UNIVERSITY OF RWANDA

GENOTYPIC DISTRIBUTION OF PENILE AND ANAL HIGH-RISK HUMAN PAPILLOMAVIRUS AMONG MEN WHO HAVE SEX WITH MEN IN RWANDA

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LEWIS NIYOTWAGIRA

GENOTYPIC DISTRIBUTION OF PENILE AND ANAL HIGH-RISK HUMAN PAPILLOMAVIRUS AMONG MEN WHO HAVE SEX WITH MEN IN RWANDA

BY

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In the Department of Biology, School of Science College of Science and Technology

at

The University of Rwanda

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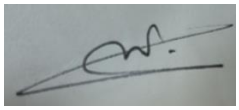
Kigali-Rwanda, 2025.

DECLARATION

I declare that this Dissertation is the result of my work and has not been submitted for any other degree at the University of Rwanda or any other Institution

Declaration on the use of the anti-plagiarism checker

Lewis NIYOTWAGIRA



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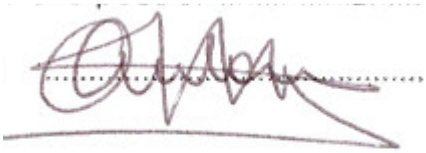

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DEDICATIONS

This dissertation is dedicated to:

- To the Almighty GOD
    - The University of Rwanda, College of Science and Technology, School of Science, Department of Biology
    - The European Union and ENABEL, a Belgian international cooperation agency, through the KWIGIRA project, which funded a Master of Science in Biotechnology at the University of Rwanda.
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ABSTRACT

This study investigated the prevalence, genotype distribution, and risk factors associated with HR-Human papillomavirus (hr-HPV) infections among men who have sex with men (MSM) in Rwanda. A cross-sectional study was conducted on 120 participants with a mean age of 27.2 years. Anal and penile swab samples were collected and genotyped to detect HPV infections and determine genotype diversity. Data Analysis was performed using STATA software. The overall prevalence of hr-HPV was 28.3% in anal samples and 39.2% in penile samples. High-risk genotypes, particularly HPV-16, predominated in both sites, while multiple genotype co-infections were observed in a significant proportion of participants. HIV status was identified as a critical factor influencing hr-HPV infection patterns. HIV-positive participants exhibited significantly higher prevalence rates of penile hr-HPV infection and multiple genotype co-infections compared to HIV-negative individuals. Although no significant difference was observed between anal and penile hr-HPV prevalence, both sites were confirmed as important reservoirs for hr-HPV. Importantly, all detected genotypes were high-risk types, underscoring the oncogenic potential of circulating hr-HPV strains in this population.

Contents

LIST OF ABBREVIATION

HPV: Human Papilloma Virus

MSM: Men who have Sex with Men

LR: Low Risk

HR: High Risk

AIN: anal intraepithelial neoplasia

STIs: Sexually Transmitted Infections

HrHPV: High Risk Human Papillomavirus Virus

ART: Anti-retroviral Therapy

WHO: World Health Organization

PCR: Polymerase Chain Reaction

SPSS: Statistical Package for the Social Sciences**.**

LIST OF TABLES

CHAPTER 1: INTRODUCTION OF THE STUDY

1.1. INTRODUCTION

Human papillomavirus (HPV) is among the most prevalent sexually transmitted infections globally, with over 200 different genotypes discovered so far (1). Among these About 40 genotypes target the anogenital region, and they are generally divided into low-risk and high-risk categories according to their cancer-causing ability. High-risk HPV strains, like HPV-16 and HPV-18, are closely linked to the onset of anogenital malignancies, including cancers of the anus, penis, cervix, and oropharynx. In contrast, low-risk strains, such as HPV-6 and HPV-11, usually lead to non-cancerous growth like genital warts (2).

The impact of HPV infection is unevenly greater in specific groups, such as men who have sex with men (MSM), because of behavioral, immune system, and societal influences (3). Men who have sex with men (MSM) exhibit a notably greater rate of anal HPV infection than heterosexual men, which is linked to a higher likelihood of developing anal intraepithelial neoplasia and anal cancer (4). HPV infection of the penis also plays a role in the development of penile intraepithelial neoplasia and invasive penile carcinoma, although in previous studies, it exhibited less frequently than anal cancer (5). In addition, men who have sex with men (MSM) face a higher likelihood of acquiring HIV, which works in combination to heighten vulnerability to persistent HPV infection and its advancement to cancerous conditions (6).

1.2. HPV infection and genotypic distribution

The genetic variation of HPV differs by region and across various population groups, affecting the patterns of HPV-associated illnesses and the success of immunization efforts(7). Worldwide, HPV-16 is the most common high-risk strain identified in anal and penile infections among men who have sex with men (MSM); however, other types like HPV-18, HPV-31, HPV-33, and HPV-45 also contribute significantly (8). Identifying the particular genotype patterns within a specific population is essential for customizing prevention measures, such as choosing appropriate vaccines and designing effective screening initiatives (9).

In Rwanda and across sub-Saharan Africa, data regarding the distribution of HPV genotypes among MSM remains limited. This growing recognition of at-risk key populations highlights a lack of detailed information on the occurrence of anal and penile hr-HPV infections in MSM

(10). This knowledge gap poses a challenge to implementing effective public health strategies intended to lower HPV-related illness and death rates within this vulnerable population.

1.3. Significance of Studying Anal and Penile HPV in MSM in Rwanda

In Rwanda, MSM encounter major obstacles in accessing healthcare services, largely due to societal stigma, prejudice, and restrictive legal frameworks (11). This leads to insufficient diagnosis and inadequate treatment of HPV and other sexually transmitted infections, thereby sustaining their spread and contributing to negative health consequences (12). Recent research has revealed a high rate of HIV among Rwandan MSM, suggesting a possible co-occurrence with hr-HPV infections that may elevate the risk of developing cancer (13).

