	28/11.0 (314)	2.19 (0.92-5.20)	**3.15 (1.32-7.50)**
Cleansed vagina in past 3 months[e]		$P = 0.02$	$P = 0.11$
No	68/29.8 (300)	1	1
Yes	51/26.6 (169)	0.56 (0.35-0.90)	0.69 (0.43-1.10)

[a]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.

[b]Girls are assumed to be continually at risk and can acquire > 1 infection at each visit. Observation time after gaps > 180 days contributes to the analysis; therefore, the total number of infections (119) is different from that in Table 1.

[c]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).

[d]'Don't know' responses considered missing data and not included in analysis.

[e]Vaginal cleansing is cleaning inside the vagina with water, soap or other products using fingers or a cloth.

but the particularly high incidence in our study may be driven by a high HPV prevalence in the male partners of these young women.[6,7,16–18] However, the incidence in our cohort is higher than in other studies in young women in East Africa: in sexually active women in Uganda (median age 20 years), HPV incidence was 30.5/100 pys,[19] and 74/100 pys in women in Mwanza, Tanzania.[17] The latter study was performed in the same region, but participants were older (median age 18), and all had reported previous sex. These comparative findings support the suggestion that incidence is highest around the time of first sex.

Comparing the incidence of individual genotypes in our Tanzanian study with a study in women in the USA aged 16-23 years, 5% of whom reported never having had sex[20]: in our participants, HPV6, -11 and -18 incidences were 3-fold higher. However, a lower rate was seen for HPV16 in our study (2.3/100 pys) compared with the study in the USA (5.4/100 pys). This is in keeping with findings that HPV-16 is less common in SSA than in other regions including the USA.[6,21]

Not working was associated with increased HPV incidence compared with being employed or in vocational training. Girls not working may be at increased risk of engaging in sex in exchange for gifts or money or of 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 local studies in older women.[25] Knowing a partner for 6 or more months before sex was associated with a more than 3-fold risk of incident HPV compared with knowing a partner for under 1 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, reported condom use at most recent 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 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 self-administered swabs since speculum examination was undesirable in girls who had not passed sexual debut. Over 90% were β-globin positive, indicating adequate sampling.[28] Further, a previous study in Uganda demonstrated good HPV-genotype correlation in self-administered and clinician-administered swabs.[29]

Unobserved intervals (without vaginal swab results) of over 180 days were removed from the analysis. However,

Table 4. Clearance of new HPV infections[a] 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)	–	–
Socioeconomic 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 most recent 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 3 months		$P = 0.14$	$P = 0.14$
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[b]	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[b]	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[b]	–	–	–

(Continued)

Table 4. Continued

	Number cleared/ person-years (rate/100 pys)	Crude HR (95% CI)	Adjusted HR (95% CI)
Partner used vaseline for sex			
No	21/23.0 (91.3)	–	–
Yes	0/1.0 (0.0)	–	–
Time had known most recent partner before sex		$P = 0.13$	$P = 0.89$
< 1 month	7/7.4 (94.9)	1	1
1-6 months	17/17.2 (98.6)	2.05 (0.64-6.53)	0.98 (0.30-3.15)
6+ months	2/3.8 (52.1)	0.82 (0.17-4.01)	0.72 (0.09-5.54)
Cleansed vagina in past 3 months[c]		$P = 0.02$	$P = 0.30$
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)

[a]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 latest available HPV result before the gap. All *P*-values are from likelihood ratio tests.

[b]'Don't know' responses considered missing data and not included in analysis.

[c]Vaginal cleansing is cleaning inside the vagina with water, soap or other products using fingers or a cloth.

sensitivity analyses, where girls were assumed to be uninfected with a new genotype during those intervals, gave similar results [incidence among all 'sexually active' girls was 158/100 pys, (95% CI: 123–203)]. Samples negative or missing for a given genotype, but which had been taken between two samples positive for that genotype, were classified as positive since studies describing long-term persistence have demonstrated sporadic detection of the same genotype early in the course of a persistent infection.[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 attending the final visit accepted an HIV test; therefore HIV-positive girls may have been included in the analysis. However, national estimates indicate a very low HIV prevalence in 15-19-year-old girls in Tanzania (1.3%).[33]

Median time from first reported sex to acquisition of any HPV was 5 months. This is longer than 2.4 months reported in college students in the USA tested 3-monthly.[34] Differences in the types of relationships formed (marriage vs 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 with 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 compared with these previous studies, and was dependent on the point at which girls reported sexual debut.[14,15,36] Clearance events may have been falsely observed through lack of detection of HPV due to self-sampling. As discussed earlier, the presence of β-globin was considered necessary to ensure adequate vaginal sampling and will have reduced this risk. A short duration of infection could 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 with the USA.[37] High levels of endocervical T lymphocytes identified in those women 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 girls were either HIV-negative or of 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]

Supplementary Data

Supplementary data are available at *IJE* online.

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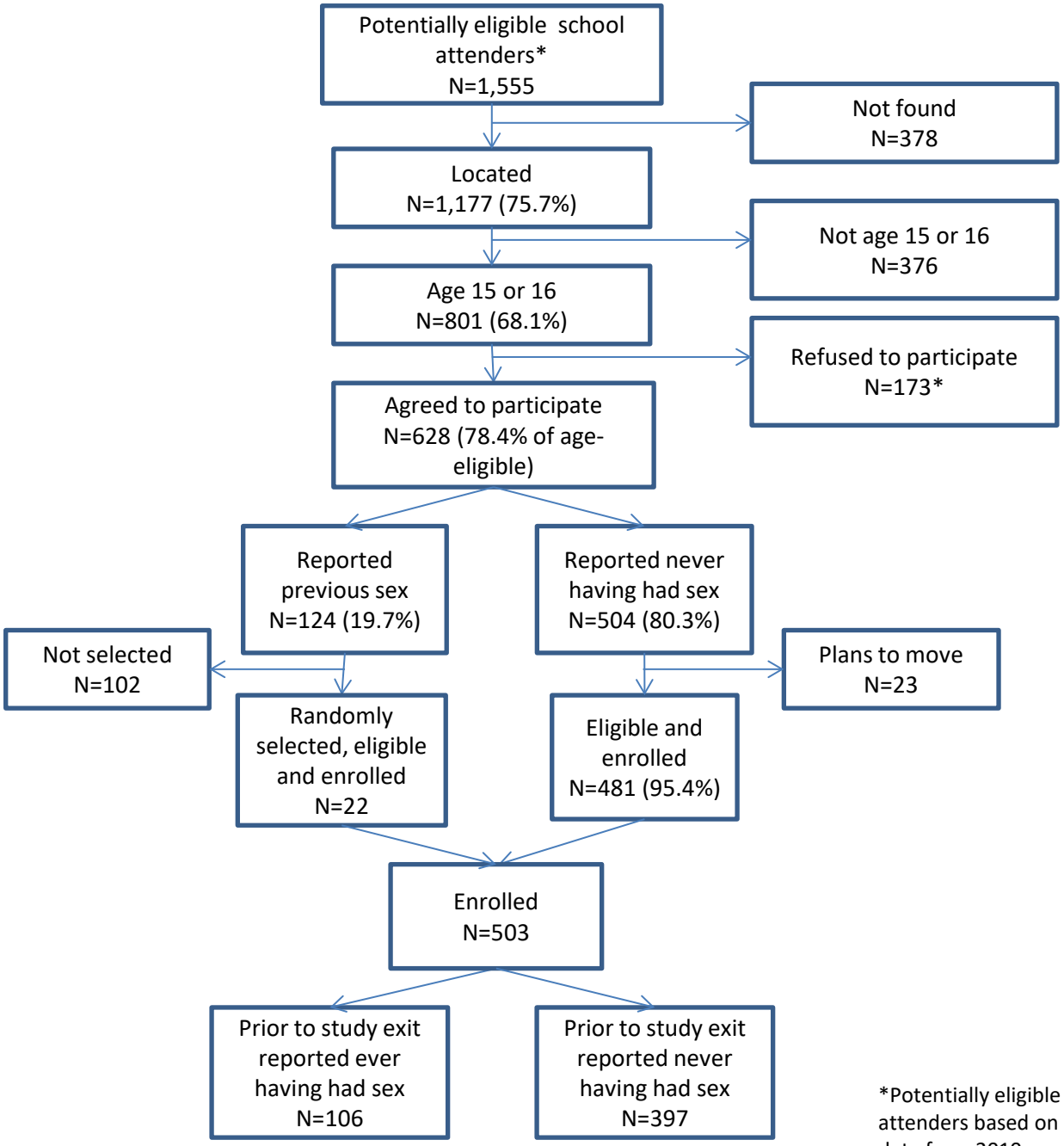

*Potentially eligible school attenders based on mapping data from 2010

Supplementary information

Statistical methods

A detailed analysis plan was produced before data analysis. For genotype-specific incidence, the date of infection was defined as the midpoint between the last negative and first positive sample for that genotype. Since the incidence rate was very high and most infections were of short duration, girls with periods longer than 180 days with missing HPV results were censored at the date of the last available HPV result before the interval with missing data.

For the analyses of overall incidence of all new HPV infections, all new HR HPV, and all new LR HPV, 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 treated as gaps in the observation time and removed from the analysis; however, observation time after the gap contributed to the analysis.

For the analysis of factors associated with the incidence of new HPV infections among all sexually active girls, we used 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. 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 to this core model sequentially and retained if associated at $p<0.10$. Time-varying behavioural factors were then added sequentially, and retained if they remained associated at $p<0.10$. This

strategy allowed us to assess the effects of variables at each level of the framework, adjusted for more distal variables.

Clearance of a genotype-specific HPV infection was defined as two consecutive negative samples, or one negative and one missing sample, for the genotype. In situations where a girl tested negative for a genotype between two positive samples for the same genotype, the intervening negative was considered to be a false negative. 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 date of their last sample.

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

APPENDIX 5. Paper: Durability of immunogenicity at 5 years after a single dose of HPV vaccine compared with 2 doses in Tanzanian girls aged 9-14 years: results of the long-term extension of the DoRIS randomised trial

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Surname/Family Name	Baisley		
Thesis Title	HPV epidemiology and HPV vaccination in East Africa		
Primary Supervisor	Deborah Watson-Jones		

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Please list the paper's authors in the intended authorship order:	Watson-Jones D, Changalucha J, Maxwell C, Whitworth H, Mutani P, Kemp TJ, Kamala B, Indangasi J, Constantine G, Hashim R, Mwanzalima D, Wiggins R, Mmbando D, Connor N, Pavon MA, Lowe B, Kapiga S, Mayaud P, de Sanjosé S, Dillner J, Hayes R, Lacey CJ, Pinto L, Baisley K

Stage of publication	**Undergoing revision**

SECTION D – Multi-authored work

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)	This paper describes the M60 results from the DoRIS trial. I am the joint Principal Investigator (PI), along with Deborah Watson-Jones, on the DoRIS trial. In collaboration Deborah Watson-Jones, I co-developed the initial ideas, designed the study, secured the funding and developed the study protocol and questionnaires. I am also the DoRIS trial statistician, and had overall responsibility for the trial data management and statistical analyses. For this paper, I analysed the data, interpreted the results, contributed to the drafting of the manuscript, along with first author, Deborah Watson-Jones, and responded to the reviewers during the peer-review process. I am the senior and corresponding author.

SECTION E

Student Signature	[redacted]
Date	15 October 2024

Supervisor Signature	[redacted]
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Durability of immunogenicity at 5 years after a single dose of HPV vaccine compared with 2 doses in Tanzanian girls aged 9-14 years: results of the long-term extension of the DoRIS randomised trial

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Abstract

Background

The World Health Organization has recommended that one dose of human papillomavirus (HPV) vaccine may be given to individuals aged 9-20 years to prevent HPV infection. Estimating durability of immune responses after a single dose in the target age for vaccination is important. We report immunogenicity results up to five years post-dose in Tanzanian girls.

Methods

Tanzanian schoolgirls (N=930) aged 9-14 years old were enrolled into an open-label, randomised controlled trial (the DoRIS trial) of 1, 2 or 3 doses of either the 2-valent vaccine (Cervarix®) or 9-valent vaccine (Gardasil-9®). HPV16/18-specific seropositivity, antibody geometric mean concentrations (GMC) and antibody avidity were measured annually through Month (M)36. Participants in the 1 and 2 dose arms were followed annually in a long-term extension of the DoRIS trial to M108; the primary outcome was HPV16/18-specific seropositivity comparing 1 dose with 2 doses.

Results

Single-dose seropositivity for HPV16 IgG antibodies at M60 with either vaccine was >99% and non-inferior to 2 doses. 98% of girls in the 1 dose 2-valent vaccine arm, and 93% of those in the 1 dose 9-valent arm, were seropositive for HPV18 at M60; however, the non-inferiority criteria for HPV18 seropositivity comparing 1 dose with 2 doses were not met. Although HPV16 and HPV18 antibody GMCs after 1 dose were lower than those observed after 2 doses, antibody GMCs in the 1 dose arms remained stable from M12 to M60. There was no evidence of a difference between the 1 dose and 2 dose arms in HPV16 or HPV18 antibody avidity at M36, for either vaccine.

Conclusions

A single dose of HPV vaccine in girls aged 9-14 years continues to provide stable immune responses five years after vaccination, although ongoing surveillance for potential waning immunity after a single dose is needed. Participants are being followed to 9 years post-vaccination.

Research in Context

Evidence before this study

The single dose HPV vaccination schedule has been shown to provide protection against persistent HPV16/18 infection for up to 11 years, in the context of observational studies of women who did not complete their multidose vaccination schedules in two randomised trials (Costa Rica Vaccine Trial (CVT), and IARC/India HPV vaccine trials). The first randomised controlled trial of single-dose HPV vaccine efficacy, conducted in females aged 15–20 years in Kenya (KEN SHE), showed an efficacy against incident persistent HPV16/18 infection of >97% at 36 months. The DoRIS trial in Tanzania, the first randomised trial of the single-dose schedule in girls in the target age range for HPV vaccination (9-14 years), showed that >98% of girls who received one dose were seropositive for HPV16/18 IgG antibodies at 24 months, and had antibody concentrations that were non-inferior to those in one dose recipients in the KEN SHE trial. WHO approved the off-label use of a single dose schedule in females and males aged 9-20 years, on the basis of these studies. However, data on durability of immune responses in young adolescents (aged <15 years) are lacking. To search for studies of long-term follow-up after a single dose of HPV vaccine in young girls, we searched PubMed using the terms "human papillomavirus" AND "vaccine" AND ("immunogenicity" OR "efficacy" OR "effectiveness") AND "single dose" AND "long-term". We limited the search to articles published since 10 August 2020 (the date of the last review of the evidence for single dose HPV vaccination, published by the Single-Dose HPV Vaccine Evaluation Consortium). This search identified one publication showing sustained antibody concentrations up to 10 years among girls who received a single dose of HPV vaccine at age 10-14 years in the India/IARC trial. A study in Fiji of women who were vaccinated through the national HPV vaccination programme at age 9-12 years showed 81% vaccine effectiveness of one dose against prevalent HPV16/18 infection over 8 years. No other studies of long-term follow-up of the single dose regimen in individuals who were vaccinated at age <15 years were identified.

Added value of this study

Here we present the immunogenicity results from the DoRIS trial after 5 years of follow-up. We show that HPV16 seropositivity 5 years after a single dose of HPV vaccine was >99%, and comparable to that in the two-dose arms. HPV18 seropositivity at 5 years was lower in

the single dose arms than the two dose arms, but was still high (>93%). HPV16/18 antibody concentrations after a single dose reached a plateau at 12 months and remained stable up to 5 years. The antibody trajectories over time after a single dose are similar to those observed in studies of a single dose in older females from different geographical locations in whom efficacy has been demonstrated.

Implications of all the available evidence

To our knowledge, this is the first randomised controlled trial of the single dose regimen to show that a single dose of HPV vaccine in girls aged 9-14 years produces durable antibody responses that remain stable up to 5 years. This is also the first study of long-term immunogenicity of a single dose of 9-valent vaccine. These data, combined with single dose efficacy data from the KEN SHE trial, and the CVT and IARC/India studies, continue to support the recent WHO recommendation for a single dose HPV vaccine regimen.

Introduction

Effective prophylactic vaccines to prevent infection with human papillomavirus (HPV) infection, the primary cause of cervical cancer, have been available for over 15 years. The World Health Organization (WHO) targets for cervical cancer elimination include 90% of girls being fully vaccinated by 15 years by 2030.[1] However, in 2022 only 21% of girls in the target age group for HPV vaccine (9-14 years) were estimated to be fully vaccinated with the recommended multidose schedules.[2] Challenges to HPV vaccine introduction, uptake and delivery include the costs of vaccinating girls and the capacity to introduce and sustain a multidose vaccination programme.[3,4]

Potential advantages of a single dose HPV vaccine regimen include reduced costs, ease of delivery and potentially increased acceptability. Observational studies that initially provided evidence for the efficacy and immunogenicity of one dose came from the Costa Rica Vaccine Trial (CVT) which offered the 2-valent vaccine Cervarix®, and the IARC/India trial which offered the 4-valent vaccine, Gardasil®.[5,6] In these studies, some participants did not complete their full multidose schedule and were followed up as observational cohorts. Single dose recipients had high HPV16/18 seropositivity but lower geometric mean concentrations (GMC) of HPV16 and HPV18 IgG antibodies compared with two or three doses. However, all doses had similar efficacy against incident or persistent HPV16/18 infection.[7,8] This protection was sustained up to 9 years in the IARC/India study and 11 years in the CVT.[9,10]

Data are now available from two randomised trials of single dose HPV vaccination. The KEN SHE trial, the first randomised trial of single dose efficacy, enrolled 15-20 year-old sexually active Kenyan females who were randomly allocated to either a single dose of 2-valent vaccine (Cervarix®), 9-valent vaccine (Gardasil-9®) or control vaccine (meningococcal vaccine).[11] The trial reported 97.8% and 98.8% efficacy against persistent HPV16/18 infection for the 2-valent and 9-valent vaccines, respectively, at 36 months post-vaccination.[12] The DoRIS trial, the first randomised trial of the single dose regimen in the target age group for HPV vaccination, compared immune responses in 9-14 year-old Tanzanian girls after 1, 2 or 3 doses of the same two HPV vaccines as offered in the KEN SHE trial.[13] At 24 months, over 99% of girls in the 1 dose arms were seropositive for anti-HPV16 antibodies and non-inferiority of HPV16 seropositivity was demonstrated for 1 dose compared with 2 or 3 doses for both vaccines.[14] Over 98% of girls in the 1 dose arms of

both vaccines were anti-HPV18 antibody positive at month (M)24, although the pre-defined non-inferiority criteria were not met for HPV18 seropositivity. Single dose antibody responses peaked at M1 and then fell slightly before plateauing between M12 and M24. Similar observations have been recorded in the CVT and India/IARC studies where single dose antibody concentrations plateaued and remained stable up to 11 years.[10,15] Immunobridging comparisons of antibody GMCs in the 1 dose arms in the DoRIS trial showed that HPV16/18 GMCs and seropositivity for both vaccines were non-inferior to those in the 1 dose groups in the KEN SHE, CVT and India/IARC studies, where single dose efficacy has been demonstrated.[16,17]

The KEN SHE and DoRIS trials contributed to the evidence that led to the recent recommendation by WHO for a 1 dose schedule in individuals aged 9-20 years.[18] However, a limitation of the results was the relatively short follow-up period in young girls. Data on the long-term durability of immune responses when a single dose is given to the girls in the target age range for vaccination are needed. These will provide important evidence of likely ongoing protection with a single dose regimen over time that could inform policy makers still considering a single dose programme. Here we present the 5-year immunogenicity data from the DoRIS trial long-term follow-up, comparing antibody responses after 1 or 2 doses of the 2-valent or 9-valent vaccines in Tanzanian girls. We also report on antibody avidity, a measure of how strongly antibody binds to its target antigen**,** at 3 years.