Furthermore, with the rollout of HPV vaccines that target multiple high-risk strains, determining the prevalent HPV types within the MSM population is crucial for assessing vaccine reach and effectiveness (14).

Existing vaccines mainly focus on HPV-16, HPV-18, and several other cancer-causing strains; however, the circulation of HPV genotypes not included in the vaccine may influence its overall effectiveness (15). Hence, monitoring HPV genotypes in anal and penile regions will generate valuable data to inform vaccination strategies and screening programs specifically designed for MSM in Rwanda.

1.4. Study rationale and objectives

Considering the scarcity of data on hr-HPV occurrence and genotype variation among MSM in Rwanda, this study seeks to address key knowledge gaps by assessing the prevalence, genotype patterns, and co-infection rates of anal and penile HPV infections. The findings will support public health officials in developing focused HPV prevention and management initiatives for MSM, a group facing an elevated risk of hr-HPV-associated cancers.

1.5. Problem statement

Human papillomavirus (HPV) continues to be one of the most widespread sexually transmitted infections worldwide, with over 200 genotypes identified to date (16). Several of these types, particularly the high-risk strains such as HPV-16 and HPV-18, are closely linked to the development of cancers affecting the anogenital and oropharyngeal regions (17). MSM are at heightened risk of HPV infection due to a combination of behavioral practices, immune-related vulnerabilities, and structural health disparities (18). Global research has consistently reported high prevalence rates of anal and penile HPV infections in MSM, especially among those also living with HIV (13).

Despite growing global recognition of HPV's burden in male populations, public health surveillance and prevention initiatives have historically concentrated on women, particularly in low- and middle-income nations (19). In sub-Saharan Africa, and Rwanda specifically, HPV research and intervention programs have primarily focused on cervical cancer prevention through female-targeted vaccination and screening (20). MSM, however, remain underrepresented in HPV studies and prevention strategies, largely due to persistent social stigma, legal exclusion, and insufficient epidemiological data (9).

At present, there is a major gap in knowledge regarding HPV infection patterns among MSM in Rwanda (20). There is virtually no published data on the prevalence of anal and penile HPV, the range of circulating genotypes in this group, or the extent of multiple genotype co-infections (20). This lack of context-specific evidence limits the capacity of health authorities and policy designers to craft inclusive and evidence-based HPV prevention strategies, such as expanding vaccination to male populations or implementing targeted screening among MSM (21).

Gaining insight into the genotypic profiles of HPV in both anal and penile areas is essential, as these anatomical sites may harbor different HPV types or combinations, influencing disease progression and vaccine impact (22).

In light of these challenges, the current study aims to address a critical gap by evaluating the prevalence and genotype diversity of anal and penile HPV among MSM in Rwanda. By also exploring the occurrence of multiple genotype infections, this research will provide vital data to

support inclusive, population-specific public health initiatives and guide future HPV control efforts in high-risk male populations.

1.6. Research Hypothesis

General Hypothesis(H1)

There is a high prevalence of anal and penile hr-HPV infections among men who have sex with men (MSM) in Rwanda, with a predominance of high-risk genotypes such as HPV-16 and 18 and frequent multiple hr-HPV genotype co-infections.

1.7. Research Questions

This study is guided by the following research questions:

1. What is the prevalence of anal and penile hr-HPV infections among MSM in Rwanda?
2. What are the genotypic distributions of hr-HPV in anal and penile samples collected from MSM?
3. What is the frequency of multiple hr-HPV genotype co-infections in anal and penile sites among MSM?
4. Is there a difference in HPV infection patterns between HIV-infected and un-infected MSM?

CHAPTER 2: LITERATURE REVIEW

2.1. Overview of Human papillomavirus (HPV)

Human papillomavirus (HPV) is a non-enveloped virus with a double-stranded DNA genome, classified under the Papillomaviridae family. It ranks among the most widespread sexually transmitted infections worldwide, impacting both men and women (23). Over 200 unique HPV genotypes have been recognized, with no fewer than 40 types known to infect the anogenital area and various other mucosal surfaces (24). HPV is mainly spread through sexual activity, including anal, vaginal, and oral intercourse, as well as through direct skin-to-skin contact (25).

In individuals with a healthy immune system, HPV infections are usually temporary and show no symptoms. Nevertheless, ongoing infection with specific high-risk HPV strains can result in cancerous changes and contribute to the emergence of several types of cancer, especially cervical, anal, penile, and oropharyngeal cancers (17). The progression of HPV infection typically begins with the virus entering the basal epithelial cells, followed by episomal replication, integration into the host's DNA, and, in high-risk cases, advancement toward cancer development (26).

2.2. HPV Genotypes and Oncogenic potential

HPV genotypes are grouped according to similarities in their DNA sequences and their potential to cause cancer. They are generally divided into low-risk (LR) and high-risk (HR) categories. Low-risk types, like HPV-6 and HPV-11, are mainly linked to non-cancerous growth such as genital warts and recurrent respiratory papillomatosis (27). On the other hand, high-risk genotypes, especially HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45 are closely linked to intraepithelial lesions and aggressive forms of cancer (28). HPV-16 is the most cancer-causing genotype, responsible for around 50% of all cervical cancer cases and a notable share of anal and penile cancers (9). The insertion of high-risk HPV DNA into the host genome interferes with control genes like E2, leading to elevated expression of viral oncogenes E6 and E7, which deactivate the tumor-suppressor proteins p53 and Rb (15). This cancer-promoting process is the basis for the development of malignancy seen in long-lasting high-risk HPV infections.

2.3. Epidemiology of HPV in Men

While the impact of HPV-related diseases in women has been extensively researched, the patterns of HPV infection in men have been comparatively underexplored. Research indicates that the rate of genital HPV infection in men is similar to, or even exceeds, that observed in

women (29). In contrast to cervical cancer screening available for women, there is no standard HPV screening protocol for men, which leads to the condition being underdiagnosed and its impact underestimated (16). The prevalence of HPV in men differs based on anatomical location, sexual practices, age, circumcision status, and immune system health. The penis, scrotum, perineal area, and anal canal are all susceptible areas for HPV infection (28). Among heterosexual men, HPV infection most frequently affects the penis, whereas in men who have sex with men (MSM), anal HPV infection is more widespread, primarily due to engagement in receptive anal sex (30).

Men harboring HPV can serve as silent carriers, passing the virus to their partners without showing symptoms. Infection of the penis with HPV may result in penile intraepithelial neoplasia (PIN) and invasive penile cancer, whereas anal HPV infection has been associated with the onset of anal intraepithelial neoplasia (AIN) and anal malignancy (31).

2.4. HPV Infection among Men who have sex with Men (MSM)

Men who have sex with men (MSM) constitute a high-risk group for HPV infection because of both behavioral and biological determinants. Numerous studies have documented a markedly greater occurrence of anal HPV among MSM in comparison to heterosexual males (24). Engaging in receptive anal sex, having multiple sexual partners, initiating sexual activity at an early age, and co-existing sexually transmitted infections (STIs) are major contributing risk factors (32).

A global meta-analysis conducted in China estimated that the combined prevalence of anal HPV infection is approximately 57% among HIV-negative MSM, rising to as high as 92% in HIV-positive MSM (4). HPV infection is also recognized as a contributing risk factor in the onset of penile cancer. Recent meta-analyses indicate that HPV accounts for over 75% of penile intraepithelial neoplasia cases and more than half of penile cancer incidences, with HPV-16 being the most frequently detected type in these malignancies (22).

The rate and genotype patterns of HPV among MSM populations vary across regions, with research from Europe, Asia, North America, and Latin America reporting different degrees of exposure to both high-risk and low-risk strains (25). Nevertheless, information from sub-Saharan Africa, Rwanda included, is still limited, which hampers regional insight and the development of focused interventions (13).

2.5. Anal and Penile HPV Infections: Prevalence and Risk factors

Among MSM, anal HPV infection is powerfully linked to an elevated risk of developing anal intraepithelial neoplasia (AIN) and anal cancer, especially when high-risk genotypes are present and in cases of HIV-related immune suppression (19). Factors that increase the possibility of anal HPV infection include early initiation of sexual activity, having several sexual partners, engaging in unprotected receptive anal sex, and the presence of other sexually transmitted infections (STIs) (33). HIV infection further elevates the risk of ongoing HPV infection and the advancement to severe precancerous lesions (34).

Although penile HPV infection has been moderately under-researched, it presents considerable risks for developing penile intraepithelial neoplasia and invasive penile carcinoma (35).

Comparable risk factors such as being uncircumcised, tobacco use, having frequent sexual partners, and inadequate genital hygiene have been linked to increased HPV prevalence in the penile region (35,36). Simultaneous infection with multiple HPV genotypes at a single anatomical location has been commonly observed, and these co-infections are believed to elevate the risk of chronic infection and cancer development (37).

Studies have demonstrated that MSM frequently experience simultaneous anal and penile HPV infections, highlighting the importance of targeted screening and genotype identification by anatomical site (38).

A recent study in Rwanda found that the prevalence of high-risk HPV (hrHPV) was greater in the penile region than the anal region among both MSM living with HIV and HIV-negative MSM, with significantly higher rates observed in MSM living with HIV. Across Africa, anal and penile hrHPV prevalence likely differs by location, influenced by factors such as HIV status and sexual behavior (13).

2.6. HPV Genotypic Distribution in MSM population Worldwide

The pattern of HPV genotypes among MSM differs widely depending on geographic location, demographic factors, and HIV status. Worldwide, HPV-16 remains the most frequently detected high-risk genotype in MSM, followed by HPV-18, HPV-31, HPV-33, and HPV-52 (39). These high-risk strains are involved in the majority of HPV-related cancers among MSM, especially anal cancer.

A major multicenter study conducted in the United States identified HPV-16 in more than 25% of anal specimens from HIV-positive MSM and in 15% of samples from HIV-negative MSM, Similarly, research from Europe and Latin America has shown high prevalence of HPV-16 and HPV-18, along with significant detection of HPV-52, HPV-58, and HPV-66, reflecting regional differences in genotype distribution (17,40).

Low-risk types, especially HPV-6 and HPV-11, are frequently identified as well, often co-occurring with high-risk genotypes and HIV-positive MSM generally exhibit greater genotype variation, likely as a result of weakened immune function, which promotes persistent infections and a higher vulnerability to multiple HPV strains (18).

Comprehending the distribution of HPV genotypes is essential for shaping effective screening and immunization strategies. For example, the bivalent and quadrivalent HPV vaccines mainly protect against HPV-16 and -18 (with the quadrivalent also covering HPV-6 and -11), whereas the nonavalent vaccine offers wider protection by including five more high-risk types: HPV-31,33, -45, -52, and 58 (41). Awareness of genotype distribution within local MSM groups is therefore essential for enhancing the effectiveness of public health efforts.

2.7. HPV Co-infection and Multiple Genotypes

Simultaneous infection with several HPV genotypes is frequently observed in MSM, especially in anal specimens. The rate of multiple infections varies from 30% to more than 70%, depending on HIV status and geographic influences as well as participation in sex group (28). These mixed infections commonly involve both high-risk and low-risk HPV types, and their medical importance stems from their link to a heightened chance of long-term infection (21,42).