Methods

Study design

DoRIS [Dose Reduction Immunobridging and Safety Study of two HPV vaccines in Tanzanian Girls; NCT02834637] is an open-label, randomised, non-inferiority immunobridging trial comparing the immune responses and safety of 1, 2 and 3 doses of the 2-valent virus-like particle (VLP) HPV vaccine (Cervarix®, GSK Biologicals, Wavre [manufacturing site] and Rixensart [marketing authorisation holder]) and the 9-valent VLP HPV vaccine (Gardasil-9®, Merck Sharp & Dohme, Haarlem). All participants were followed until M36. Participants in the 1 and 2 dose arms were invited to join a long-term follow-up extension of the DoRIS trial, where they will be followed to 9 years (M108). The 3 dose arms were not invited for long-term follow-up because most countries have

discontinued provision of 3 dose regimens in this age group and there are extensive data on 3 dose schedules from earlier clinical trials. The trial and its extension were approved by the ethics committees of the Tanzanian Medical Research Coordinating Committee and the London School of Hygiene & Tropical Medicine, with regulatory approval from the Tanzania Medicines and Medical Devices Authority.

Participants

As previously described, 930 girls were enrolled into the main DoRIS trial from primary and secondary schools in Mwanza, Tanzania between March 2017–January 2018.[13,14] Girls were eligible if they were aged 9-14 years, healthy, HIV-negative, planning to reside in Mwanza for 36 months and willing to give informed assent. Girls who had received a prophylactic HPV vaccine, who had a history of genital warts, cervical lesions or past treatment following positive cervical cancer screening, or who were pregnant, immunocompromised, or unwell were excluded. Written or fingerprinted informed parental or guardian consent and written/fingerprinted assent from potential participants were obtained before screening and vaccination. At the end of the main trial, girls in the 1 and 2 dose arms and their parents or guardians were given information about the extension. Girls who were under 18 years were asked for written or fingerprinted assent, with written/fingerprinted consent from their parent or guardian. Participants who were aged 18 years or older were asked for written/fingerprinted consent.

Randomisation

Participants were randomly allocated (1:1:1:1:1:1) to one of 6 trial arms: 3 doses given over 6 months, 2 doses given 6 months apart, or a single dose, of either the 2-valent vaccine or the 9-valent vaccine. The randomisation list was computer-generated by an independent statistician, using random permuted block sizes of 12, 18 and 24. Allocation concealment from the study team and participants was accomplished using sequentially numbered sealed opaque envelopes. Once allocated, participants and clinic staff were made aware of the participant's trial arm.

Procedures

Full details of the trial procedures have been described previously.[13] In brief, eligibility screening was done within 30 days before randomisation. At screening, girls (or parent if the

girl was aged <12 years) had to pass a test of understanding. Three attempts were allowed before considering a girl to be a screen failure. At the day (D)0 enrolment visit, participants were randomly allocated to trial arm, and blood samples were collected for baseline immunogenicity. Two nurse-assisted, self-administered vaginal swabs were collected for baseline HPV DNA testing and genotyping with the Anyplex II HPV 28 detection assay (Seegene, Seoul), at the Catalan Institute of Oncology, Barcelona.

Participants were vaccinated according to their study arm, and attended the clinic 1 month after each vaccination visit to record any adverse events (AEs). Whole blood samples of approximately 15-20 mL were collected for immunological assays at D0, M1, M7, M12, M24, M36 and M60, and will be collected at M84 and M108. During the trial extension, to help ensure a high rate of retention, participants are contacted or sent an SMS reminder about the trial every 3 months and visited at home at least annually. Participants in the extension will be offered HIV testing at M84 and M108.

HPV16 and HPV18 serum IgG concentrations were measured using an L1 VLP ELISA at the HPV Serology Laboratory of the Frederick National Laboratory for Cancer Research in Maryland, USA.[19] Antibody seropositivity was defined as a concentration equal to or greater than the assay threshold: 1.309 IU/mL for HPV16 and 1.109 IU/mL for HPV18. The HPV16- and HPV18-specific antibody avidity index (AI) in the ELISA was determined at M12, M24 and M36 as previously described, and will be measured again at M84 and M108.[14] For the ELISA testing, the same lot of HPV16 and HPV18 VLPs, positive control, negative control, and internal reference standard were used for all DoRIS samples from M0 to M60, although samples were tested in different calendar years. In addition, the same positive control acceptability range was used. When testing the M60 samples, for both HPV16 and HPV18, the laboratory retested a subset of 19 seronegative and 24 seropositive samples from earlier timepoints, to evaluate drift of the assay results over time. All seronegative samples were found to be seronegative on re-testing. The re-test results from the seropositive samples were not significantly different from the original results, for either HPV16 ($p=0.34$, by Wilcoxon signed rank test for matched pairs) or HPV18 ($p=0.77$). A description of the immunological assays at each timepoint is provided in Supplementary Table 1.

Outcomes

The primary outcomes of the main DoRIS trial related to HPV16 and HPV18 antibody responses at M24 and have been reported previously.[14,16] The primary outcome of the extended follow-up was to demonstrate non-inferiority of immune responses to 1 dose of HPV vaccine compared with 2 doses of the same vaccine by evaluating HPV16/18-specific seropositivity at M60. Secondary outcomes being reported here include evaluation of the stability of immune responses, comparing HPV16/18-specific antibody GMCs at M60 with those at earlier timepoints within the same arm, and evaluation of HPV16/18 antibody avidity at M36, comparing 1 dose with 2 doses of the same vaccine.

Statistical analysis

The sample size for the main DoRIS trial was based on the co-primary objectives at M24 of demonstrating non-inferiority of HPV16/18 seropositivity comparing 1 dose with 2 or 3 doses, and non-inferiority of GMCs in the immunobridging analyses.[13] The sample size for the trial extension was not prespecified; all participants in the 1 and 2 dose arms could enrol. Retention at M36 was more than 95%; therefore, we expected to enrol around 150 per arm in the extension. Allowing a 10% loss to follow-up over 24 months, we would have around 135 girls per arm at M60. If the true proportion seropositive is the same in each arm, with 135 girls per arm, the study would have >90% power to demonstrate that the lower limit of the 95% confidence interval (CI) for the difference [1 dose minus 2 dose] is above -5%, indicating that seropositivity with the one-dose schedule was not decreased by more than 5.0%.

For all immunogenicity outcomes, the primary analysis was conducted in the per-protocol population, defined as girls who received the allocated doses of vaccine within the protocol-defined window and who were HPV antibody and DNA negative at enrolment for the specific genotype under analysis. Participants who missed visits or withdrew from the trial could still be included in the per-protocol analysis, as long as they met these criteria. As a sensitivity analysis, the analyses were repeated in all participants who received at least one dose of HPV vaccine, irrespective of their baseline antibody or HPV DNA status (total vaccinated cohort).

We tabulated the number and proportion of girls who were seropositive for HPV16/18- specific antibodies at M60. For each vaccine type and HPV genotype, we calculated the difference (1 dose minus 2 doses) in the proportion seropositive and the 95% CI for the difference using the

exact method of Chan and Zhang.[20] Non-inferiority of seropositivity was concluded if the lower bound of the two-sided 95% CI for the difference was above –5%.

For the evaluation of antibody GMC and avidity, we first log_{10}-transformed HPV genotype-specific antibody concentrations and antibody AIs; those below the assay cut-off were given a value of half the cut-off before log transformation. The arithmetic mean log_{10} antibody concentration, and log_{10} AI, and their 95% CIs were calculated for each arm, assuming a normal distribution. Normality was assessed graphically using normal plots.

We assessed stability of the immune responses by estimating the fold change in HPV16 and HPV18 GMCs between M60 and the earlier time points (M36, M24 and M12). For each HPV genotype and vaccine type, we fitted a linear mixed effects model with log_{10} antibody concentration as the response variable, dose group, time point, and a dose group-time interaction term as fixed effects, and participant as a random effect to account for correlation of repeated measurements within participants. The change over time in HPV16 or HPV18 log_{10} concentrations (e.g. M60 minus M36) was estimated from this model, and the GMC ratio (M60/M36) and its 95% CI were obtained by back-transformation.

We compared HPV16/18 antibody AI at M36 between the 1 and 2 dose groups by calculating the difference in HPV genotype-specific log_{10} AI (1 dose minus 2 doses) and its 95% CI; the geometric mean AI ratio and its 95% CI were obtained by back-transformation.

Role of the funding source

The funders of this study did not have any role in the study design, data collection and analysis, data interpretation or writing of this report.

Results

Of the 930 girls originally enrolled into the main DoRIS trial, 620 were randomly allocated to the 1 and 2 dose arms and were eligible to enrol in the long-term follow-up extension to the trial. Enrolment into the extension was between March 2021 and August 2022. Of the 620 eligible participants, 598 (96.5%) were enrolled (Figure 1); the remaining had withdrawn or were lost to follow-up by M36 (N=13) or refused consent (N=9). Of the 598 enrolled, 595 (99.5%) received their scheduled doses within the protocol-defined window. One girl in the two-dose 2-valent arm received her 6-month dose one day early, one girl in the two-dose 9-

valent arm received the 2-valent vaccine in error instead of a second dose of 9-valent vaccine, and one girl in the one-dose 9-valent arm received a dose of the 4-valent HPV vaccine through the Tanzanian national HPV vaccination programme between M24 and M36. These 3 girls were excluded from the per-protocol analyses but included in the total vaccinated cohort analyses.

Among the 598 girls enrolled in the extension, baseline characteristics at enrolment to the main trial were similar between the trial arms (Table 1). One girl was positive for HPV16 and HPV18 DNA at baseline, and 32 girls (5.4%) were HPV16 seropositive and 55 (9.2%) were HPV18 seropositive at baseline. There was some evidence of a difference between arms in the proportion with any HPV DNA (p=0.02), or any high-risk HPV DNA (p=0.06), at baseline, with prevalence in the 1 dose arm of the 9-valent vaccine being slightly higher than in the other arms (Table 1).

All 598 participants attended the M60 visit. In the per-protocol analysis, we included 289/303 (95.4%) and 273/295 (92.5%) participants in the 1 and 2 dose arms, respectively, in the analysis of HPV16 antibody responses, and 273/303 (90.1%) and 267/295 (90.5%) in the analysis of HPV18. In the 1 dose arms, all participants except one in the 2-valent vaccine arm (99.7%) were seropositive for HPV16 antibodies at M60, and 261 (95.6%) were seropositive for HPV18 (Table 2). In the 2 dose arms, all participants were seropositive for HPV16, and all except two in the 9-valent vaccine arm (99.3%) were seropositive for HPV18. Non-inferiority of HPV16 antibody seropositivity at M60 was met for 1 dose compared with 2 doses of both vaccines. Non-inferiority of HPV18 seropositivity was not met for either vaccine.

Similar results were seen at M36, with 287/288 (99.7%) participants in the 1 dose arms being seropositive for HPV16, and non-inferiority of seropositivity comparing 1 dose with 2 doses being met for both vaccines (Supplementary Table 2). A slightly higher proportion of participants in the 1-dose arms were seropositive for HPV18 at M36 than M60 (268/272, 98.5% vs 95.4%, respectively). Non-inferiority of HPV18 seropositivity comparing 1 dose with 2 doses at M36 was met for the 2-valent vaccine but not the 9-valent vaccine.

In the per-protocol analysis of both vaccines and HPV genotypes, antibody GMCs in the 1 dose arms remained relatively constant from M12 through M60, with little evidence of a difference between M60 and earlier time points (Table 3; Figure 2). In contrast, HPV16 and

HPV18 antibody GMCs in the 2 dose arms of both vaccines peaked at M7 and then slowly declined thereafter. HPV16/18 antibody GMCs at M60 in the 2 dose arms, although significantly higher than in the 1 dose arms, were around 20% lower than at M36, and 65-70% lower than at M12.

In the per-protocol analysis, there was no evidence of a difference between the 1 and 2 dose arms in HPV16 and HPV18 geometric mean antibody AI at M36 for either vaccine (Table 4; Supplementary Figure 1). Geometric mean AI ratios were around 1.0, with the lower limit of the 95%CI above 0.90 for all comparisons.

Immunogenicity results among the total vaccinated cohort were similar to those in the per-protocol analysis, for both vaccines and both HPV genotypes (Supplementary Tables 3 and 4; Supplementary Figure 2).

There were 40 serious adverse events (SAEs) experienced by 31 of 620 girls (5.0%) originally enrolled in the 1 and 2 dose arms (including 22 who were not enrolled in the extension) up to M60 (Supplementary Tables 5 and 6). Hospitalisation for malaria was the most common SAE (34 events, 25 girls). A 10-year-old girl in the 2-dose 9-valent vaccine arm who had received her first dose four months previously unfortunately died from severe malaria. There was no evidence of a difference in the number of SAEs between arms and no SAE was considered to be related to the vaccine

Discussion

This is the first randomised trial to assess the long-term durability of antibody responses after a single dose in girls in the primary target age for HPV vaccination. This is also the first study of long-term immunogenicity of a single dose of 9-valent vaccine in any age group. We showed that HPV16/18 immune responses plateau at around 12 months and remain relatively constant to 5 years after a single dose of either the 2-valent or 9-valent HPV vaccine. HPV16 seropositivity rates at 5 years after a single dose were non-inferior to those after 2 doses for both vaccines. Although we did not demonstrate non-inferiority of HPV18 seropositivity, 98% of participants in the 1 dose 2-valent vaccine arm, and 93% in the 1 dose 9-valent vaccine arm, were HPV18 seropositive at 5 years. HPV 16/18 antibody avidity at 3 years post-vaccination did not differ between the 1 dose and 2 dose arms, for either vaccine.

Our results are consistent with findings in older females in the CVT and IARC/India studies, where antibody concentrations after a single dose of the 2-valent vaccine or the 4-valent vaccine followed similar trajectories over time and have been stable for a decade.[10,15] In the CVT, 97% of single dose recipients were seropositive for HPV16 and 93% were seropositive for HPV18 at 11 years. In the India trial, HPV16 and HPV18 seropositivity among single dose recipients at 10 years was 96% and 97%, respectively. Both studies have shown long-term (up to 11 years) single dose efficacy against persistent HPV16/18 infection.

Our findings that HPV16/18 antibody avidity in the 1 dose arms was comparable to that after 2 doses are similar to a study in the Netherlands of girls who were vaccinated with the 2-valent vaccine at the age of 12 years through the national programme.[21] In that study, HPV16 antibody avidity at 5 years did not differ between those who received only a single dose and those who received 2 doses, and HPV18 antibody avidity was higher in the single dose recipients. In the CVT, where women were vaccinated at age 18-25 years, HPV16 antibody avidity was 5-10% lower among women who received 1 dose than 3 doses, but was relatively constant over time to 11 years.[22] Avidity is believed to reflect the quality of antibodies post vaccination following affinity maturation and antigen-driven B-cell selection. Our results, and those of the Netherlands and CVT studies, suggest that a single dose of HPV VLP vaccine, irrespective of its adjuvant, can generate robust and stable immune responses through B-cell activation. Several biological mechanisms by which a single dose can produce stable antibody responses have been proposed, linked to the repetitive structure and spacing of the VLP epitopes which trigger a particularly effective cascade of immune responses.[23]

As we previously observed at M24, HPV16/18 antibody GMCs at M60 were higher with the 2-valent vaccine than the 9-valent vaccine. These findings are similar to those in the KEN SHE trial which compared the same two HPV vaccines as the DoRIS trial, and to other studies that compared the 2-valent and 4-valent vaccines.[17,24,25] Despite the differences in antibody GMCs, the vaccines have similar extremely high efficacy against persistent HPV16/18 infection. In addition, as we also observed at M24, HPV18 antibody GMCs and seropositivity at M60 were lower than those for HPV16, for both vaccines. These results were not unexpected and have been reported in other studies. A trial of 3 doses of the 9-valent vs 4-valent vaccines among women aged 16-26 years found that 18% in the 9-valent arm and 23% in the 4-valent arm no longer had detectable HPV18 antibodies at 3.5 years.[26] Similarly, an extended follow-up of women who received 3 doses of the 4-valent vaccine

found that 35% were no longer HPV18 seropositive at 5 years, despite sustained efficacy against HPV18 infection.[27] The mechanism for protection among women who become HPV18 seronegative several years after vaccination is still unclear. It is uncertain whether a minimum serum antibody concentration must be maintained for protection, or whether exposure to the virus can activate memory B cells to produce neutralising antibody locally in the genital tract. The results from these efficacy studies suggest that some of the vaccine's protection is likely to be mediated through immune memory.