From a mechanistic perspective, concurrent infections may weaken localized immune defenses and support sustained viral replication but don’t enhance precancerous lesions (21). Research has shown that individuals harboring multiple HPV infections are at a greater risk of developing high-grade squamous intraepithelial lesions (HSIL) compared to those infected with only one genotype (43).

Although co-infections are also found in penile specimens, they generally occur less often than in anal samples. However, their occurrence remains significant, particularly among groups engaged in high-risk sexual practices (28). Co-infections can potentially influence the rate at

which the virus is cleared and may diminish the effectiveness of vaccines, especially when non-vaccine genotypes are predominant (44).

Considering the possibility of combined cancer-causing effects, assessing the occurrence and distribution of HPV co-infections at both anal and penile locations is a vital aspect of thorough HPV monitoring.

2.8. HPV and HIV Co-infection in MSM

HIV infection significantly impacts the course of HPV by weakening the immune system, heightening vulnerability to HPV infection, slowing down viral elimination, and promoting long-term persistence and advancement to precancerous conditions (45). Multiple studies have established that HIV-positive MSM exhibit greater prevalence, wider genotype diversity, and increased persistence of anal and penile HPV infections compared to their HIV-negative peers (6,22). Among HIV-positive individuals, the occurrence of high-risk HPV strains such as HPV-16 and HPV-18 is markedly higher. This group also faces a substantially increased likelihood of developing high-grade anal intraepithelial neoplasia and anal cancer, with some research indicating up to a 30-fold greater risk compared to the general population (46).

Although antiretroviral therapy (ART) helps to partially restore immune function, it does not completely remove the risk of HPV-associated complications. Ongoing HPV infections and high-grade squamous intraepithelial lesions (HSIL) can still develop even with effective viral suppression (47). Therefore, HIV-positive MSM need tailored screening and immunization approaches, such as anal cytology and high-resolution anoscope when available (48).

Dual infection with HIV and HPV presents significant public health challenges, as each virus may enhance the transmission of the other. For example, mucosal injury caused by HPV lesions can heighten the risk of acquiring HIV, while immune suppression from HIV infection accelerates the progression of HPV (13). These interactions highlight the importance of coordinated strategies for managing both HIV and HPV among MSM populations.

2.9. HPV in sub-Saharan Africa and Rwanda current knowledge

Although the worldwide patterns of HPV among MSM have been extensively investigated, data from sub-Saharan Africa, especially regarding MSM communities, remains scarce. The majority of studies in the region have concentrated on cervical HPV in women, resulting in a lack of insight into the impact of HPV among male populations (28).

Although existing research is limited, it indicates a significant burden of HPV infection accompanied by considerable genotype variation. In Cape Town, MSM exhibited a high occurrence of both low-risk and high-risk HPV strains, with multiple genotype infections frequently observed (28). In Bamako, Mali, close to 90% of HIV-positive MSM were found to be infected with high-risk HPV types, with many individuals harboring multiple concurrent infections (18).

In Rwanda, most HPV-related research has primarily focused on preventing cervical cancer and rolling out HPV vaccination programs for adolescent girls, which results to a significant gap in data concerning anal and penile HPV infections among MSM, a highly-risk population (10,13).

A study focusing on HIV in MSM in Kigali revealed elevated HIV rates and behavioral risk factors that also increase susceptibility to HPV, indicating a probable overlap between the two infections (13).

This lack of HPV genotypic surveillance in Rwandan MSM hinders the development of evidence-based interventions, including vaccination campaigns, STI screening, and cancer prevention programs. Therefore, localized research on HPV genotypic distribution in MSM is urgently needed to inform national public health strategies.

2.10. Vaccination and Prevention strategies for HPV in MSM

Vaccination against HPV is the most efficient method of primary prevention for HPV-associated illnesses. The World Health Organization (WHO) advises immunizing all adolescents, with particular attention to including males and high-risk groups like men who have sex with men (MSM) (49). There are currently three primary types of HPV vaccines: the bivalent (targeting HPV-16 and 18), the quadrivalent (covering HPV-6, -11, -16, and -18), and the nonavalent (protecting against HPV-6, -11, -16, -18, -31, -33, -45, -52, and -58) formulations (31).

Clinical studies have shown that HPV vaccines are highly effective in preventing genital warts, anal intraepithelial neoplasia (AIN), and high-grade squamous intraepithelial lesions (HSIL) in males, especially when given before initial exposure to the virus (15). HPV vaccination effectively reduces anal HPV infections and related lesions in MSM, especially those who are HIV-negative. However, vaccine uptake remains low in many areas due to barriers like stigma, limited awareness, high costs, and poor access (50).

Since 2011, Rwanda's national HPV immunization initiatives have focused on girls aged 12 to 14, attaining high levels of coverage (20). However, males and MSM have not been part of the standard vaccination program. Considering the significant impact of HPV in MSM and the proven effectiveness of vaccinating females, extending vaccine access to include MSM could be a cost-efficient approach to lowering the burden of HPV-related illnesses.

Other preventive measures involve comprehensive sexual health education, encouraging condom use, and routine screening for anal and penile abnormalities among high-risk MSM. Combining STI services with HPV testing and counseling could enhance early identification and treatment of HPV-related health issues.

2.11. Research gaps and rationale for the current study

Despite growing global recognition of the HPV burden among MSM, significant knowledge gaps persist, particularly in low- and middle-income countries like Rwanda. There is a lack of data on:

- The prevalence of anal and penile HPV infection among MSM in Rwanda.
- The genotypic distribution of HPV strains in this population.
- The frequency and pattern of multiple HPV genotype co-infections.
- The interaction between HPV and HIV among Rwandan MSM.

These gaps hinder effective public health planning and policy formulation. Without local epidemiological data, vaccine programs, screening guidelines, and educational initiatives cannot be adequately tailored to the needs of MSM.

The current study addresses these gaps by systematically analyzing anal and penile HPV infections in MSM in Rwanda. By determining prevalence, identifying genotypes, and assessing co-infection patterns, this study will provide evidence to support the inclusion of MSM in HPV control strategies and inform future research and healthcare delivery.

2.12. OBJECTIVES

2.12.1. General Objective:

To determine the genotypic distribution of penile and anal hr-HPV infections among MSM in Rwanda.

2.12.2. Specific Objectives:

- To determine the prevalence and Genotypic distribution of hr-HPV infection in penile and anal sites among MSM in Rwanda.
- To assess the frequency of multiple hr-HPV genotype co-infections among MSM.
- To compare the genotypic distribution of hr-HPV infections between HIV-infected and un-infected MSM.

CHAPTER 3: METHODOLOGY

3.1. Study Design

This study employed a cross-sectional, laboratory-based design to determine the prevalence and genotypic distribution of human papillomavirus (HPV) infections in the penile and anal regions among men who have sex with men (MSM) in Rwanda.

3.2. Study Area

The research was conducted in Kigali, Rwanda, primarily recruiting participants from healthcare centers, community-based organizations, and sexual health clinics that serve the MSM population.

3.3. Study Population

The targeted population consists of biologically male individuals aged 18 years and above who self-identify as men who have sex with men (MSM) and are residing in Kigali.

3.4. Inclusion Criteria

MSM aged ≥18 years

Willing to provide informed consent

Willing to undergo penile and anal sample collection

Available for data collection and lab testing procedures

3.5. Exclusion Criteria

Presence of severe genital or anal pathology interfering with sample collection

Refusal to participate or withdraws consent

3.6. Sample Size Determination

The sample size for this study was calculated to estimate the prevalence of anal and penile hr-HPV infection among MSM in Rwanda with an acceptable margin of error and confidence level.

The sample size (n) was calculated using the following formula for estimating a proportion in a population:

$$n = z^2 \times p \times (1-p) / d^2$$

Where:

- n = required sample size
- Z = Z-score corresponding to the desired confidence level (1.96 for 95% confidence)
- p = estimated prevalence of HPV infection among MSM (from previous studies or pilot data)
- d = margin of error (precision), typically set at 5% or 0.05

Assuming an estimated prevalence (p) of 10% based on regional HPV prevalence studies among MSM, a 95% confidence level (Z=1.96Z = 1.96Z=1.96), and a margin of error (d) of 5%, the calculation is as follows:

$n = (1.96^2) \times 0.10 \times (1-0.10) / (0.05)^2 = 3.8416 \times 0.10 \times 0.90 / 0.0025 = 0.3457 / 0.0025 = 138.3$

To account for non-response or unusable samples, a 10% contingency is added:

$N \text{ adjusted} = n / 1 - 0.10 = 138.3 / 0.90 = 153.7 \approx 154n$

However, due to logistical and resource constraints, a sample size of **120 participants** was selected for this study as a feasible target, balancing statistical power and available

3.7. Sampling Technique

Participants were recruited through purposive and snowball sampling methods via trusted community-based organizations working with key populations. This ensures privacy and increased participant trust.

3.8. Data Collection Tools and Procedures

3.8.1. Questionnaire

A structured interviewer-administered questionnaire was used to collect data on:

Demographics (age and residence)

Medical history (HIV status)

3.8.2. Biological Sample Collection

Anal Swab: A sterile Dacron swab was inserted ~3–5 cm into the anal canal and rotated gently.

Penile Swab: Another sterile swab was used to collect epithelial cells from the penile shaft and glans.

Samples were placed in PreservCyt medium and stored at 2–8°C, then transported to the molecular biology laboratory for analysis.

3.9. Laboratory Analysis

HPV genotyping was carried out using the AmpFire® HPV Genotyping Detection a fast, real-time assay based on isothermal amplification technology that enables simultaneous detection and identification of 14 high-risk genotypes and HPV-53 directly from clinical specimens. The test targets the following types: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, -68, and 53.

To begin HPV genotyping, 1X Lysis Buffer was prepared according to standard protocol and can be stored at room temperature for up to six months. Each cervical cell sample was mixed briefly, and 1 mL transferred to a 1.5 mL tube, then centrifuged at maximum speed for 10 minutes. After discarding the supernatant without disturbing the pellet, 100 µL of 1X Lysis Buffer was added, and the pellet was resuspended by vortexing. The entire content transferred to a PCR tube, sealed, and incubated at 95°C for 20 minutes, followed by cooling and brief centrifugation. Four Master Mixes were then prepared using specific Primer Mixes. Each Master Mix (20 µL) was dispensed into N+2 tubes (or a 96-well PCR plate), and 5 µL of each sample were added into corresponding tubes for genotyping. Negative and positive controls were included similarly. After mixing and centrifuging, the tubes or plate were loaded into a real-time PCR machine for amplification. Following the run, all reaction tubes or plates were carefully removed and discarded as hazardous waste.

3.10. Data Management and Analysis

3.10.1. Data Entry and Cleaning

All data were coded using unique Participant IDs and entered into Microsoft Excel and then exported to SPSS for analysis.

3.10.2. Statistical Analysis

Descriptive Statistics with STATA: Frequency and percentages of HPV infection, genotype types, and anatomical sites.

CHAPTER 4: RESULTS

4.1. Participant Characteristics

4.1.1. Demographic Characteristics of the Study Population

A total of 120 participants were included in the study. The mean age was 27.2 years with a standard deviation of 5.17 years, indicating a relatively young population. All participants were male.