Combined with our immunobridging results, where immune responses at 2 years after a single dose were non-inferior to those in the KEN SHE, CVT, and IARC/India studies where efficacy against persistent HPV infection had been demonstrated, our long-term immunogenicity results at 5 years suggest that a single dose of the vaccine given to girls aged 9-14 years is likely to protect against HPV 16/18 infection once girls pass sexual debut. Ongoing data from the CVT and India/IARC studies also confirm that, in those observational cohorts, efficacy in the single dose recipients is sustained for up to a decade.

Study strengths include excellent retention at 5 years post-vaccination and enrolment of girls from a malaria-endemic setting in a country which has a very high prevalence and incidence of HPV infection and high rates of cervical cancer.[28,29,30] Our results are therefore generalisable to other parts of sub-Saharan Africa and other high-burden countries. The immunological assays for this study were conducted in the same laboratory that evaluated earlier immune responses from the DoRIS trial, using the same lot of ELISA VLPs, standards and critical reagents, with retesting of samples from earlier timepoints, to minimise potential assay variability. The laboratory is also performing HPV immunological assays for other studies of the single dose schedule, allowing comparison of results across these studies.[31]. The inclusion of HPV16/18 antibody avidity is important for confirming the robustness of the immune response to a single dose.

Our study has several limitations. Our sample size does not allow us to directly evaluate efficacy in the DoRIS trial, although we have bridged our immune response to those in studies with efficacy results. We did not demonstrate non-inferiority of HPV18 seropositivity when comparing 1 dose with 2 doses, although over 93% of girls in the 1 dose arms remained HPV18 seropositive and the clinical significance of the loss of seropositivity is unclear. Our data on durability are based on 5 years of follow-up; however, based on our other studies in Mwanza, the median age of sexual debut among females in this region is around 17 years.[30,

32] We are continuing follow-up of participants to 9 years post-vaccination, which will provide further information on the durability of single-dose immune responses in this age group, at a time point where most participants are likely to have passed sexual debut.

In conclusion, a single dose of the 2-valent or 9-valent vaccine in healthy African girls living in a malaria-endemic region and who are in the primary target age for vaccination continues to result in sustained, stable antibody responses 5 years post-vaccination. Antibody kinetics are similar to those observed in other studies in older females from different geographies in whom efficacy has been demonstrated. These data, combined with recent efficacy data on single dose regimens from the KEN SHE randomised controlled trial and long term follow-up data from India and Costa Rica continue to support the recent WHO recommendation for a single dose HPV vaccine regimen.[18] The potential savings and vaccine supplies that could become available if single dose vaccination programmes are introduced may permit catch-up vaccination for those who failed to be vaccinated in previous years and may allow vaccination of multi-age cohorts, at least for a limited time. Ongoing surveillance for potential waning immunity following a single dose is important and follow-up of the existing single dose study cohorts, including DoRIS participants, is underway. Encouragingly, as of March 2024, 38 countries have introduced HPV vaccination using a single dose regimen or made a recommendation to switch their current HPV vaccination programmes to a single dose HPV vaccination strategy.[33] Our data may reassure countries considering a potential HPV vaccination programme with a single dose strategy.

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Contributors

DWJ, KB and JC conceived the study. DWJ, KB, RJH, CJL, JC, HW, PM, LP, SdS and JD developed the protocol and were involved in the interpretation of the results. DWJ and KB were responsible for funding acquisition. DWJ, KB and JC were joint principal investigators and CM, HW, LP, JI, SK, PM, SdS, JD, RJH and CJL were coinvestigators. DWJ, JC, CM, HW, SK, PM, BK, JI, BL, DM, GC, BK and NC were responsible for the on-site conduct of the study and participated in monitoring and supervision of data collection. KB was responsible for the study statistical analysis and RH for data management of the study. JI, LP, TJK, RW, CJL, SdS, JD, MAP and BL were responsible for laboratory aspects of the study. DM was the trial pharmacist and was responsible for study vaccine dispensing. PM, BK and GC were involved in the medical care of the participants. DWJ drafted the manuscript and all authors commented and contributed to the final version. The corresponding author had full access to the data in the study and had responsibility for the final decision to submit the manuscript for publication.

Declaration of interests

DWJ reports grants from the Bill and Melinda Gates Foundation (BMGF) during the DoRIS trial, and a grant from GSK Biologicals in 2007 for a previous study unrelated to this submitted work. KB reports grants from BMGF during the conduct of the DoRIS trial, and grant support from Merck Pharmaceuticals outside the submitted work. Other authors declare no competing interests.

Data Sharing

De-identified participant data presented in this manuscript can be made available after publication following written request to the London School of Hygiene & Tropical Medicine and the Mwanza Intervention Trials Unit (MITU), Tanzania. Requests must be accompanied by an analysis plan which will be reviewed by the MITU Data Sharing Committee and lead investigators for each trial. Requesting researchers will be required to sign a Data Access Agreement if approval is given.

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Table 1. Baseline characteristics[1] of participants in DoRIS trial long-term follow-up

	1 dose Cervarix®	2 doses Cervarix®	1 dose Gardasil-9®	2 doses Gardasil-9®	Total
	N=151	N=146	N=152	N=149	N=598
Median (IQR) age (years)	10 (9-12)	10 (9-12)	10 (9-12)	11 (10-13)	10 (9-12)
Age group					
9-10 years	83 (55.0%)	74 (50.7%)	88 (57.9%)	66 (44.3%)	311 (52.0%)
11-12 years	39 (25.8%)	39 (26.7%)	39 (25.7%)	45 (30.2%)	162 (27.1%)
13-14 years	29 (19.2%)	33 (22.6%)	25 (16.4%)	38 (25.5%)	125 (20.9%)
Years lived in Mwanza					
Entire life	114 (75.5%)	116 (79.5%)	115 (75.7%)	119 (79.9%)	464 (77.6%)
>5 years	19 (12.6%)	17 (11.6%)	18 (11.8%)	17 (11.4%)	71 (11.9%)
≤5 years	18 (11.9%)	13 (8.9 %)	19 (12.5%)	13 (8.7 %)	63 (10.5%)
School type					
Primary	121 (80.1%)	117 (80.1%)	125 (82.2%)	117 (78.5%)	480 (80.3%)
Secondary	30 (19.9%)	29 (19.9%)	27 (17.8%)	32 (21.5%)	118 (19.7%)
Passed menarche					
Yes	19 (12.6%)	18 (12.3%)	17 (11.2%)	20 (13.4%)	74 (12.4%)
Ever cleansed vagina					
Yes	15 (9.9 %)	14 (9.6 %)	14 (9.2 %)	11 (7.4 %)	54 (9.0 %)
Ever had sex					
Yes	1 (0.7 %)	2 (1.4 %)	1 (0.7 %)	4 (2.7 %)	8 (1.3 %)
HPV16 DNA positive					
Yes	0 (0.0 %)	0 (0.0 %)	1 (0.7 %)	0 (0.0 %)	1 (0.2 %)
HPV18 DNA positive					
Yes	0 (0.0 %)	0 (0.0 %)	1 (0.7 %)	0 (0.0 %)	1 (0.2 %)
Any high-risk HPV genotype DNA					
Yes	0 (0.0 %)	2 (1.4 %)	6 (3.9 %)	2 (1.3 %)	10 (1.7 %)
Any HPV genotype DNA					
Yes	0 (0.0 %)	2 (1.4 %)	7 (4.6 %)	2 (1.3 %)	11 (1.8 %)
HPV16 seropositive					
Yes	6 (4.0 %)	9 (6.2 %)	7 (4.6 %)	10 (6.7 %)	32 (5.4 %)
HPV18 seropositive					
Yes	13 (8.6 %)	10 (6.8 %)	16 (10.5%)	16 (10.7%)	55 (9.2 %)

[1]Characteristics at enrolment to the main DoRIS trial (i.e. before vaccination), among girls in the 1 dose and 2 dose arms who consented to long-term follow-up

Table 2. Comparisons of antibody seropositivity at M60 after 1 or 2 doses of HPV vaccine in DoRIS trial (per-protocol cohort[1])

	1 dose		2 doses		Difference in seropositivity[2] (exact 95% CI)
	N	Seropositive[2] (%)	N	Seropositive[2] (%)	1 dose – 2 dose
Month 60					
Cervarix®					
HPV-16	145	144 (99.3%)	136	136 (100.0%)	-0.7% (-4.6, 3.1)
HPV-18	138	135 (97.8%)	135	135 (100.0%)	-2.2% (-7.1, 1.5)
Gardasil-9®					
HPV-16	144	144 (100.0%)	137	137 (100.0%)	0
HPV-18	135	126 (93.3%)	132	130 (98.5%)	-5.2% (-12.1, 0.5)

[1]DoRIS participants who were ELISA antibody negative and DNA negative at baseline (pre-vaccination) for the HPV genotype under analysis. [2]Seropositivity defined as antibody concentrations above the laboratory determined cut-off (HPV16 = 1.309 IU/mL; HPV18 = 1.109 IU/mL).

Table 3. Stability of geometric mean concentrations (GMC) from M12 to M60 in DoRIS trial (per protocol cohort[1])

	1 dose		2 doses	
	N[1]	**GMC[2] (95% CI) (IU/mL)**	**N[1]**	**GMC[2] (95% CI) (IU/mL)**
Cervarix®				
HPV 16				
Month 12	147	19.4 (16.6, 22.7)	140	267.6 (231.9, 308.8)
Month 24	148	22.9 (19.9, 26.4)	141	162.7 (141.1, 187.7)
Month 36	146	20.7 (17.9, 23.9)	141	121.5 (107.4, 137.4)
Month 60	145	20.5 (17.3, 24.3)	136	97.6 (85.8, 111.0)
GMC ratio[3] (M60 /M12) (95% CI)		*1.06 (0.96, 1.17)*		*0.36 (0.33, 0.40)*
GMC ratio[3] (M60 /M24) (95% CI)		*0.90 (0.82, 0.99)*		*0.59 (0.54, 0.66)*
GMC ratio[3] (M60 /M36) (95% CI)		*1.00 (0.90, 1.10)*		*0.80 (0.72, 0.88)*
HPV 18				
Month 12	140	8.6 (7.3, 10.0)	139	96.0 (83.1, 110.9)
Month 24	141	9.9 (8.5, 11.5)	140	50.0 (43.4, 57.8)
Month 36	139	9.3 (8.0, 10.7)	140	40.1 (34.9, 46.1)
Month 60	138	9.7 (8.2, 11.6)	135	35.1 (30.4, 40.4)
GMC ratio[3] (M60 /M12) (95% CI)		*1.14 (1.02, 1.26)*		*0.36 (0.33, 0.40)*
GMC ratio[3] (M60 /M24) (95% CI)		*0.98 (0.89, 1.09)*		*0.70 (0.63, 0.77)*
GMC ratio[3] (M60 /M36) (95% CI)		*1.05 (0.95, 1.17)*		*0.87 (0.78, 0.96)*
Gardasil-9®				
HPV 16				
Month 12	145	13.2 (11.5, 15.0)	142	252.8 (219.2, 291.5)
Month 24	145	13.7 (11.9, 15.8)	141	124.9 (107.2, 145.5)
Month 36	142	13.2 (11.6, 15.1)	140	82.7 (70.7, 96.8)
Month 60	144	13.1 (11.3, 15.3)	137	66.8 (55.9, 79.7)
GMC ratio[3] (M60 /M12) (95% CI)		*1.00 (0.92, 1.09)*		*0.27 (0.24, 0.29)*
GMC ratio[3] (M60 /M24) (95% CI)		*0.96 (0.88, 1.04)*		*0.54 (0.49, 0.59)*
GMC ratio[3] (M60 /M36) (95% CI)		*0.99 (0.91, 1.08)*		*0.82 (0.75, 0.89)*
HPV 18				
Month 12	136	5.2 (4.5, 6.1)	137	58.8 (50.2, 68.9)
Month 24	136	5.7 (4.9, 6.8)	136	29.3 (24.7, 34.7)
Month 36	133	5.8 (4.9, 6.7)	135	20.9 (17.6, 24.9)
Month 60	135	5.3 (4.3, 6.4)	132	16.6 (13.6, 20.3)

GMC ratio[3] (M60 /M12) (95% CI)	*1.01 (0.92, 1.11)*	*0.29 (0.26, 0.32)*
GMC ratio[3] (M60 /M24) (95% CI)	*0.92 (0.84, 1.01)*	*0.57 (0.52, 0.63)*
GMC ratio[3] (M60 /M36) (95% CI)	*0.91 (0.83, 1.00)*	*0.80 (0.73, 0.88)*

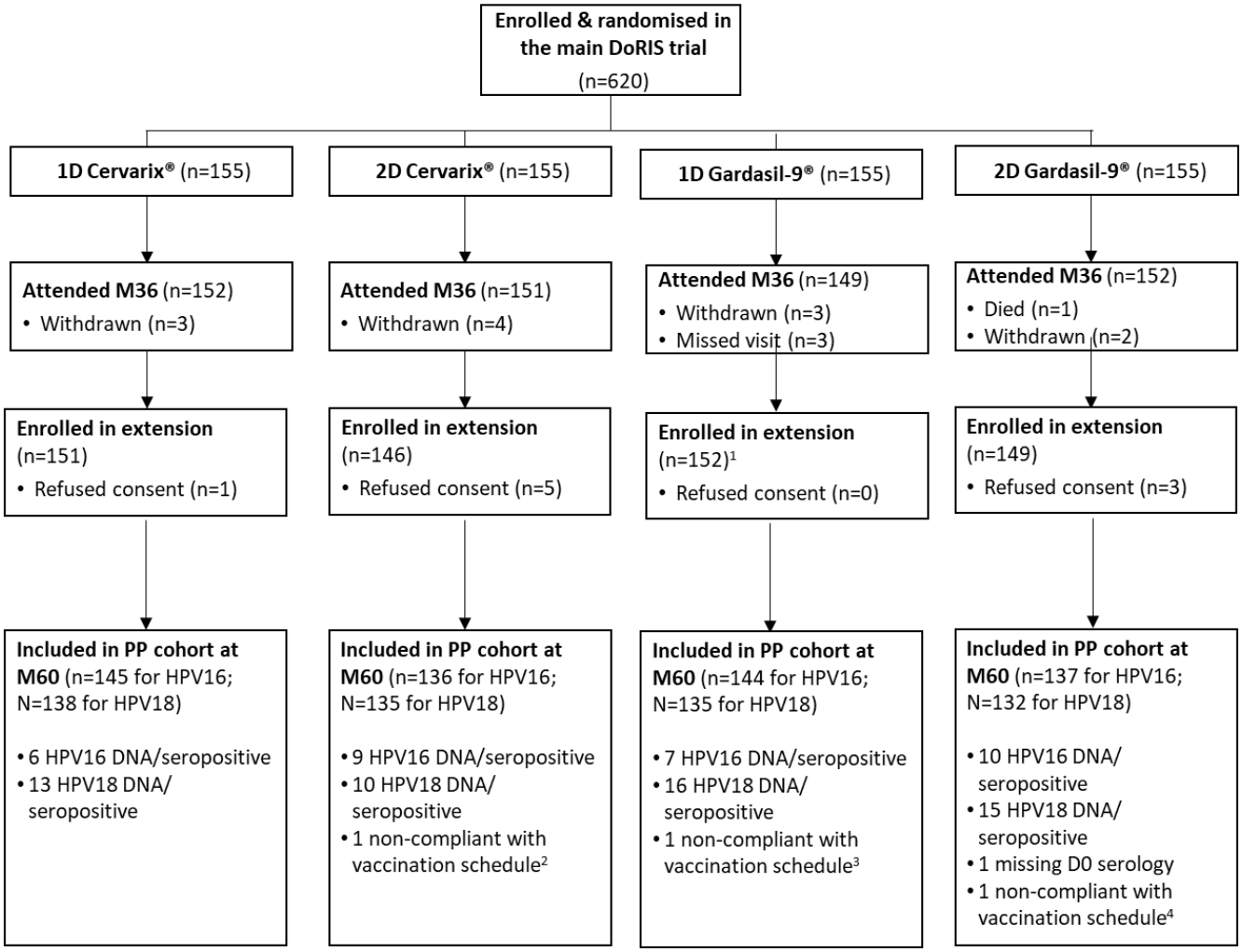
[1]DoRIS participants who were ELISA antibody negative and DNA negative at baseline (pre-vaccination) for the HPV genotype under analysis. [2]ELISA serum antibody geometric mean concentration (GMC). [3]Estimated with linear mixed effects model with log antibody concentration as the response and dose group, time point, and a dose group-time interaction term as fixed effects, and participant as a random effect to account for correlation of repeated measurements within participants.