Table 1: DATA COLLECTION TOOL

Characteristic	*Value*
Sample Size	120
Age (Mean ± SD)	27.2 ± 5.17
Sex (Male)	120 (100%)

4.1.2. HIV Status

Out of the 120 participants, 83.3% (n=100) were HIV-negative while 16.7% (n=20) were HIV-positive.

Table 2: Descriptive Characteristics of Study Participants:

Variable	Frequency	Percentage (%)
HPV Results (Penile Swab)		
- Negative	62	68.1%
- Positive	29	31.9%
HPV Results (Anal Swab)		
- Negative	66	72.5%
- Positive	25	27.5%
HIV Test Results		
- Negative	59	64.8%
- Positive	32	35.2%
Age Group		
- 20–34 years	35	38.5%
- 35–44 years	34	37.4%
- 45 years and above	22	24.2%
Sex		

- Male	91	100%

Table 3 HIV Status Distribution

HIV Status	Frequency	Percentage
Negative	100	83.3%
Positive	20	16.7%

The study enrolled 120 MSM with a mean age of 27.2 years (SD 5.2). Majority (51.7%) originated from Kigali City. All participants were biologically male.

Table 4: Residence of the participants

Birth City	Frequency (n)	Percentage (%)
Kigali City	62	51.7
Southern	18	15.0
Western	14	11.7
Northern	10	8.3
Eastern	7	5.8
Bujumbura	4	3.3
Other	5	4.2

4.2. hr-HPV Prevalence

The prevalence of hr-HPV infection differed significantly by HIV status at both anal and penile sites. Overall, hr-HPV was detected in 28.3% of participants at the anal site and 39.2% at the penile site. Among HIV-negative individuals, the anal hr-HPV prevalence was 25.0%, compared to a notably higher 45.0% in HIV-positive individuals. Similarly, at the penile site, hr-HPV prevalence was 34.0% in HIV-negative participants but rose sharply to 65.0% among those who were HIV-positive. These differences were statistically significant ($p < 0.05$), indicating that HIV-positive status is associated with a higher likelihood of hr-HPV infection at both anatomical sites.

Table 5: hr-HPV Prevalence by Site and HIV Status

Site	HIV Status	HPV Positive (n)	Total (n)	Prevalence (%)	95% CI
Anal	Overall	34	120	28.3	21.0 – 37.0
Anal	Negative	25	100	25.0	18.0 – 34.0
Anal	Positive	9	20	45.0	26.0 – 66.0
Penile	Overall	47	120	39.2	31.0 – 48.0
Penile	Negative	34	100	34.0	25.0 – 44.0
Penile	Positive	13	20	65.0	43.0 – 82.0

HIV-positive participants had significantly higher HPV prevalence at both sites ($p<0.05$). **Prevalence and Distribution of hr-HPV Infections in Anal and Penile Samples by HIV Status shows that Anal HPV Infection**: Out of 120 participants, 34 (28.3%) tested positive and Penile HPV Infection: 47 (39.2%) tested positive.

4.3. Distribution of Total Number of hr-HPV Genotypes Detected

The number of HPV genotypes detected in each individual ranged from 0 to 5 or more.

The total number of HPV genotypes detected per individual varied from zero to five or more. Notably, 40.8% of the participants had no detectable HPV genotypes, indicating a large proportion without infection or with undetectable viral presence. Among those with HPV, the majority had one genotype (27.5%), while smaller proportions harbored multiple genotypes: 8.3% had two genotypes, 9.2% had three, 7.5% had four, and 6.7% had five or more genotypes. This distribution highlights that while single-genotype infections are common, a significant subset of individuals are infected with multiple HPV genotypes, which may have implications for disease risk and progression.

Table 6: Genotype frequencies

Number of Genotypes	Frequency	Percentage (%)
0	49	40.83
1	33	27.50
2	10	8.33
3	11	9.17
4	9	7.50

5+	8	6.67

4.4. Genotype Distribution

4.4.1. Anal hr-HPV Genotypes

A total of 85 genotype detections were made in the anal samples. The most frequent genotype was HPV-16 (16.5%), followed by HPV-53 (9.4%) and HPV-31 (8.2%).

A total of 85 HPV genotype detections were identified from anal samples collected in the study. Among these, HPV-16 was the most prevalent genotype, accounting for 16.5% of all detections. This was followed by HPV-53 at 9.4% and HPV-31 at 8.2%, indicating these genotypes are the predominant strains circulating in the anal region of the participants.

Other genotypes detected with notable frequency included HPV-52 (7.1%) and HPV-58 (5.9%). Several genotypes, including HPV-51, HPV-66, and HPV-56, were each detected 4 times, representing 4.7% individually. A group of genotypes such as HPV-39, HPV-59, HPV-45, HPV-35, and HPV-59 appeared 3 times each (3.5%), while genotypes like HPV-18, HPV-33, HPV-35, HPV-31, HPV-68, and HPV-18 were found twice each (2.4%). Finally, some genotypes including HPV-52, HPV-33, HPV-68, HPV-53, HPV-39, and HPV-66 were detected only once, making up 1.2% each.

This distribution suggests a diverse spectrum of HPV genotypes present in the anal region, with a dominance of high-risk types such as HPV-16 and HPV-31, which are known to be associated with higher oncogenic potential.

Table 7 : Distribution of anal HPV genotypes

Genotype	**Frequency**	**Percentage (%)**
HPV-16	14	16.47
53	8	9.41
HPV-31	7	8.24
52	6	7.06
58	5	5.88
51, 66, 56	4 each	4.71 each
39, HPV-59, 45, 35, 59	3 each	3.53 each
HPV-18, 33, HPV-35, 31, 68, 18	2 each	2.35 each

HPV-52, HPV-33, HPV-68, HPV-53, HPV-39, HPV-66		1 each	1.18 each

4.4.2. Penile hr-HPV Genotypes

The penile samples showed a total of 89 genotype detections. HPV-16 was the most common (20.2%), followed by genotype 51 (9.0%) and HPV-59, HPV-39, and 53 (each about 6.7%).