Table 4. Comparisons of geometric mean (GM) antibody avidity index (AI) at M36 after 1 or 2 doses of HPV vaccine in DoRIS trial (per-protocol cohort[1])

	1 dose		2 doses		Geometric mean AI ratio (95% CI)
	N	GM avidity index[2]	N	GM avidity index[2]	1 dose / 2 dose
Month 36					
Cervarix®					
HPV-16	146	2.99 (2.91, 3.09)	141	3.06 (3.01, 3.12)	0.98 (0.95, 1.01)
HPV-18	139	1.80 (1.74, 1.87)	140	1.83 (1.78, 1.88)	0.98 (0.93, 1.02)
Gardasil-9®					
HPV-16	142	2.92 (2.84, 3.01)	140	2.98 (2.92, 3.04)	0.98 (0.95, 1.01)
HPV-18	133	2.04 (1.98, 2.11)	135	2.08 (2.03, 2.13)	0.98 (0.94, 1.02)

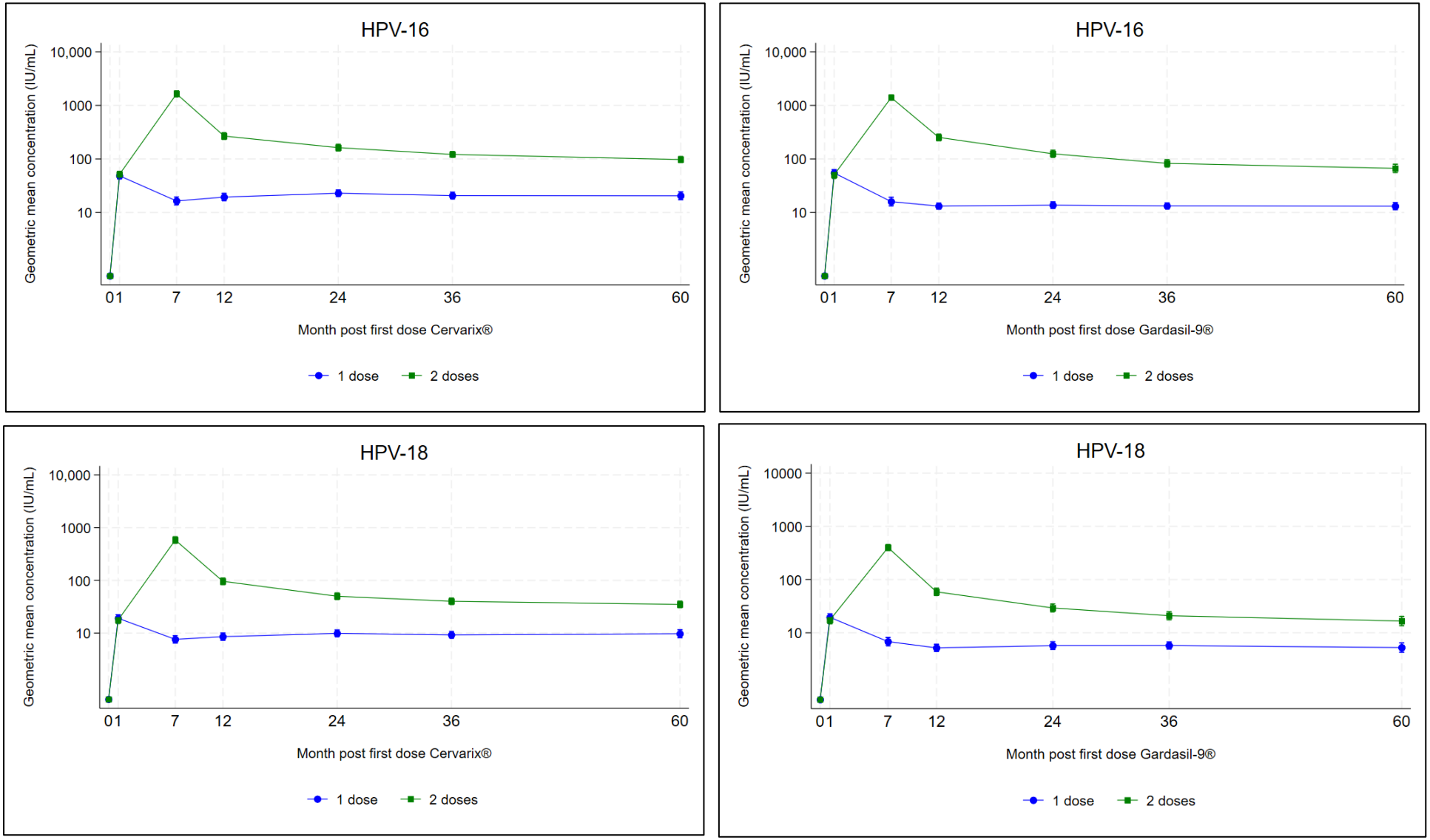
[1]DoRIS participants who were ELISA antibody negative and DNA negative at baseline (pre-vaccination) for the HPV genotype under analysis. [2]Geometric mean avidity index.

Figure 1. Flow chart for DoRIS trial long-term follow-up to Month 60



[1]The 3 participants who missed the M36 visit were contacted by the study team and invited to enrol in the extension; all consented to enrol and attended M60. [2]Received last dose out of window (1 day early). [3]Vaccinated through the Tanzanian national programme between M24 and M36. [4]Received 2-valent vaccine in error.

Figure 2. HPV16 (top) and HPV18 (bottom) specific antibody geometric mean concentrations (IU/mL) over time by number of doses of 2-valent (left) or 9-valent (right) vaccine and study visit (per-protocol cohort)



Supplementary Table 1. Laboratory assays used in DoRIS trial

Assay	Marker	Timepoint	Sample type	Laboratory
L1 VLP ELISA	HPV-16/18 IgG antibody concentrations	M0, 1, 7, 12, 24, 36, 60, 84 & 108	Serum	Frederick National Laboratory for Cancer Research, USA
ELISA-based avidity	HPV 16/18 IgG antibody avidity index	M0, 12, 24 & 36, 84 & 108	Serum	Frederick National Laboratory for Cancer Research, USA
HPV Multiplex immunoassay	HPV-6, 11, 16, 18, 31, 33, 45, 52 & 58 IgG antibody concentrations	M0, 12, 24, 36, 60, 84 & 108	Serum	Frederick National Laboratory for Cancer Research, USA
Pseudovirion (PsV) Luminex	HPV-6, 11, 16, 18, 31, 33, 45, 52 & 58 IgG antibody concentrations	M0, 1, 7, 12, 24, 36, 60, 84 & 108	Serum	Karolinska Institute, Sweden
Memory B cell ELISPOT	HPV 16/18-specific memory B cell response	M0, 1, 7, 12, 24 & 36	PBMC	Centre for Immunology and Infection, York, UK
qPCR[1]	Malaria	M0, 1, 2 & 6	Dried blood spot	LSHTM, UK
Roche linear array	HPV DNA	M0	Vaginal swab	Catalan Institute of Oncology, Barcelona, Spain
HIV rapid test[2]	HIV serostatus	M0	Serum	NIMR Mwanza, Tanzania

[1]Quantitative PCR. [2]Two rapid tests (serial testing), with second test done only if first test is reactive; rapid tests repeated if discordant. Participants with persistently discordant results tested by ELISA.

Supplementary Table 2. Comparisons of antibody seropositivity at M36 post HPV vaccination with 1 or 2 doses of HPV vaccine in DoRIS trial (per protocol cohort[1])

	1 dose		2 doses		Difference in seropositivity[2] (exact 95% CI)
	N	Seropositive[2] (%)	N	Seropositive[2] (%)	1 dose – 2 dose
Month 36					
Cervarix®					
HPV-16	146	145 (99.3%)	141	141 (100.0%)	-0.7% (-3.9, 2.0)
HPV-18	139	137 (98.6%)	140	139 (99.3%)	-0.7% (-4.6, 2.7)
Gardasil-9®					
HPV-16	142	142 (100.0%)	140	140 (100.0%)	0
HPV-18	133	131 (98.5%)	135	135 (100.0%)	-1.5% (-5.5, 1.3)

[1]DoRIS participants who were ELISA antibody negative and DNA negative at baseline (pre-vaccination) for the HPV genotype under analysis. [2]Seropositivity defined as antibody concentrations above the laboratory determined cut-off (HPV16 = 1.309 IU/mL; HPV18 = 1.109 IU/mL).

Supplementary Table 3. Comparisons of antibody seropositivity at M36 and M60 post HPV vaccination with 1 or 2 doses of HPV vaccine in DoRIS trial (total vaccinated cohort[1])

	1 dose			2 doses			Difference in seroconversion[3] (exact 95% CI)
	N	Seropositive[2] (%)	Seroconverted[3] (%)	N	Seropositive[2] (%)	Seroconverted[3] (%)	1 dose – 2 dose
Cervarix®							
Month 36							
HPV16	152	151 (99.3%)	145 (95.4%)	151	151 (100.0%)	142 (94.0%)	1.4% (-4.1, 7.0)
HPV18	152	150 (98.7%)	137 (90.1%)	151	150 (99.3%)	140 (92.7%)	-2.6% (-9.3- 4.0)
Month 60							
HPV16	151	150 (99.3%)	144 (95.4%)	146	146 (100.0%)	137 (93.8%)	-0.7% (-4.4- 2.7)
HPV18	151	148 (98.0%)	135 (89.4%)	146	146 (100.0%)	136 (93.2%)	-2.0% (-6.5, 1.5)
Gardasil-9®							
Month 36							
HPV16	149	149 (100.0%)	143 (96.0%)	152	152 (100.0%)	141 (92.8%)	3.2% (-2.4, 9.0)
HPV18	149	147 (98.7%)	132 (88.6%)	152	152 (100.0%)	135 (88.8%)	-0.4% (-8.0, 7.1)
Month 60							
HPV16	152	152 (100.0%)	145 (95.4%)	149	149 (100.0%)	138 (92.6%)	0
HPV18	152	143 (94.1%)	127 (83.6%)	149	147 (98.7%)	130 (87.2%)	-4.6% (-10.7, 0.4)

[1]DoRIS participants who received at least one dose of vaccine, irrespective of their HPV DNA or serostatus at baseline. [2]Antibody concentrations above the laboratory determined cut-off (HPV16 = 1.309 IU/mL; HPV18 = 1.109 IU/mL). [3]Seroconversion defined as antibody concentrations above the laboratory determined cut-off at the relevant visit, among girls who were seronegative at baseline

Supplementary Table 4. Stability of geometric mean concentrations (GMC) between M12 to M60 in DoRIS trial (total vaccinated cohort[1])

	1 dose		2 doses	
	N[1]	**GMC[2] (95% CI) (IU/mL)**	**N[1]**	**GMC[2] (95% CI) (IU/mL)**
Cervarix®				
HPV 16				
Month 12	153	19.5 (16.7, 22.7)	150	267.2 (232.9, 306.7)
Month 24	154	22.7 (19.8, 26.1)	151	162.8 (142.1, 186.5)
Month 36	152	20.6 (17.9, 23.7)	151	121.7 (108.2, 136.9)
Month 60	151	20.5 (17.4, 24.1)	146	96.0 (85.0, 108.5)
GMC ratio[3] (M60 /M12) (95% CI)		*1.06 (0.96, 1.16)*		*0.36 (0.32, 0.39)*
GMC ratio[3] (M60 /M24) (95% CI)		*0.91 (0.83, 1.00)*		*0.58 (0.53, 0.64)*
GMC ratio[3] (M60 /M36) (95% CI)		*1.00 (0.91, 1.10)*		*0.78 (0.71, 0.86)*
HPV 18				
Month 12	153	8.3 (7.1, 9.6)	150	92.7 (80.0, 107.3)
Month 24	154	9.6 (8.3, 11.1)	151	50.8 (44.2, 58.3)
Month 36	152	9.0 (7.8, 10.4)	151	40.4 (35.3, 46.2)
Month 60	151	9.5 (8.0, 11.2)	146	35.0 (30.5, 40.1)
GMC ratio[3] (M60 /M12) (95% CI)		*1.14 (1.03, 1.26)*		*0.38 (0.34, 0.42)*
GMC ratio[3] (M60 /M24) (95% CI)		*0.99 (0.89, 1.09)*		*0.69 (0.62, 0.76)*
GMC ratio[3] (M60 /M36) (95% CI)		*1.05 (0.95, 1.17)*		*0.86 (0.78, 0.96)*
Gardasil-9®				
HPV 16				
Month 12	152	13.5 (11.8, 15.5)	154	248.8 (217.3, 284.8)
Month 24	152	14.1 (12.2, 16.3)	153	122.8 (106.2, 142.0)
Month 36	149	14.0 (12.1, 16.2)	152	82.5 (71.0, 95.8)
Month 60	152	13.8 (11.8, 16.1)	149	66.0 (55.9, 78.0)
GMC ratio[3] (M60 /M12) (95% CI)		*1.02 (0.93, 1.11)*		*0.27 (0.25, 0.29)*
GMC ratio[3] (M60 /M24) (95% CI)		*0.98 (0.90, 1.07)*		*0.54 (0.50, 0.59)*
GMC ratio[3] (M60 /M36) (95% CI)		*0.99 (0.90, 1.08)*		*0.81 (0.74, 0.88)*
HPV 18				
Month 12	152	5.4 (4.7, 6.3)	154	58.2 (50.1, 67.7)
Month 24	152	6.0 (5.2, 7.0)	153	29.0 (24.7, 34.0)
Month 36	149	6.1 (5.3, 7.1)	152	20.9 (17.8, 24.6)
Month 60	152	5.7 (4.7, 6.8)	149	16.7 (13.8, 20.3)
GMC ratio[3] (M60 /M12) (95% CI)		*1.04 (0.95, 1.14)*		*0.29 (0.27, 0.32)*

GMC ratio[3] (M60 /M24) (95% CI)	*0.94 (0.86, 1.03)*	*0.58 (0.53, 0.64)*
GMC ratio[3] (M60 /M36) (95% CI)	*0.92 (0.84, 1.01)*	*0.81 (0.74, 0.89)*

[1]DoRIS participants who received at least one dose of vaccine, irrespective of their HPV DNA or serostatus at baseline. [2]ELISA serum antibody geometric mean concentrations (GMC).
[3]Estimated with linear mixed effects model with log antibody concentration as the response and dose group, time point, and a dose group-time interaction term as fixed effects, and participant as a random effect to account for correlation of repeated measurements within participants.

Supplementary Table 5. Number of participants with at least one serious adverse event, and number of events, by trial arm from enrolment to Month 60 (total vaccinated cohort)

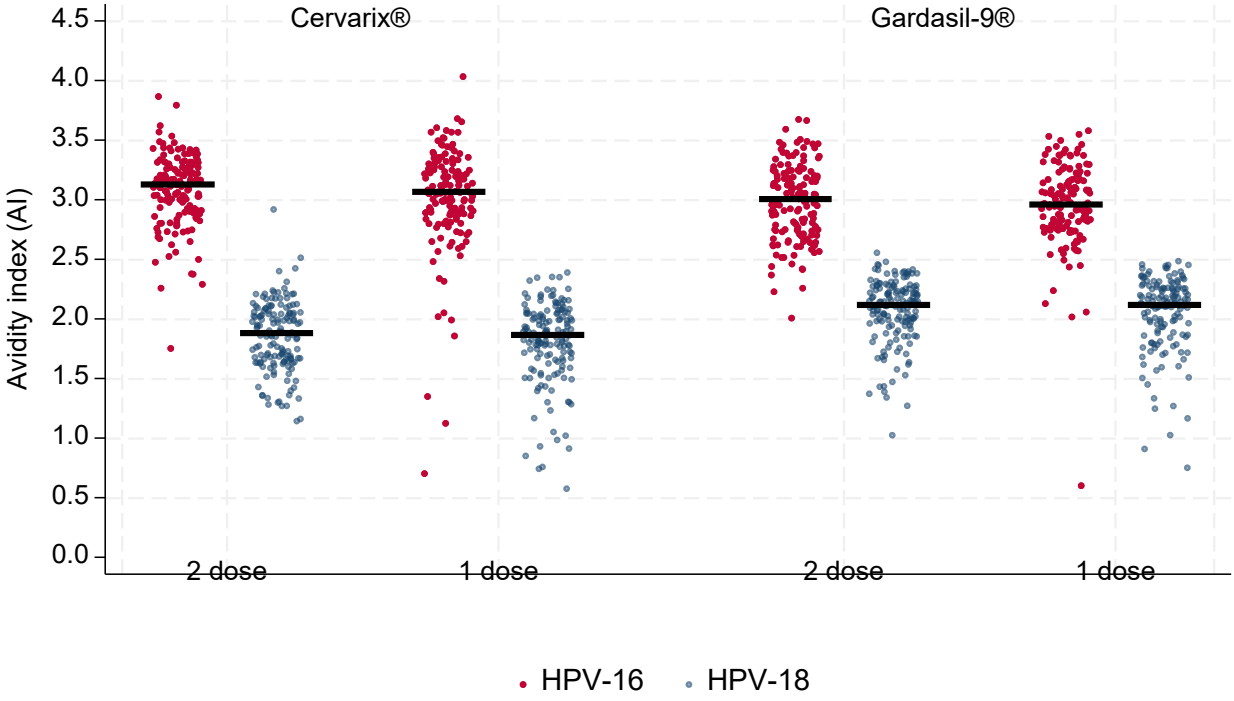
		1 dose Cervarix® (N=155)	2 doses Cervarix® (N=155)	1 dose Gardasil-9® (N=155)	2 doses Gardasil-9® (N=155)	Total (N=620)[1]
All SAEs	Number of girls (%)	10 (6.5 %)	4 (2.6 %)	8 (5.2 %)	9 (5.8 %)	31 (5.0 %)
	(Number of events)	*(17)*	*(4)*	*(8)*	*(11)*	*(40)*
Components of SAEs						
Death	Number of girls (%)	0 (-)	0 (-)	0 (-)	1 (0.6 %)	1 (0.2 %)
	(Number of events)	*(0)*	*(0)*	*(0)*	*(1)*	*(1)*
Hospitalisation	Number of girls (%)	10 (6.5 %)	3 (1.9 %)	7 (4.5 %)	8 (5.2 %)	28 (4.5 %)
	(Number of events)	(17)	(3)	(7)	(10)	(37)
Life-threatening condition	Number of girls (%)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Persistent disability	Number of girls (%)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Congenital abnormality	Number of girls (%)	0 (-)	1 (0.6 %)	0 (-)	0 (-)	1 (0.2 %)
	(Number of events)	*(0)*	*(1)*	*(0)*	*(0)*	*(1)*
Other medically important event	Number of girls (%)	0 (-)	0 (-)	1 (0.6 %)	0 (-)	1 (0.2 %)
	(Number of events)	*(0)*	*(0)*	*(1)*	*(0)*	*(1)*

[1]Includes 22 participants who were not enrolled in the long-term follow-up extension (13 participants who were lost to follow-up/withdrawn by M36 and 9 who did not consent)