A total of 89 HPV genotype detections were identified from penile samples. The most prevalent genotype was HPV-16, representing 20.2% of detections. This was followed by genotype 51 at 9.0%, and genotypes HPV-59, HPV-39, and HPV-53, each accounting for approximately 6.7% of detections. Other genotypes such as HPV-45, HPV-66, HPV-53, HPV-59, and HPV-18 were each detected 4 times (4.5%), while HPV-66, HPV-31, HPV-52, and HPV-45 appeared 3 times each (3.4%). Additionally, genotypes like HPV-35, HPV-56, and HPV-58 were detected twice each (2.3%), and a variety of genotypes including HPV-16, HPV-31, HPV-54, HPV-51, HPV-66, HPV-68, and HPV-39 were each found once (1.1%). These findings illustrate a diverse distribution of HPV genotypes in the penile region, with HPV-16 predominating, consistent with its known high oncogenic risk.

Table 8: Penile genotype distribution

Genotype	**Frequency**	**Percentage (%)**
HPV-16	18	20.22
51	8	8.99
59, HPV-39	6 each	6.74 each
HPV-45, 66, 53, HPV-59, 18	4 each	4.49 each
HPV-66, HPV-31, HPV-52, 52, 45	3 each	3.37 each
35, HPV-56, 56, 58	2 each	2.25 each
16, 31, 54, HPV-16, HPV-51, HPV-66, 68, 39	1 each	1.12 each

4.5. Association Between HIV and hr-HPV Infections

4.5.1. Anal hr-HPV Infection by HIV Status

Among the study participants, HIV-negative individuals were more frequently HPV-negative (75) compared to HPV-positive (25) at the anal site. In contrast, HIV-positive individuals had a lower number of HPV-negative cases (11) and a smaller number of HPV-positive cases (9).

However, the difference in HPV infection status between HIV-negative and HIV-positive groups was not statistically significant (Chi-square = 2.3721, p = 0.1235). This indicates that, within this sample, HIV status was not significantly associated with HPV infection presence.

Table 9: Association Between HIV Status and HPV Infection Among Study Participants

HIV Status	HPV Negative	HPV Positive
Negative	75	25
Positive	11	9

- **Chi-square = 2.3721**, **p = 0.1235** → *Not statistically significant*

4.5.2. Penile hr-HPV Infection by HIV Status

The data show that among HIV-negative participants, 66 were HPV-negative and 34 were HPV-positive at the penile site. In contrast, among HIV-positive individuals, fewer were HPV-negative (7), while a higher proportion were HPV-positive (13). The difference in penile HPV infection status between HIV-negative and HIV-positive groups was statistically significant (Chi-square = 5.4841, p = 0.0192). This indicates that HIV-positive participants had a significantly higher prevalence of penile HPV infection compared to HIV-negative individuals, suggesting an association between HIV infection and increased susceptibility to penile HPV infection

Table 10: Penile hr-HPV Infection Status by HIV Status Among Study Participants

HIV Status	HPV Negative	HPV Positive
Negative	66	34
Positive	7	13

- **Chi-square = 5.4841**, **p = 0.0192** → *Statistically significant*

4.5.3. Multiple hr-HPV Infections by HIV Status

The data reveal that among HIV-negative participants, 75 had no multiple hr-HPV infections, while 25 had multiple infections. Conversely, among HIV-positive participants, only 7 had no multiple infections, whereas 13 had multiple hr-HPV infections. The difference in the prevalence of multiple HPV infections between HIV-negative and HIV-positive groups was highly statistically significant (Chi-square = 10.5443, p = 0.0012). This indicates that HIV-positive individuals are significantly more likely to have multiple hr-HPV infections compared to HIV-negative individuals, suggesting that HIV infection may increase susceptibility to co-infection with multiple HPV genotypes.

Table 11: Distribution of Multiple HPV Infections by HIV Status Among Study Participants

HIV Status	No Multiple Infection	Multiple Infection
Negative	75	25
Positive	7	13

- **Chi-square = 10.5443**, **p = 0.0012** → *Highly significant*

4.6. Prevalence of site (Anal and Penile) hr-HPV Infections

Among the 120 samples collected, HPV infection was detected in **28.3%** of anal swab samples and **39.2%** of penile swab samples. This indicates that HPV prevalence is higher at the penile site compared to the anal site. The 95% confidence intervals 21% to 37% for anal samples and 31% to 48% for penile samples suggest that this difference is meaningful and potentially clinically significant. These findings highlight the importance of screening and prevention efforts at both anatomical sites, with particular attention to the higher burden observed in penile infections.

Table 12: Site-Specific Prevalence of hr-HPV Infections in Anal and Penile Samples

(N = 120)

Site	HPV Positive (n)	Total (n)	Prevalence (%)	95% CI
Anal	34	120	28.3%	(21%, 37%)
Penile	47	120	39.2%	(31%, 48%)

The prevalence of HPV infection was higher in penile swab samples (39.2%) compared to anal swabs (28.3%). The confidence intervals suggest that this difference is notable and clinically relevant.