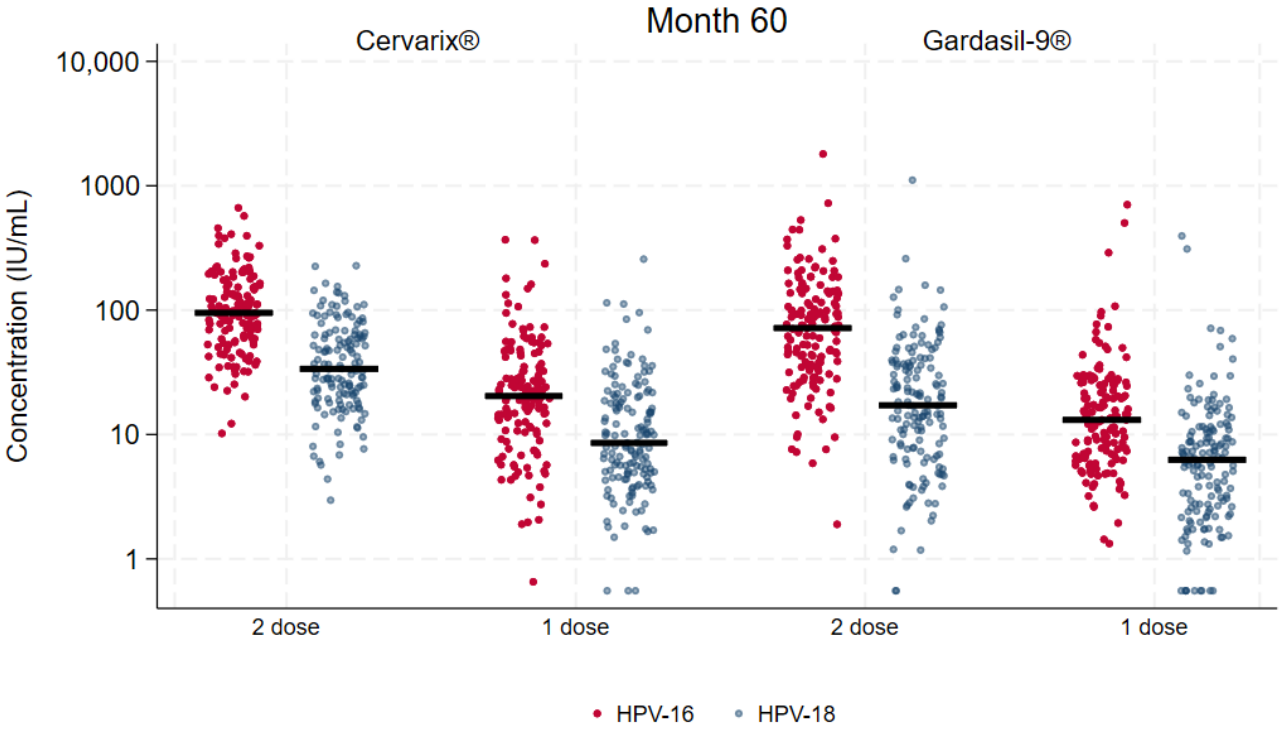
Supplementary Table 6. Serious adverse event by diagnosis and trial arm, from enrolment to Month 60 visit (total vaccinated cohort)

Number of events	1 dose Cervarix®	2 doses Cervarix®	1 dose Gardasil-9®	2 doses Gardasil-9®	Total
Severe malaria	15	3	6	11	35
Urinary tract infection	0	0	0	0	0
Gastroenteritis	0	0	1	0	1
Amoebiasis	0	0	0	0	0
Peptic ulcer disease	1	0	0	0	1
Dehydration due to fever	1	0	0	0	1
Anaemia	0	0	0	0	0
Vasovagal syncope	0	0	0	0	0
Snake bite	0	0	0	0	0
Spontaneous abortion	0	0	1	0	1
Caesarean section	0	0	0	0	0
Congenital anomaly	0	1	0	0	1
Total events	**17**	**4**	**8**	**11**	**40**

Supplementary Figure 1. Distribution of HPV-16 and HPV-18 antibody avidity index (AI) at 36 months by arm. Each data point represents a single individual and the line through the data points represents the median AI



Supplementary Figure 2. Distribution of HPV-16 and HPV-18 antibody concentrations (IU/mL) at 60 months by arm (total vaccinated cohort). Each data point represents a single individual and the line through the data points represents the median concentration



APPENDIX 6. Summary of studies designed to formally evaluate single-dose HPV vaccine schedule

Trial (registration)	Vaccines	Evidence type	N enrolled	Age	Location	Start/end dates	Primary endpoint	Brief description
DoRIS (NCT02834637)	Cervarix & Gardasil-9	Immunogenicity	930	Girls 9-14 years old	Tanzania	February 2017 – March 2027	Non-inferiority of HPV16/18 antibody seropositivity comparing 1 dose with 2 and 3 doses; non-inferiority of HPV16/18 antibody concentrations comparing 1 dose with historical cohorts who received 1 dose in whom efficacy has been demonstrated	Randomised to 1, 2, or 3 doses of Cervarix or Gardasil-9; follow to 36 months. 1 and 2 dose arms in trial extension to 108 months.
KEN SHE (NCT03675256)	Cervarix & Gardasil-9 (with meningitis control)	Efficacy and immunogenicity	2275	Women 15-20 years	Kenya	December 2018 – December 2025	Efficacy of 1 dose in prevention of incident 6-month persistent HPV16/18 infection	Randomised to 1 dose of Cervarix, Gardasil-9 or control vaccine; follow to 54 months
HANDS (NCT03832049)	Gardasil-9	Immunogenicity	1720	Girls 4-8 years and 9-14 years; women 15-26 years	The Gambia	September 2019 – June 2024	Non-inferiority of HPV vaccine genotype antibodies concentrations 4 weeks after last dose, comparing 1 and 2 doses with 3 doses	Girls aged 4-8 years and 9-14 years randomised to 1 or 2 doses; women aged 15-26 years given 3 doses; follow to 36 months
Primavera (NCT03728881)	Cervarix & Gardasil	Immunogenicity	1240	Girls 9-14 years; Women 18-25 years	Costa Rica	April 2019 – February 2024	Non-inferiority of HPV16/18 antibody concentrations comparing 1 dose with 3 doses	Girls aged 10-14 years given 1 dose of Cervarix; women aged 18-25 years given 3 doses of Gardasil; follow to 36 months
ESCUDDO (NCT03180034)	Cervarix & Gardasil-9	Efficacy and immunogenicity	28,000	Girls 12-16 years	Costa Rica	November 2017 – August 2025	non-inferiority of 1 vs 2 doses in prevention of incident 6-month persistent HPV16/18 infection	Randomised to 1 or 2 doses of Cervarix or Gardasil-9; follow to 60 months
PRISMA (NCT05237947)	Cervarix & Gardasil-9 (with diphtheria/ tetanus/ pertussis control vaccine)	Efficacy and immunogenicity	5000	Women 18-30 years	Costa Rica	March 2022 – May 2026	Efficacy of 1 dose in prevention of incident 6-month persistent HPV16/18 infection	Randomised to 1 dose of Cervarix, Gardasil-9 or control vaccine; follow to 60 months
Thailand impact study (NCT03747770)	Gardasil	Effectiveness	~18,000 (vaccination); ~9000 (surveys)	Girls ~13-14 years (Grade 8);	Thailand	December 2018 – June 2023	Effectiveness of 1 dose in reducing HPV16/18 prevalence, compared with unvaccinated girls of same grade	Girls in grade 8 given 1 or 2 doses; Girls in grade 10 & 12 for cross-sectional surveys

Trial (registration)	Vaccines	Evidence type	N enrolled	Age	Location	Start/end dates	Primary endpoint	Brief description
				Girls 15-18 years (Grades 10 and 12)				Evaluation of effectiveness through repeat cross-sectional prevalence surveys at 2 and 4 years
HOPE	Cervarix	Effectiveness	4807 received single dose; 3950 in prevalence surveys	Girls ~9 years (Grade 4) (vaccination); Girls ~15-16 years (Grade 10) (surveys)	South Africa	February 2019 (start of single-dose catch up); final survey 2023	Population impact of 1 dose catch-up in protection against HPV16/18 infection Population impact of the national 2 dose programme in protecting against HPV16/18 infection	Girls in grade 4 receive 2 doses through national programme; Girls in grade 10 receive a single dose in 'catch-up' programme. Evaluation of effectiveness through repeat cross-sectional prevalence surveys

APPENDIX 7. Paper: Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose in young women aged 15–20 years in Kenya (KEN SHE): an immunobridging analysis of randomised controlled trials

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Surname/Family Name	Baisley		
Thesis Title	HPV epidemiology and HPV vaccination in East Africa		
Primary Supervisor	Deborah Watson-Jones		

If the Research Paper has previously been published please complete Section B, if not please move to Section C.

SECTION B – Paper already published

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SECTION D – Multi-authored work

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)	This paper describes the immunobriding of the DoRIS results with those from the KEN SHE trial. I am the joint Principal Investigator (PI), along with Deborah Watson-Jones, on the DoRIS trial. In collaboration Deborah Watson-Jones, I co-developed the initial ideas, designed the study, secured the funding and developed the study protocol and questionnaires. I am also the DoRIS trial statistician, and had overall responsibility for the trial data management and statistical analyses. I wrote the protocol and analysis plan for the immunobriding study presented in this paper, analysed the data and interpreted the results. I am the first and corresponding author, wrote the first draft of the manuscript, and was responsible for replying to reviewers'comments during the peer-review process.

SECTION E

Student Signature	[redacted]
Date	15 October 2024

Supervisor Signature	[redacted]
Date	17 October 2024

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Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose in young women aged 15–20 years in Kenya (KEN SHE): an immunobridging analysis of randomised controlled trials

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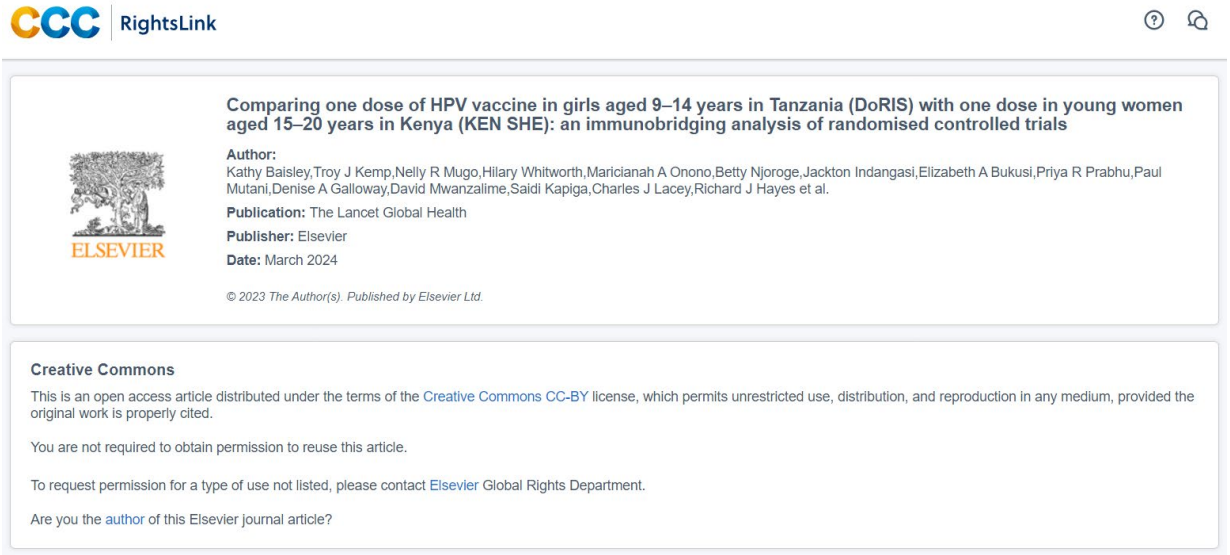

Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose in young women aged 15–20 years in Kenya (KEN SHE): an immunobridging analysis of randomised controlled trials

Kathy Baisley, Troy J Kemp, Nelly R Mugo, Hilary Whitworth, Maricianah A Onono, Betty Njoroge, Jackton Indangasi, Elizabeth A Bukusi, Priya R Prabhu, Paul Mutani, Denise A Galloway, David Mwanzalime, Saidi Kapiga, Charles J Lacey, Richard J Hayes, John Changalucha, Ligia A Pinto, Ruanne V Barnabas, Deborah Watson-Jones

Summary

Background The first randomised controlled trial of single-dose human papillomavirus (HPV) vaccine efficacy, the Kenya single-dose HPV-vaccine efficacy (KEN SHE) trial, showed greater than 97% efficacy against persistent HPV16 and HPV18 infection at 36 months among women in Kenya. We compared antibody responses after one dose of HPV vaccine in the Dose Reduction Immunobridging and Safety Study (DoRIS), the first randomised trial of the single-dose regimen in girls aged 9–14 years, the target age range for vaccination, with those after one dose of the same vaccine in KEN SHE.

Methods In the DoRIS trial, 930 girls aged 9–14 years in Tanzania were randomly assigned to one, two, or three doses of the 2-valent vaccine (Cervarix) or the 9-valent vaccine (Gardasil-9). The proportion seroconverting and geometric mean concentrations (GMCs) at month 24 after one dose were compared with those in women aged 15–20 years who were randomly assigned to one dose of the same vaccines at the same timepoint in KEN SHE. Batched samples were tested together by virus-like particle ELISA for HPV16 and HPV18 IgG antibodies. Non-inferiority of GMC ratios (DoRIS trial:KEN SHE) was predefined as a lower bound of the 95% CI less than 0·50.

Findings Month 24 HPV16 and HPV18 antibody GMCs in DoRIS were similar or higher than those in KEN SHE. 2-valent GMC ratios were 0·90 (95% CI 0·72–1·14) for HPV16 and 1·02 (0·78–1·33) for HPV18. 9-valent GMC ratios were 1·44 (95% CI 1·14–1·82) and 1·47 (1·13–1·90), respectively. Non-inferiority of antibody GMCs and seropositivity was met for HPV16 and HPV18 for both vaccines.

Interpretation HPV16 and HPV18 immune responses in young girls 24 months after a single dose of 2-valent or 9-valent HPV vaccine were comparable to those in young women who were randomly assigned to a single dose of the same vaccines and in whom efficacy had been shown. A single dose of HPV vaccine, when given to girls in the target age range for vaccination, induces immune responses that could be effective against persistent HPV16 and HPV18 infection at least two years after vaccination.

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See **Comment** page e360

For the KiSwahili translation of the abstract see **Online** for appendix 1

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Introduction

Cervical cancer is the leading cause of cancer-related morbidity and mortality among women in much of sub-Saharan Africa. It is caused by infection with oncogenic human papillomavirus (HPV) genotypes, which is almost entirely preventable through prophylactic vaccination. As part of its global strategy for cervical cancer elimination, WHO has set a target of 90% of girls worldwide being vaccinated against HPV by the age of 15 years by 2030.[1] However, in 2021, only 21% of girls aged 15 years were estimated to be vaccinated against HPV, largely because many countries had not yet introduced HPV vaccination programmes.[2] A major barrier to introduction, particularly for low-income and middle-income countries, has been the costs and logistical challenges of delivering the vaccine as a multi-dose schedule.[3] A global shortage of HPV vaccine also contributed to delays in its introduction for some countries.

Over the past decade, accumulating observational evidence has suggested that a single dose of HPV vaccine produces durable immune responses and

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For more on **cervial cancer in sub-Saharan Africa** see https://gco.iarc.fr/

Research in context

Evidence before this study

A review of the evidence for single dose human papillomavirus (HPV) vaccination was published in November, 2020, by the Single-Dose HPV Vaccine Evaluation Consortium, which summarised papers published until Aug 10, 2020. Results included eight observational studies that arose from three randomised trials (ie, Costa Rica Vaccine Trial (CVT), PATRICIA, and IARC/India HPV vaccine trials) in which participants received fewer than their allocated doses, showing that single dose antibody concentrations stabilised around 12 months after vaccination at a plateau level that was maintained and provided protection against persistent HPV infection for up to 11 years. Immunogenicity results were also available from 11 observational studies of women who received fewer than three doses through national HPV vaccination programmes, similarly showing that antibody concentrations after one dose were considerably higher than for natural infection and were sustained over time. Lastly, in 21 observational studies of vaccine effectiveness against HPV infection or cervical abnormalities among partially vaccinated women, ten found some evidence of effectiveness of one dose. On July 16, 2023, we searched PubMed for papers published since Aug 10, 2020, using the terms "human papillomavirus" AND "vaccine" AND ("immunogenicity" OR "efficacy" OR "effectiveness") AND "single dose". This search identified ten studies, five of which extended results from the CVT and IARC/India/ trials. The others included a study of women in Fiji who were vaccinated through a national HPV vaccination campaign in 2008–09, which showed 81% vaccine effectiveness of one dose against prevalent HPV16 and HPV18 infection. A non-randomised immunogenicity trial in the USA of a delayed second dose of Gardasil-9 among girls and boys aged 9–11 years showed that HPV16 and HPV18 antibody concentrations remained stable up to 24 months after one dose. The remaining three papers reported the results of the KEN SHE trial, the first randomised trial of single dose HPV vaccine efficacy, in females aged 15–20 years in Kenya, and the immunogenicity and immunobridging results of the DoRIS trial in Tanzania, the first randomised trial of the one-dose schedule in girls in the target age range for HPV vaccination (ie, aged 9–14 years). The KEN SHE trial showed one dose vaccine efficacy of 97·5%. The DoRIS trial showed that more than 98% of girls who received one dose were seropositive for HPV16 and HPV18 IgG antibodies at 24 months, had antibody concentrations that were stable over time, and who were non-inferior to those who had received one dose in the CVT and India/IARC trials.

Added value of this study

Here we present an immunobridging study comparing immune responses in the DoRIS trial with those in the KEN SHE trial. Both trials were conducted in east Africa, a region with one of the highest cervical cancer rates worldwide, and included enrolment of participants from malaria-endemic localities. Since evaluating HPV vaccine efficacy in young girls is difficult because of the time needed to accrue HPV infection endpoints, immunobridging studies are valuable for inferring protection in the young population. The trials used the same two HPV vaccines; this study provides the first immunobridging efficacy results for the 9-valent vaccine. We show that HPV16 and HPV18 antibody concentrations and seropositivity at 24 months after a single dose of HPV vaccine in girls aged 9–14 years in the DoRIS trial were non-inferior to those in young females aged 15–20 years in the KEN SHE trial. These results are salient for low-income settings where the cost and logistical advantages of a single dose regimen are particularly important.

Implications of all the available evidence

These results from the first two randomised trials of the single-dose schedule provide the strongest available evidence that one dose of HPV vaccine induces immune responses in young girls that are comparable with those seen in young women in whom efficacy has been shown, and are sustained for up to two years after vaccination. These data add to the observational evidence showing efficacy of a single dose up to 11 years, and further support the recent WHO recommendation for a single dose HPV vaccine schedule, providing a promising strategy towards achieving cervical cancer elimination.

protection against HPV infection and cervical cancer precursor lesions. Observational studies nested within three large HPV vaccine trials in which some participants did not complete their allocated dose schedules (ie, the Costa Rica Vaccine trial (CVT), the PATRICIA trial, and the IARC/India trial) have shown comparable efficacy against persistent HPV infection, a necessary precursor for cervical cancer, in females who received one dose compared with those who received two or three doses.[4–6]

Recently, these observational findings have been confirmed in two of the first randomised controlled trials of one dose of HPV vaccine, both in sub-Saharan Africa. The Kenya single-dose HPV-vaccine efficacy (KEN SHE) trial, conducted among sexually-active women aged 15–20 years, found that a single dose of the 2-valent vaccine (Cervarix, GlaxoSmithKline Biologicals, Rixensart, Belgium) or of the 9-valent vaccine (Sanofi Pasteur MSD, Lyon, France) provided 97·5% or higher efficacy against incident 6-month persistent HPV16 and HPV18 infection, compared with a control vaccine.[7] The Dose Reduction Immunobridging and Safety Study (DoRIS) trial, conducted among girls aged 9–14 years (the target age group for HPV vaccination) in Tanzania, found that more than 98% of participants were seropositive for antibodies to HPV16 and HPV18 at 24 months after vaccination with either the 2-valent or 9-valent vaccines, irrespective of whether they received one, two, or three doses, and antibody concentrations after a single dose were stable for up to 3 years.[8,9] HPV16 and HPV18

antibody concentrations after one dose of HPV vaccine in DoRIS were found to be non-inferior to those who had received one dose in the CVT and IARC/India trials, in whom efficacy has been shown for up to 11 years or more.[10] Given the strength of the available evidence, WHO recently amended their recommendations regarding HPV vaccination to allow either one-dose or two-dose schedules among individuals who are immunocompetent up to 20 years of age.[11]

Here we aimed to report the results of immunobridging the DoRIS to KEN SHE trials, comparing immune responses after one dose in young girls in Tanzania with those in young women in Kenya. These results provide several important advances over previous immunobridging comparisons. First, we are bridging immune responses in DoRIS to a randomised trial with direct and rigorous evidence of efficacy rather than to observational studies, thus providing the strongest available evidence of efficacy of the single-dose regimen in young girls. The KEN SHE and DoRIS trials used the same two vaccines, so provide the first immunobridging comparison for the 9-valent vaccine. The trials were both conducted in east Africa, which has one of the highest rates of cervical cancer in the world, and where the cost and logistical advantages of a single-dose regimen are particularly important. Lastly, the DoRIS trial and one of the KEN SHE trial sites were in malaria-endemic areas; malaria has been shown to affect the immune responses to some vaccines.[12,13] The first trial of HPV vaccine in Africa showed some evidence of higher antibody concentrations in participants with malaria than in participants without malaria.[14] In 2014, WHO called for HPV vaccine trials in malaria-endemic areas as a research priority.[15]

Methods

Study design

The DoRIS trial (NCT02834637) is the first randomised trial to evaluate the immunogenicity of a single-dose regimen in girls in the target age range for vaccination. The trial commenced enrolment in February, 2017, and has been described in detail previously.[16] In brief, it is an ongoing unblinded, individually randomised controlled trial comparing reduced-dose schedules of two HPV vaccines among 930 healthy, HIV-negative Tanzanian schoolgirls aged 9–14 years (appendix 2 p 1). Girls were randomly allocated to one of six groups comprising three doses, two doses, or a single dose of the GlaxoSmithKline 2-valent or Merck 9-valent vaccine. All participants were followed up to month 36; girls in the one dose and two dose groups have been enrolled in a trial extension and will be followed up to 9 years.

Immunobridging objectives

The overall aim of the immunobridging study is to compare vaccine-induced HPV-specific immune responses 24 months after one dose in young girls in the DoRIS trial with those after one dose in young women in the KEN SHE trial. Our hypothesis was that HPV16 and HPV18 antibody responses in girls aged 9–14-years after a single dose of HPV vaccine would be non-inferior to those observed in young women aged 15–20-years.

The primary objective was to show non-inferiority of HPV16 and HPV18 antibody geometric mean concentrations (GMCs) at month 24 after vaccination, when comparing one dose of HPV vaccine in the DoRIS trial with one dose of the same vaccine in KEN SHE. The secondary objective was to show non-inferiority of HPV16 and HPV18 seropositivity. The month 24 timepoint was chosen because studies have shown that immune responses after a single dose in females aged 10–25 years reach a plateau around 12 months, after which they remain stable up to 11 years.[6,17] Results from the DoRIS trial showed that antibody concentrations after one dose declined between month 1 and month 7 then reached a plateau by month 12 and remained stable up to month 36.[8]

The KEN SHE trial

KEN SHE is the first randomised controlled efficacy trial of a single dose of HPV vaccine. The trial enrolled 2275 healthy, HIV-negative, sexually active young women aged 15–20 years in Kenya between December, 2018, and November, 2019.[18] Women were randomly allocated to one of three groups, comprising a single dose of the GlaxoSmithKline 2-valent HPV vaccine, the Merck 9-valent HPV vaccine, or meningococcal vaccine (appendix 2 p 1). Women enrolled in the main trial were invited to participate in the immunobridging substudy at the time of enrolment; all women were invited until the target enrolment of 910 participants was reached.

The primary efficacy analysis was at month 18, with the final analysis at month 36 evaluating durability. Vaccine efficacy of the 2-valent vaccine against incident persistent HPV16 and HPV18 infection was 97·5% (95% CI 81·7–99·7) at month 18 and 97·5% (90·0–99·4) at month 36.[7,19] 9-valent vaccine efficacy at the same timepoints was 97·5% (95% CI 81·6–99·7) and 98·8% (91·3–99·8), respectively.

Sample selection

The immunobridging study used blood samples from all girls in the one dose groups in the DoRIS trial who attended the month 24 visit within a window of 22–28-months after vaccination. For the KEN SHE trial, we took a random sample of 155 participants in the immunobridging substudy in each HPV vaccine group, from those who had blood samples available from month 0 and month 24, and the month 24 sample was taken within the 22–28-month window.

See Online for appendix 2

Laboratory methods

Antibodies to HPV16 and HPV18 were measured by type-specific virus-like particle ELISA assay at the

Frederick National Laboratory for Cancer Research HPV Immunology Laboratory in Frederick, MD, USA (appendix 2 p 2). Antibody concentrations greater than or equal to the lower limit of detection for each assay were pre-specified to indicate seropositivity (HPV16 ≥1·309 international units [IU]/mL and HPV18 ≥1·109 IU/mL). HPV DNA genotyping was done using Anyplex II HPV28 (Seegene, Seoul, South Korea) at the Catalan Institute of Oncology, Barcelona, Spain (DoRIS) and the University of Washington East Africa STI Laboratory, Mombasa, Kenya (KEN SHE).

Sample size

Our sample size calculations were based on an expected 130 girls in each group in the DoRIS trial available for the per-protocol analysis at month 24, assuming a 20% loss to follow-up across 36 months and 5% HPV seropositive or DNA positive at enrolment in the DoRIS trial. With 130 in each group from each trial, if the true GMC ratio (DoRIS:KEN SHE) between groups is 1·0, we had more than 90% power to show that the lower limit of the 95% CI for the GMC ratio was above 0·50, indicating that the one dose schedule in DoRIS did not decrease HPV16 and HPV18 antibody GMC by more than 50% compared with KEN SHE. The non-inferiority margin of 0·50 was defined a priori on the basis of that used in other HPV vaccine trials.[20,21] We assumed an SD of 0·50–0·60 log_{10} anti-HPV concentration,[20,22] and used a one-sided non-inferiority test at the 2·5% level.

Statistical analysis

The primary immunobridging analysis was in the per-protocol cohort: participants who received one dose of HPV vaccine and who were HPV antibody negative and DNA negative at enrolment for the genotype under analysis. Secondary analyses included all participants who received one dose, irrespective of baseline antibody or HPV DNA status.

Separate analyses were done for each vaccine type. HPV antibody concentrations were log_{10}-transformed; concentrations less than the assay cutoff were given a value of half the cutoff before log transformation. Arithmetic mean log_{10} antibody concentrations and 95% CIs were calculated for each group, assuming a normal distribution.

The difference in HPV genotype-specific log_{10} concentrations at month 24 between the two groups (DoRIS minus KEN SHE) and its 95% CI were calculated; the GMC ratio and its 95% CI were obtained by back-transformation. Non-inferiority of the antibody response was concluded if the lower bound for the two-sided 95% CI for the GMC ratio was above 0·50. Linear regression with a fixed term for the study groups was used to obtain p values; p values less than 0·05 were considered statistically significant.

The number and proportion of participants in each group who were seropositive for HPV16-specific and HPV18-specific antibodies at month 24 was tabulated. Seropositivity for a particular HPV genotype was defined as an antibody level higher than the assay cutoff. For each vaccine and HPV genotype, we calculated the difference (one dose of DoRIS minus one dose of KEN SHE) in the proportion of seropositive individuals and estimated the 95% CI for the difference using the exact method of Chan and Zhang.[23] Non-inferiority of seropositivity was concluded if the lower bound of the two-sided 95% CI for the difference was higher than –5%.

For the primary outcomes, success was required for both HPV16 and HPV18 to conclude non-inferiority for each vaccine; therefore, no multiplicity adjustment was made to account for the testing of multiple HPV genotypes. Missing data were minimal (<1%) so a complete case analysis was used. SAS (version 9.1) and Stata (version 17) were used for all analyses.

Role of the funding source

The funders of this study did not have any role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

The DoRIS trial enrolled 930 participants (155 per group); 154 (99%) participants in the one dose 2-valent vaccine group and 152 (98%) in the one dose 9-valent vaccine group attended the month 24 visit within the 22–28-month timeframe so were included in the immunobridging analysis. The KEN SHE trial enrolled 302 participants in the immunobridging substudy in the one dose 2-valent vaccine group, and 303 in the one dose 9-valent vaccine group. Of those, 287 (95%) and 278 (92%), respectively, attended the month 24 visit within the required timeframe; 154 were randomly sampled from each group.

	One dose DoRIS (Cervarix; n=154)	One dose KEN SHE (Cervarix; n=154)	One dose DoRIS (Gardasil-9; n=152)	One dose KEN SHE (Gardasil-9; n=154)
Age, years	10 (9–12)	18·5 (17–19)	10 (9–12)	18 (17–19)
Age group, years				
9–14	154 (100%)	0	152 (100%)	0
15–20	0	154 (100%)	0	154 (100%)
HPV16 seropositive at baseline	6 (4%)	40 (26%)	7 (5%)	25 (16%)
HPV18 seropositive at baseline	13 (8%)	49 (32%)	16 (11%)	30 (19%)
HPV16 DNA positive (cervical) at baseline	0	14 (9%)	1 (<1%)	9 (6%)
HPV18 DNA positive (cervical) at baseline	0	6 (4%)	1 (<1%)	3 (2%)
In per-protocol analysis				
HPV16*	148 (96%)	109 (71%)	145 (95%)	121 (79%)
HPV18*	141 (92%)	99 (64%)	136 (89%)	123 (80%)
Age, years†	10 (9–12)	19 (17–19)	10 (9–12)	18 (17–19)

Data are n (%) or median (IQR). HPV=human papillomavirus. *Numbers in the per-protocol analysis for each HPV genotype. †Median (IQR) age in the per-protocol population does not differ between the subgroup for the HPV16 analysis and that for the HPV18 analysis, for any of the four groups.

Table 1: **Demographic characteristics at baseline among one dose groups in the DoRIS and KEN SHE trials**

Owing to protocol-specified differences in eligibility requirements, DoRIS participants were younger (median [IQR] 10 years [9–12]) than those in KEN SHE (18·5 years [17–19]; table 1). In addition, baseline HPV16 and HPV18 seropositivity and DNA positivity was higher in KEN SHE than in DoRIS, consistent with the older age range and that all KEN SHE participants were sexually active. Therefore a larger proportion of KEN SHE participants than DoRIS participants were excluded from the per-protocol analyses.

In the per-protocol analysis of the 2-valent vaccine, 147 (99%) of 148 participants in the DoRIS trial and 109 (100%) of 109 participants in the KEN SHE trial were seropositive for IgG antibodies to HPV16, and 139 (99%) of 141 and 97 (98%) of 99, respectively, for HPV18 (table 2). HPV16 and HPV18 antibody GMCs after one dose of the 2-valent vaccine were similar in DoRIS (p=0·39) and in KEN SHE (p=0·91; figure 1). Non-inferiority of antibody concentrations for the 2-valent vaccine was met for both HPV genotypes, with GMC ratios (DoRIS:KEN SHE) of 0·90 (95% CI 0·72 to 1·14) for HPV16 and 1·02 (0·78 to 1·33) for HPV18 (figure 2). Non-inferiority was also met for seropositivity, with a difference of –0·7% (95% CI –3·9 to 3·0) for HPV16 and 0·1% (–3·2 to 4·1) for HPV18.

In the per-protocol analysis of the 9-valent vaccine, 144 (99%) of 145 participants in the DoRIS trial and 120 (99%) of 121 participants in the KEN SHE trial, were seropositive for IgG antibodies to HPV16, and 133 (98%) of 136 and 113 (92%) of 123 for HPV18 (table 2). HPV16 and HPV18 antibody GMCs after one dose of the 9-valent vaccine were significantly higher in DoRIS than in KEN SHE (p=0·002 and p=0·004, respectively). Non-inferiority of antibody concentrations was met for the 9-valent vaccine for both HPV genotypes, with GMC ratios of 1·44 (95% CI 1·14 to 1·82) for HPV16, and 1·47 (1·13 to 1·90) for HPV18 (figure 2). Non-inferiority was also met for seropositivity, with a difference of 0·1% (95% CI –3·2 to 4·1) for HPV16 and 5·9% (0·5 to 12·5) for HPV18.

In the total vaccinated cohort, non-inferiority of antibody GMCs and seropositivity was shown for the 2-valent and 9-valent vaccines, for both HPV genotypes (table 3).

	N*	GMC† (95% CI), IU/mL	IQR	Seropositive‡ n (%)
HPV16 IgG antibody				
DoRIS (Cervarix)	148	22·9 (19·9 to 26·4)	14·7 to 40·0	147 (99%)
KEN SHE (Cervarix)	109	25·3 (21·0 to 30·6)	13·0 to 43·2	109 (100%)
GMC ratio, DoRIS:KEN SHE (95% CI)	..	..	0·90 (0·72 to 1·14)	..
Difference in seropositive, DoRIS–KEN SHE (95% CI)	..	..	–0·7% (–3·9 to 3·0)	..
DoRIS (Gardasil-9)	145	13·7 (11·9 to 15·8)	8·9 to 21·4	144 (99%)
KEN SHE (Gardasil-9)	121	9·5 (7·8 to 11·5)	4·8 to 19·1	120 (99%)
GMC ratio, DoRIS:KEN SHE (95% CI)	..	..	1·44 (1·14 to 1·82)	..
Difference in seropositive, DoRIS–KEN SHE (95% CI)	..	..	0·1% (–3·2 to 4·1)	..
HPV18 IgG antibody				
DoRIS (Cervarix)	141	9·9 (8·5 to 11·5)	5·7 to 17·7	139 (99%)
KEN SHE (Cervarix)	99	9·7 (7·6 to 12·4)	4·3 to 21·8	97 (98%)
GMC ratio, DoRIS:KEN SHE (95% CI)	..	..	1·02 (0·78 to 1·33)	..
Difference in seropositive, DoRIS–KEN SHE (95% CI)	..	..	0·6% (–3·5 to 6·0)	..
DoRIS (Gardasil-9)	136	5·7 (4·9 to 6·8)	3·0 to 10·8	133 (98%)
KEN SHE (Gardasil-9)	123	3·9 (3·2 to 4·8)	1·8 to 8·4	113 (92%)
GMC ratio, DoRIS:KEN SHE (95% CI)	..	..	1·47 (1·13 to 1·90)	..
Difference in seropositive, DoRIS–KEN SHE (95% CI)	..	..	5·9% (0·5 to 12·5)	..

GMC=geometric mean concentration. HPV=human papillomavirus. IU=international unit. *DoRIS and KEN SHE participants who were ELISA antibody negative and DNA negative at baseline (pre-vaccination) for the HPV genotype under analysis. †ELISA serum antibody GMC. ‡Seropositivity defined as concentrations greater than the laboratory determined cutoff (HPV16 1·309 IU/mL and HPV18 1·109 IU/mL).

Table 2: **Comparisons of GMCs and seropositivity rates at month 24 post-single dose HPV vaccination between DoRIS and KEN SHE (per-protocol population)***

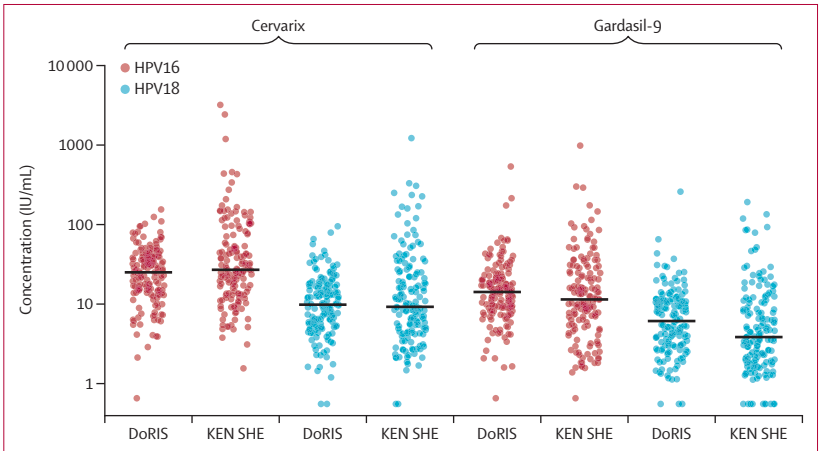

Figure 1: **Distribution of HPV16 and HPV18 antibody concentrations at 24 months after a single dose of HPV vaccine, by group (total vaccinated population)**
Each data point represents a single individual and the line through the data points represents the median concentration. HPV=human papillomavirus. IU=international unit.

Discussion

We found that immune responses 24 months after a single dose of two different HPV vaccines in girls in the target age range for vaccination were non-inferior to those in young women in Kenya who were randomly assigned to a single dose of the same vaccine and in whom efficacy was shown. These results from the first two randomised trials of the single-dose schedule provide the strongest available evidence that one dose of HPV vaccine induces immune responses in young girls that are comparable to those seen in young women in whom efficacy has been shown. In April, 2022, WHO's Strategic Advisory Group of Experts (SAGE) recommended that HPV vaccine dose schedules be updated to allow countries to choose a one-dose or two-dose schedule for individuals aged 9–20 years.[24] In December, 2022, WHO published a new position paper, stating that a single-dose schedule can provide comparable efficacy and durability

of protection as two doses and confirming the recommendations of SAGE.[11]

The SAGE recommendations were based in part on evidence from the KEN SHE and DoRIS trials. These trials are particularly important because they were conducted in a region with one of the highest HPV infection and cervical cancer rates in the world. In the control group of KEN SHE, the incidence of persistent infection with 9-valent HPV vaccine genotypes at month 18 (9·2 per 100 person-years) was around 30% higher than has been reported in other trials.[19] The HPV16 and HPV18 vaccine efficacy of higher than 97% observed in the KEN SHE trial is comparable to the three-dose efficacy observed in several licensure trials, and provides strong evidence for single-dose protection. The results of the DoRIS trial provide further insight on vaccine-induced antibodies in young girls up to 36 months after vaccination with a single dose.[9]

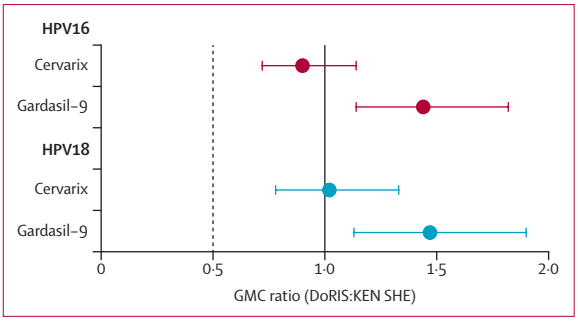

Figure 2: **Ratio of GMCs (DoRIS:KEN SHE) and 95% CIs, at 24 months after a single dose of HPV vaccine (per-protocol population)**
The black dotted line represents the non-inferiority margin. The solid black line is a GMC ratio of 1 (ie, no difference between the two groups). GMC=geometric mean concentration. HPV=human papillomavirus.

Immunobridging studies provide a valuable strategy to evaluate reduced-dose schedules in young girls, the primary target group for HPV vaccination but in whom evaluating efficacy is difficult because of the time needed to accrue virological or disease endpoints. In immunobridging studies, HPV genotype-specific antibody concentrations in a new population group are compared with those in a population for which efficacy has been shown; if antibody concentrations are shown to be non-inferior, then efficacy is also assumed to be comparable. This approach was taken for the original licensure of HPV vaccines in girls aged 9–14 years, and the approval of a two-dose schedule in this age range.[25,26] Antibody concentrations are a recommended endpoint for immunobridging because protection conferred by virus-like particle HPV vaccines is considered to be mediated primarily by neutralising antibodies.[27] Total IgG as measured by the ELISA assay has been shown to correlate well with HPV16 and HPV18 neutralisation assays, even at the lower antibody concentrations observed after a single dose.[28] However, the minimum antibody concentration needed for protection has not been established.

Age is a key determinant of antibody responses following HPV vaccination, with young girls having

	N*	GMC† (95% CI), IU/mL	IQR	Seropositive‡ n (%)	Seroconverted§ n (%)
HPV16 IgG antibody					
DoRIS (Cervarix)	154	22·7 (19·8 to 26·1)	14·5 to 39·8	153 (99%)	147 (95%)
KEN SHE (Cervarix)	154	32·3 (26·5 to 39·3)	13·5 to 71·1	154 (100%)	114 (74%)
GMC ratio, DoRIS:KEN SHE (95% CI)	..	..	0·70 (0·55 to 0·89)	..	..
Difference in seropositive, DoRIS–KEN SHE (95% CI)	..	..	–0·6% (–3·7 to 1·8)	..	..
DoRIS (Gardasil-9)	152	14·1 (12·2 to 16·3)	8·8 to 21·8	151 (99%)	144 (95%)
KEN SHE (Gardasil-9)	154	12·3 (10·1 to 14·9)	5·4 to 28·4	153 (99%)	128 (83%)
GMC ratio, DoRIS:KEN SHE (95% CI)	..	..	1·15 (0·90 to 1·46)	..	..
Difference in seropositive, DoRIS–KEN SHE (95% CI)	..	..	0 (–3·1 to 3·0)	..	..
HPV18 IgG antibody					
DoRIS (Cervarix)	154	9·6 (8·3 to 11·1)	5·5 to 17·5	152 (99%)	139 (90%)
KEN SHE (Cervarix)	154	11·8 (9·5 to 14·6)	4·7 to 25·2	152 (99%)	102 (66%)
GMC ratio DoRIS:KEN SHE (95% CI)	..	..	0·81 (0·63 to 1·05)	..	..
Difference in seropositive, DoRIS–KEN SHE (95% CI)	..	..	0 (–3·5 to 3·5)	..	..
DoRIS (Gardasil-9)	152	6·0 (5·2 to 7·0)	3·0 to 10·9	149 (98%)	133 (88%)
KEN SHE (Gardasil-9)	154	4·7 (3·9 to 5·8)	1·9 to 10·5	144 (94%)	114 (74%)
GMC ratio, DoRIS:KEN SHE (95% CI)	..	..	1·27 (0·99 to 1·64)	..	..
Difference in seropositive, DoRIS–KEN SHE (95% CI)	..	..	4·5% (–0·1 to 9·9)	..	..

GMC=geometric mean concentration. HPV=human papillomavirus. IU=international unit. *All participants (irrespective of ELISA antibody or HPV DNA status at baseline). †ELISA serum antibody GMC. ‡Seropositivity defined by the laboratory determined cutoff (HPV16 1·309 IU/mL and HPV18 1·109 IU/mL). §Seroconversion defined as concentrations greater than the laboratory determined cutoff among participants who were seronegative for the HPV genotype at baseline.

Table 3: **Comparisons of GMC and seropositivity rates at month 24 post-single dose HPV vaccination between DoRIS and KEN SHE (total vaccinated population)***

significantly higher antibody GMCs than young women. Notably, HPV16 and HPV18 GMCs after a single dose of the 2-valent vaccine in DoRIS were similar or lower to those in KEN SHE, despite the older age in KEN SHE (median age 18·5 years *vs* 10 years). This was particularly pronounced in the total vaccinated population, for which HPV16 antibody concentrations in DoRIS were significantly lower than those in KEN SHE. These findings are similar to those in our immunobridging study of DoRIS with CVT, in which antibody GMCs in the total vaccinated population after one dose of the 2-valent vaccine were lower (although not statistically significant) in DoRIS than CVT, despite the older age in CVT (median age 20 years).[10] One explanation could be that vaccination boosts an individual's response to previous natural infection, or that boosting of vaccine antibody responses occurred during subsequent sexual exposure to HPV. The KEN SHE participants were all sexually active and 38% had evidence of current or previous HPV16 and HPV18 infection at enrolment. In contrast, only 1·5% of all DoRIS participants reported having passed sexual debut by month 24, and 11% had any evidence of HPV16 and HPV18 infection at enrolment. In the IARC/India trial (girls aged 10–18 years), a small increase in HPV16 and HPV18 antibody GMCs in the single-dose recipients was noted between month 36 and month 120; the authors speculated that this might have reflected a boosting effect as girls became sexually active.[17] In contrast, HPV16 and HPV18 antibody GMCs after a single dose of 9-valent vaccine were higher in DoRIS than in KEN SHE, even in the total vaccinated population, consistent with their younger age. The reasons for these differences in age effect between vaccines are unknown; however, a higher proportion of 2-valent than 9-valent recipients in KEN SHE had evidence of HPV infection at baseline (45% *vs* 30%, p<0·01). A further explanation is that the potent adjuvant of the 2-valent vaccine[22] could over-ride the age effect seen after one dose of the 9-valent vaccine.

In both trials, HPV16 and HPV18 antibody GMCs were higher after the 2-valent than the 9-valent vaccine. These results are similar to other studies that compared the 2-valent and 4-valent vaccines.[22,29] Despite this, both vaccines have similar high efficacy against persistent HPV16 and HPV18 infection and disease. HPV18 antibody GMCs and seropositivity were lower than those for HPV16, for both vaccines. This finding has been reported in other studies, despite high clinical efficacy against HPV18-related persistent infection and related sequelae.[20,28,30]

Strengths of our study include the immunobridging of results from two different HPV vaccines in girls in the target age range for vaccination to those from the first randomised trial of the efficacy of the single-dose regimen. We bridged our immune responses at 24 months after vaccination, after the one dose antibody concentrations had reached a plateau. Both trials were conducted in a region with extremely high HPV infection and cervical cancer rates, and where vaccination is most needed. The trials were also conducted in areas where malaria is endemic and has the potential to affect the immune response. We tested the samples from both trials in the same laboratory, using the same internationally standardised and well validated assay, allowing reproducibility of results and comparison with other studies. Both trials had excellent retention at the relevant visits for this study.

Limitations of our study include that it was restricted to participants who were HIV negative. The efficacy of reduced dose HPV vaccine regimens in women living with HIV is still uncertain, and WHO continues to recommend that immunocompromised individuals receive three doses when possible.[11] Another important question is whether a single dose would provide protection in women who are vaccinated at ages older than 20 years, and among those who are infected with HPV vaccine genotypes. Although data from national HPV vaccination programmes have shown some effectiveness of one dose in women vaccinated up to the age of 30 years, the highest effectiveness is in younger age groups.[31] Estimates of effectiveness vary depending on whether the analysis includes a buffer period (lag time) to allow prevalent HPV infections to clear, which is likely to be of greater importance when evaluating the single-dose regimen in older individuals.[31]

Nigeria introduced HPV vaccination in October, 2023, with a single-dose regimen, the first African country to do so, and Tanzania will switch to single-dose delivery in 2024. Although the single-dose schedule has the potential to greatly increase vaccine coverage, the vaccine effectiveness at the population level is unlikely to equal the greater than 97% efficacy seen in KEN SHE. A recent study in Rwanda estimated vaccine effectiveness of 70% among vaccinated women aged 17–29 years; the authors suggested this finding was likely because some women were already sexually active when vaccinated.[32]

Of note, our immunobridging study is based on only 24 months of follow-up, and our data on durability of the immune response is only up to 36 months. Since antibody concentrations in DoRIS were stable between month 24 and month 36,[9] it is likely that immune responses would be non-inferior to those in KEN SHE at month 36, for which efficacy was also shown. We are continuing follow-up of the DoRIS trial cohort to 9 years post-vaccination, which will provide further information on long-term immune responses in young girls. When comparing GMCs, we used a pre-specified non-inferiority margin of 0·50. Although a more stringent margin of 0·67 was met for the per-protocol population, it was not met for the 2-valent vaccine in the total vaccinated population. Since an immune correlate of protection is undefined, the significance of this finding is unclear. Lastly, the per-protocol population excluded a

large proportion of the KEN SHE participants, because of previous or current HPV infection at baseline, although the pre-specified non-inferiority margins were still met when these women were included.

In summary, our immunobridging results provide evidence that one dose of HPV vaccine in young girls induces antibody concentrations 24 months after vaccination that could be protective against persistent HPV16 and HPV18 infection. A single-dose HPV vaccine schedule could substantially reduce the costs and logistical challenges of vaccine delivery, alleviate vaccine supply constraints, and expand global vaccine introductions and coverage.

Contributors

KB, DW-J, LAP, NRM, and RVB developed the initial idea and design of the immunobridging study. KB, RVB, LAP, TJK, and DW-J developed the immunobridging protocol. DW-J, KB, and JC were joint principal investigators and CJL, RJH, SK, JI, PM, and HW were coinvestigators of the DoRIS trial. DW-J, JC, HW, SK, PM, DM, and JI were responsible for the on-site conduct of the DoRIS trial. RVB and NRM, together with other coinvestigators designed the KEN SHE trial. MAO, RVB, BN, EAB, and NRM oversaw the operations of the KEN SHE trial. JI, DAG, DM, PRP, LAP, and TJK were responsible for laboratory aspects of the study. KB was responsible for the statistical analysis. All authors contributed to the results interpretation. KB wrote the first draft of the manuscript and all authors critically reviewed and approved the finalised manuscript. KB and TJK directly accessed and verified the underlying data that are reported in the manuscript. KB, DW-J, TJK, LAP, and RVB had full access to the data in the study. No authors were prohibited from accessing the data. All authors had responsibility for the final decision to submit the manuscript for publication.

Equitable partnership declaration

The authors of this paper have submitted an equitable partnership declaration (appendix 3). This statement allows researchers to describe how their work engages with researchers, communities, and environments in the countries of study. This statement is part of *The Lancet Global Health's* broader goal to decolonise global health.

See Online for appendix 3

Declaration of interests

KB reports grants from the Bill & Melinda Gates Foundation during the conduct of the DoRIS trial, and a grant and vaccine donations from Merck Pharmaceuticals outside the submitted work. DW-J reports grants from the Gates Foundation and the UK Research and Innovation Medical Research Council during the DoRIS trial, and a grant and vaccine donations from Merck outside the submitted work. HW reports grants from the Gates Foundation and the UK Research and Innovation Medical Research Council during the DoRIS trial, and vaccine donations from Merck outside the submitted work. JC reports grant support from the Gates Foundation and the UK Research and Innovation Medical Research Council during the DoRIS trial. RJH reports a grant from the UK Research and Innovation Medical Research Council during the DoRIS trial. RVB reports grants from the Gates Foundation during the conduct of the KEN SHE trial, and data monitoring committee honorarium from Gilead Sciences and manuscript and abstract writing support from Regeneron Pharmaceuticals outside the submitted work. NRM reports grant support from Merck Pharmaceuticals outside the submitted work. DAG reports grants from the Gates Foundation during the conduct of the KEN SHE trial, and grants and personal fees from Merck outside the submitted work. EAB reports grants from the National Institutes of Health, the Centers for Disease Control and Prevention, and the European and Developing Countries Clinical Trials Partnership during the conduct of the KEN SHE trial; and personal fees from Gilead Sciences and personal fees from Merck outside the submitted work. All other authors declare no competing interests.

Data sharing

De-identified participant data presented in this manuscript can be made available after publication following written request to the London School of Hygiene & Tropical Medicine and the Mwanza Intervention Trials Unit, Tanzania. Requests must be accompanied by an analysis plan, which will be reviewed by the Mwanza Intervention Trials Unit Data Sharing Committee and lead investigators for each trial. Requesting researchers will be required to sign a Data Access Agreement if approval is given.

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Supplementary appendix 1

This translation in Kiswahili was submitted by the authors and we reproduce it as supplied. It has not been peer reviewed. *The Lancet*'s editorial processes have only been applied to the original in English, which should serve as reference for this manuscript.

Tafsiri hii katika Kiswahili iliwasilishwa na waandishi na tunatengeneza tena kama hutolewa. Haijapitiwa. Mchakato wa hariri wa *Lancet* umetumika tu kwa asili kwa Kiingereza, ambayo inapaswa kutumika kama kumbukumbu kwa muswada hii.

Supplement to: Baisley K, Kemp TJ, Mugo NR, et al. Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose in young women aged 15–20 years in Kenya (KEN SHE): an immunobridging analysis of randomised controlled trials. *Lancet Glob Health* 2024; **12:** e491–99.

Kulinganisha kinga mwili iliyopatikana baada ya dozi moja ya chanjo ya HPV kwa wasichana wa umri wa miaka 9 hadi 14 katika utafiti wa DoRIS nchini Tanzania na iliyopatikana baada ya dozi moja kwa wasichana wenye umri wa miaka 15 hadi 20 katika utafiti wa KEN SHE nchini Kenya

Muhtasari

Utangulizi

Utafiti wa kwanza wa majaribio wa ufanisi wa dozi moja ya chanjo ya HPV uitwao 'KEN SHE' ulionyesha ufanisi wa zaidi ya asilimia 97 katika kuzuia maambukizi yasiyoondolewa ya virusi vya HPV16 na HPV 18 miezi 36 baada ya chanjo kutolewa kwa wanawake nchini Kenya. Tumelinganisha mwitikio wa kingamwili baada ya dozi moja ya chanjo ya HPV katika utafiti wa DoRIS, utafiti wa kwanza wa dozi moja kwa wasichana wenye umri wa miaka 9 – 14, umri unaolengwa katika mpango wa kutoa chanjo ya HPV, na mwitikio wa kingamwili uliopatikana baada ya dozi moja ya chanjo ya aina hiyo hiyo katika utafiti wa KEN SHE.

Mbinu zilizotumika katika utafiti

Katika utafiti wa DoRIS, wasichana 930 wenye umri wa miaka 9 - 14, nchini Tanzania walipangwa katika makundi kwa njia ya bahati nasibu na kupata dozi moja, mbili au tatu za chanjo inayozuia aina mbili za virusi vya HPV (Cervarix) au chanjo inayozuia aina tisa za virusi vya HPV (Gardasil). Idadi ya waliotengeneza kingamwili na kiwango cha kinga mwilini miezi 24 baada ya kutolewa chanjo dozi moja ililinganishwa na ile ya wanawake wenye umri wa miaka 15 - 20 waliopangwa kwa njia ya bahati nasibu na kupewa dozi moja ya chanjo ile ile katika utafiti wa KEN SHE. Sampuli ziliwekwa katika mafungu na kupimwa kwa pamoja kwa njia ya ELISA ili kutambua sampuli ambazo zina kinga aina ya IgG dhidi ya virusi vya HPV16 na HPV18. Uwiano wa kiwango cha kinga kati ya DoRIS na KEN SHE ili usiwe duni ulipangwa kuwa kikomo cha chini kwa asilimia 95 kisiwe chini ya 0.50

Matokeo ya utafiti

Kiwango cha kinga dhidi ya HPV 16 na HPV 18 miezi 24 tangu kutolewa kwa dozi moja katika utafiti wa DoRIS kilifanana au kuwa juu ya kiwango cha kinga katika utafiti wa KEN SHE. Uwiano wa kiwango cha kinga kwa chanjo inayozuia virusi aina mbili (2-valent) ulikuwa 0.9 (95% CI 0.72 -1.14) kwa HPV 16 na 1.02 (0.78 – 1.33) kwa HPV 18. Uwiano wa kiwango cha kinga kwa chanjo inayozuia virusi aina tisa (9-valent) ulikuwa 1.44 (95% CI 1.14 – 1.82) kwa

HPV 16 na 1.47 (1.13 -1.90) kwa HPV 18. Utengenezaji wa kinga na kiwango cha kinga dhidi ya HPV 16 na HPV 18 katika utafiti wa DoRIS ikilinganishwa na ule wa KEN SHE haukuwa hafifu na ulifikia kiwango kilichowekwa kwa chanjo zote.

Tafsiri ya matokeo ya utafiti huu

Mwitikio wa kinga dhidi ya virusi vya HPV16 na HPV18 kwa wasichana wadogo miezi 24 baada ya dozi moja ya chanjo inayozuia virusi aina mbili au tisa ilikuwa sawa na ule uliopatikana kwa vijana wa kike waliopangwa kupata dozi moja ya chanjo ile ile kwa njia ya bahati nasibu na kuonyesha ufanisi. Dozi moja ya chanjo ya HPV ikitolewa kwa wasichana walio na umri uliolengwa katika utoaji wa chanjo, inasababisha mwitikio wa kinga ambao unaweza kuwa na uwezo wa kuzuia maambukizi ya HPV16 na HPV 18 yasiyoondolewa na kingamwili.

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Supplementary appendix 2

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Baisley K, Kemp TJ, Mugo NR, et al. Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose in young women aged 15–20 years in Kenya (KEN SHE): an immunobridging analysis of randomised controlled trials. *Lancet Glob Health* 2024; **12:** e491–99.

Supplementary appendix

Table of Contents

Methods

DoRIS trial

The DoRIS trial [Dose Reduction Immunobridging and Safety Study of two HPV vaccines in Tanzanian Girls; clinicaltrials.gov: NCT02834637] is the first randomised trial to evaluate immunogenicity of the single dose regimen in girls in the target age range for vaccination. The trial commenced enrolment in February 2017 and has been described in detail previously.[1] In brief, it is an ongoing unblinded, individually-randomised controlled trial comparing 1, 2 and 3 doses of two HPV vaccines among 930 healthy, HIV-negative Tanzanian schoolgirls aged 9-14 years. The trial is being conducted in Mwanza, in the Lake Victoria zone of north-western Tanzania. Girls were randomly allocated to one of 6 arms comprising 3 different dose schedules of the GSK 2-valent or Merck 9-valent vaccine: the originally recommended 3 dose schedule given at 0, 1 and 6 months (2-valent vaccine) or 0, 2 and 6 months (9-valent); 2 doses given 6 months apart; or a single dose. Before vaccination, girls were asked to collect a vaginal swab which was used to detect HPV DNA. Blood samples for HPV immune responses including IgG antibodies to HPV 16/18, antibody avidity and memory B cell responses were taken at baseline, Month (M) 1, M7, M12, M24 and M36. All participants were followed up to M36 for safety and immunogenicity evaluations. Girls in the 1 and 2 dose arms have been enrolled in a trial extension and will be followed up to 9 years (M108).

The trial was approved by the Tanzanian Medical Research Coordinating Committee and the ethics committee of the London School of Hygiene and Tropical Medicine. Written informed consent was obtained from parents/guardians, with written assent from participants.

The KEN SHE trial

KEN SHE (NCT03675256) is the first randomised controlled efficacy trial of a single dose of HPV vaccine. The trial enrolled 2275 sexually-active young women aged 15-20 years from 3 sites in Kenya (Thika, Nairobi and Kisumu) between December 2018 and November 2019.[2] Women were randomly allocated to one of 3 arms, comprising a single dose of the GSK 2-valent HPV vaccine (N=760), the Merck 9-valent HPV vaccine (N=758), or meningococcal vaccine (N=757). Women were seen at M3, M6 and then 6-monthly for 36 months; cervical swabs for HPV DNA testing were collected at each visit. Women enrolled in the main trial were invited to participate in the immunobridging sub-study at the time of enrolment; all

women were invited until the target enrolment of 910 participants was reached. Blood samples for immunogenicity were collected at enrolment, M1 and M24.

The trial was approved by the Kenya Medical Research Institute (KEMRI) Scientific and Ethics Review Unit (SERU) and the University of Washington (UW) Institutional Review Board (IRB).

Laboratory methods

Antibodies to HPV-16 and HPV-18 were measured by type-specific virus like particles (VLP) ELISA assay at the Frederick National Laboratory for Cancer Research HPV Immunology Laboratory in Maryland, USA. The DoRIS samples were originally tested at the Frederick laboratory in March 2021. For the immunobridging study, samples from the KEN SHE trial and a 20% simple random sample of the M24 DoRIS trial samples (30 per arm) were batched and tested together in April 2022 in the same laboratory, using the same assays and procedures as in 2021. The re-test results for the DoRIS samples were evaluated for between-run acceptability; concentrations were required to be within ±20% of the original results for concentrations >20 EU/mL, or within 25% of the original results, for concentrations ≤20 EU/mL. We required ≥80% of the re-test results to meet the acceptability criteria, or all DoRIS samples were to be retested. The acceptability criteria were pre-defined beforehand, and were based on United States Food and Drug Administration (US FDA) recommendations.[3] During ELISA testing, laboratory staff were blinded to the trial, HPV vaccine dose group and timepoint of the samples. Antibody concentrations greater than or equal to the lower limit of detection for each assay were pre-specified to indicate seropositivity (HPV16: ≥1.309 international units [IU]/mL; HPV18: ≥1.109 IU/mL).

The difference in antibody concentration between the original test and retest for the DoRIS samples was <20% in 88.3% of samples for HPV16 and 94.8% of samples for HPV18. Therefore, the retest results met the acceptability criteria and no further retesting was done. The original DoRIS results were used in the analyses.

In the DoRIS trial, HPV DNA genotyping at enrolment was done using the Anyplex II HPV28 (Seegene, South Korea), a multiplex, type-specific, real-time PCR-based detection assay, at the Catalan Institute of Oncology, Barcelona. In KEN SHE, HPV DNA genotyping was conducted using the same assay at the University of Washington East Africa STI Laboratory, Mombasa, Kenya.

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Supplementary appendix 3

This Equitable Partnership Declaration (EPD) was submitted by the authors, and we reproduce it as supplied. It has not been peer reviewed. *The Lancet*'s editorial processes have not been applied to the EPD.

Supplement to: Baisley K, Kemp TJ, Mugo NR, et al. Comparing one dose of HPV vaccine in girls aged 9–14 years in Tanzania (DoRIS) with one dose in young women aged 15–20 years in Kenya (KEN SHE): an immunobridging analysis of randomised controlled trials. *Lancet Glob Health* 2024; **12:** e491–99.

Equitable Partnership Declaration questions

This Equitable Partnership Declaration is a statement being published online alongside papers at *The Lancet Global Health*, as a separate appendix, to allow researchers to describe how their work engages with researchers, communities, and environments in the countries of study. This is part of our broader goal to decolonise global health, handing control and leadership of research to academics and clinicians who are based in the regions of study, and to affected communities.

Please answer all questions with as much detail as possible, noting that all included information will be published open-access and it will be freely available online to all who wish to read it. If a question does not apply to your study, please state "Not applicable".

The format of and questions in this statement are currently in a pilot phase. Please email Dr Liam Messin (Liam.Messin@lancet.com; deputy editor) and Dr Kate McIntosh (Kate.McIntosh@lancet.com; senior editor) with any feedback, particularly if you find any questions unclear.

Researcher considerations

1. Please detail the involvement that researchers who are based in the region(s) of study had during a) study design; b) clinical study processes, such as processing blood samples, prescribing medication, or patient recruitment; c) data interpretation; and d) manuscript preparation, commenting on all aspects. If they were not involved in any of these aspects, please explain why.

   This question is intended for international partnerships; if all your authors are based in the area of study, this question is not applicable.

   This should include a thorough description of their leadership role(s) in the study. Are local researchers named in the author list or the acknowledgements, or are they not mentioned at all (and, if not, why)? Please also describe the involvement of early career researchers based in the location of the study. Some of this information might be repeated from the Contributors section in the manuscript. Note: we adhere to ICMJE authorship criteria when deciding who should be named on a paper.

a) Study design: The DoRIS and KEN SHE trial investigators are committed to fostering collaborative research, and have built on successful long-term research partnerships between key institutions in Tanzania and Kenya, and institutions in the Global North. Researchers based in Tanzania (DoRIS) and Kenya (KEN SHE) were involved in the two trials from their inception as joint/site Principal Investigators or co-Investigators – including securing of funding, study design and protocol development. These individuals are all listed as authors on the paper. 11 of the 19 authors are based in East Africa. Of the 8 joint/site Principal Investigators for the two trials, 6 are based in East Africa.
b) Clinical study processes: The clinical processes for both trials were conducted and overseen by researchers in Tanzania and Kenya, including participant recruitment, questionnaire administration and clinical examinations at follow-up visits, and processing of blood samples. Although the specialist VLP ELISA assays for the

immunobridging analysis were conducted in the United States, many of the other laboratory tests were done in Tanzania or Kenya, include HPV genotyping for KEN SHE. The researchers overseeing the clinical processes are included as authors on the paper.
c) Data interpretation: Researchers based in Tanzania and Kenya had a key role in data interpretation.
d) Manuscript preparation: The author who prepared the first draft of the manuscript was based in East Africa for many years. All authors contributed to revisions of the manuscript and approved the final submitted version, including the 11 who are based in East Africa.

2. Were the data used in your study collected by authors named on the paper, or have they been extracted from a source such as a national survey? ie, is this a secondary analysis of data that were not collected by the authors of this paper. If the authors of this paper were not involved in data collection, how were data interpreted with sufficient contextual knowledge?

   The Lancet Global Health *believe contextual understanding is crucial for informed data analysis and interpretation.*

The data were collected by authors who are named on the paper, as described in the previous section.

3. How was funding used to remunerate and enhance the skills of researchers and institutions based in the area(s) of study? And how was funding used to improve research infrastructure in the area of study?

   Potentially effective investments into long-term skills and opportunities within institutions could include training or mentorship in analytical techniques and manuscript writing, opportunities to lead all or specific aspects of the study, financial remuneration rather than requiring volunteers, and other professional development and educational opportunities.

   Improvements to research infrastructure could be funding of extended trial designs (such as platform trials) and use of master protocols to enable these designs, establishment of long-term contracts for research staff, building research facilities, and local control of funding allocation.

Skills: Research capacity strengthening was built into the funding applications for both trials. DoRIS and KEN SHE study staff received training in all aspects of clinical trials, including but not limited to GCP, clinical trial management, protocol training, informed consent processes, SOP writing, reporting SAEs, sample collection and processing, quality assurance, maintenance of an

investigator site file, GCLP, immunology training, dealing with data queries, and community liaison activities. Clinical and laboratory staff have been mentored and supported to lead aspects of the trials, and to attend and present at scientific conferences. Local investigators have also completed formal training such as Masters in Public Health (MPH). The DoRIS trial has been extended to follow participants for 9 years and we have appointed one of the clinical team members to the post of trial manager for this trial extension.

Research infrastructure:

The Mwanza Intervention Trials Unit (MITU) in Mwanza (where the DoRIS trial was conducted) is supported by a major UK partner institution (LSHTM) as part of a successful and equitable long-term (>30 years) collaboration with the Tanzanian National Institute for Medical Research (NIMR). The MITU research facilities were built in 2008 with funding from the UK Medical Research Council (MRC), secured through this partnership. MITU is an integral component of NIMR Mwanza Centre and administers its own funding. Through the KEN SHE funding, infrastructure for clinics, generators, and -80 C freezers were established. Also, the pharmacies strengthened their infrastructure for vaccine trials, which were deployed during the COVID-19 pandemic.

4. How did you safeguard the researchers who implemented the study?

Please describe how you guaranteed safe working conditions for study staff, including provision of appropriate personal protective equipment, protection from violence, and prevention of overworking.

Both DoRIS and KEN SHE study staff follow standard operating procedures for safe processes at work including health and safety measures in the clinics and laboratories. Oversight of procedures, including safety, was also conducted. All equipment is subject to annual servicing and checks. Staff were provided with appropriate personal protective equipment for blood draws and laboratory processes and this was expanded during the COVID-19 pandemic to include masks, etc. Training has been given on sensitive issues such as sexual harassment in the workplace. The DoRIS trial clinic site is located in a secure compound owned by the National Institute for Medical Research in Mwanza. Premises have local security staff on duty. Hours of work are logged into the register at MITU which is monitored by HR staff and the key line manager. Staff whose duties covered weekends and public holidays are given time off in lieu or paid overtime. For KEN SHE, KEMRI guidelines were closely followed at all times, including during the COVID-19 lockdowns.

<u>Benefits to the communities and regions of study</u>

5. How does the study address the research and policy priorities of its location?

How were the local priorities determined and then used to inform the research question? Who decided which priorities to take forward? Which elements of the study address those priorities?

East Africa has one of the highest rates of cervical cancer incidence and mortality in the world. HPV vaccine scale-up has the potential to dramatically decrease cervical cancer cases in the long-term. However, at the time that the two trials were planned, neither Tanzania nor Kenya had a

national HPV vaccination programme, in part because of the high cost and logistical complexities of delivering a multi-dose schedule. The DoRIS trial followed previous work on HPV vaccine led by MITU which helped inform early Ministry of Health (MoH) policies on HPV vaccine introduction. The DoRIS and KEN SHE trials were designed in consultation with the MoH of both countries, as they were developing their national cervical cancer control strategies and discussing how HPV vaccination might be included. The results of both trials are critical for each country's decision-making regarding their HPV vaccine programmes. The Tanzanian and Kenyan MoH were kept informed and involved during all stages of the trials to ensure that the results that they needed to inform national policies and recommendations were available. The study results have directly informed policy in both countries (and beyond).

6. How will research products be shared in the community of study?

For instance, will you be providing written or oral layperson summaries for non-academic information sharing? Will study data be made available to institutions in the region(s) of study? The Lancet Global Health *encourages authors to translate the summary (abstract) into relevant languages after paper editing; do you intend to translate your summary?*

The investigators of both trials are fully committed to sharing and dissemination of study results. We believe that community and stakeholder consultations are important at every stage of a research study, in order to build and sustain trust in research and research partnerships.
We worked closely with community stakeholders throughout the trials. A community advisory board (CAB) was established, with local leaders, trial participants, parents/guardians, health workers, teachers and other community members invited to join. Regular community and stakeholder meetings were held during the trials, to disseminate findings and information on study progress. Extensive dissemination of the DoRIS results was done with schools and communities in 2023. The study results have been presented at local and national meetings, and the abstract of the manuscript will be translated into KiSwahili.

7. How were individuals, communities, and environments protected from harm?

a) *How did you ensure that sensitive patient data was handled safely and respectfully? Was there any potential for stigma or discrimination against participants arising from any of the procedures or outcomes of the study?*

The investigators of both trials have extensive experience in data protection and protection of privacy of participants. Study staff were specifically trained in preserving confidentiality of trial participants, including training in Good Clinical Practice and Human Subjects Protection. All interviews were conducted in private and confidential settings. Interview and clinical data, questionnaires, laboratory, and other trial forms were identified by unique study ID numbers, with no personal identifiers to maintain participant confidentiality. Personal identifiers (name, address) were only collected for informed consent, and for tracing of participants by the study team. A linking list with study ID numbers and personal identifiers was kept at the clinic, separate from the other documents, and was only accessible to selected research team members (such as the lead clinicians).

Participants were asked questions about sexual behaviour and genital hygiene practices that may have potentially resulted in embarrassment or distress. Interviewers were experienced in sexual and reproductive health research, and received specialised training in handling these sensitive data. We also tested for HIV, so there could have been psychological trauma from learning one's HIV status. Nurses, counsellors and other relevant staff had specialised training in counselling about HIV, STIs, cervical cancer screening and treatment, stigma, and gender-based issues.

Staff who conducted the informed consent process were fully trained with specific training around discussion of key messages and questions to ensure that potential participants understood the study before agreeing to participate.

Although every effort to protect participant privacy and confidentiality was made, it was theoretically possible that social harms could have resulted owing to an individual's participation in the study (e.g. through accidental disclosure of a participant's HIV status). Both trials had plans for assessing study-related social harms and referring participants to appropriate resources as needed.

b) *Might any of the tests be experienced as invasive or culturally insensitive?*

The collection of genital swabs for HPV testing may have been considered invasive or culturally insensitive, particularly for young girls (the DoRIS trial). In the DoRIS trial (age 9-14 years), self-administered swabs were collected, with assistance of an experienced nurse, to minimise discomfort and embarrassment. This method was developed and evaluated carefully through focus group discussions with adolescent girls. We have used this method in many of our previous studies of HPV and sexual and reproductive health in this age group, and results have been published showing high acceptability of this approach. The procedure was carefully explained with verbal, diagrammatic and written information in Swahili. In the KEN SHE trial (age 15-25 years, all sexually active), swabs were collected during a pelvic examination by an experienced clinician; procedures were explained, questions answered, and the participants were given a choice to have a chaperone present for the examination.

c) *How did you determine that work was sensitive to traditions, restrictions, and considerations of all cultural and religious groups in the study population?*

Input from key stakeholders was sought to help guide the development of the study protocol, standard operating procedures, and plans for sensitisation. Before the studies began, we held focus group discussions with community members (parents, health workers, religious leaders, teachers, sexually active young women and non-sexually active women) to explore community attitudes towards HPV vaccination and other study procedures and during the study we conducted qualitative research with parents and participants that helped inform our ongoing engagement with participants.

d) *Were biowaste and radioactive waste disposed of in accordance with local laws?*

Yes

e) *Were any structures built that would have impacted members of the community or the environment (such as handwashing facilities in a public space)? If so, how did you ensure that you had appropriate community buy-in?*

Not applicable

f) *How might the study have impacted existing health-care resources (such as staff workloads, use of equipment that is typically employed elsewhere, or reallocation of public funds)?*

The DoRIS and KEN SHE trials did not carry out data collection in existing health care facilities. Staff were specifically employed to work on the study and the study used equipment that was in place in MITU/KEMRI or purchased specifically for the study. No public funds were reallocated for the either trial.

8. Finally, please provide the title (eg, Dr/Prof, Mr/Mrs/Ms/Mx), name, and email address of an author who can be contacted about this statement. This can be the corresponding author.

Name: Kathy Baisley **Email:** kathy.baisley@lshtm.ac.uk

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