4.7. Association Between HIV Status and hr-HPV Infection (Penile Swab)

The results from the table titled *"Association Between HIV Status and HPV Infection (Penile Swab)"* indicate a strong and statistically significant relationship between HIV status and the presence of penile HPV infection. Among HIV-negative participants, only 13.6% tested positive for HPV, while the majority (86.4%) were HPV-negative. In contrast, a notably higher proportion of HIV-positive individuals (65.6%) were HPV-positive, with only 34.4% testing

negative. The p-value of less than 0.001 confirms that this association is statistically significant, meaning the observed difference is unlikely to be due to random chance. These findings suggest that individuals living with HIV are at a substantially greater risk of penile HPV infection, possibly due to factors such as immune suppression or shared modes of transmission

Table 13: HIV Status vs. hr-HPV Infection (Penile Swab)

HIV Status	***HPV Positive***	***HPV Negative***	***Total***	***p-value***
Negative	8 (13.6%)	51 (86.4%)	59	< 0.001*
Positive	21 (65.6%)	11 (34.4%)	32	

A statistically significant association was observed between HIV status and HPV infection in penile swab samples. HIV-positive participants had a much higher prevalence of HPV compared to HIV-negative individuals (**p < 0.001**).

4.8. Association Between HIV Status and hr-HPV Infection (Anal Swab)

The analysis presented in the table *"HIV Status vs. HPV Infection (Anal Swab)"* reveals a statistically significant association between HIV status and the prevalence of anal HPV infection. Among HIV-negative participants, only 11.9% tested positive for HPV, while 88.1% were negative. In contrast, more than half (56.3%) of HIV-positive individuals were HPV-positive, and only 43.8% were HPV-negative. The p-value of 0.002 indicates that this difference is statistically significant, suggesting that the higher prevalence of anal HPV infection among HIV-positive individuals is unlikely to be due to chance. This finding emphasizes the increased susceptibility of HIV-positive individuals to co-infection with HPV, possibly due to compromised immune defenses or shared transmission routes.

Table 14: HIV Status vs. HPV Infection (Anal Swab)

HIV Status	**HPV Positive**	**HPV Negative**	**Total**	**p-value**
Negative	7 (11.9%)	52 (88.1%)	59	0.002*
Positive	18 (56.3%)	14 (43.8%)	32	

A significant association was also observed between HIV status and anal HPV infection (**p = 0.002**), reinforcing the vulnerability of HIV-positive individuals to HPV co-infection.

CHAPTER 5: Discussion

The present study investigated the prevalence and genotype distribution of HPV infections among MSM in Rwanda, with particular attention to differences by HIV status and anatomical site of infection. The study population consisted of 120 young adult males, with a mean age of 27.2 years, predominantly originating from Kigali City. This demographic reflects a typical urban MSM cohort that may have unique risk profiles due to sexual behaviors and access to healthcare services.

The findings demonstrated a high prevalence of hr-HPV infections, with 28.3% of participants testing positive for anal hr-HPV and 39.2% for penile hr-HPV of the mean age 27.2 years as supported the study conducted in Serbia, which also specified that HPV in male is prevalent in age 27-29 years (51). This agrees with another study conducted by Murenzi et al (13). There was no significant difference in the HPV infection between anal and penile, suggesting that both anatomical regions are important reservoirs for HPV in this population (51). Notably, our study showed that high-risk (HR) genotypes (16 and 18) were exclusively detected in all HPV-positive samples, highlighting the oncogenic potential of circulating HPV types and reinforcing the importance of targeted screening and vaccination strategies.

HIV status was found to be a critical determinant of HPV infection patterns. HIV-positive participants had significantly higher prevalence rates of HPV at both anal (56.3%) and penile (65.6%) sites compared to HIV-negative individuals. Kovacevic et al mentioned that the most prevalent HPV cases were found in HIV positive male who had sex with males (51). Moreover, multiple HPV genotype infections were markedly more common among HIV-positive individuals ($p = 0.0012$), consistent with the hypothesis that immunosuppression facilitates persistent and concurrent infections. These findings align with previous research in China demonstrating synergistic interactions between HIV and HPV, where compromised immune responses in HIV-infected individuals contribute to higher viral persistence, increased diversity of HPV genotypes, and elevated risk of HPV-associated malignancies (32).

The genotype distribution analysis revealed HPV-16 as the most prevalent type in both anal (16.5%) and penile (20.2%) samples, followed by other high-risk genotypes such as HPV-53, HPV-31, HPV-51, and HPV-59. The predominance of HPV-16 is of particular concern, given

its established role in the pathogenesis of anal, penile, and oropharyngeal cancers. The detection of multiple high-risk genotypes further underscores the potential burden of HPV-related disease in this population. It was confirmed that among HIV positive and negative, HPV-16 was the most predominant.

Interestingly, while HIV status was strongly associated with penile HPV prevalence and multiple genotype infections, the association with anal HPV prevalence did not reach statistical significance in one analysis ($p = 0.1235$). This discrepancy may be attribute ed to the small sample size of HIV-positive participants, leading to limited statistical power, or to differences in exposure patterns and local immune responses between anatomical sites. The same study was conducted in Nigeria that the prevalence of anal HR-HPV was higher among HIV-positive than HIV-negative MSM (91.1% vs. 40.6%, $P < 0.001$) which is supportive for our findings (42).

These results highlight the need for comprehensive HPV prevention strategies among MSM in Rwanda, particularly among HIV-positive individuals who bear a disproportionate burden of high-risk and multiple HPV infections. Targeted interventions may include increased HPV vaccination coverage, routine screening for anal and penile lesions, and strengthened HIV care to mitigate immunosuppression-related vulnerability. The exclusive detection of high-risk genotypes also supports prioritizing vaccines that cover a broad range of oncogenic HPV types.

Future studies should aim to include larger and more diverse MSM populations across Rwanda to validate these findings and explore regional variations. Longitudinal research is warranted to assess the persistence of HPV infections, the progression to precancerous lesions, and the impact of antiretroviral therapy on HPV natural history. Additionally, molecular studies focusing on viral load, immune markers, and interactions between multiple genotypes could provide deeper insights into the pathogenesis of HPV in HIV-positive MSM. Evaluating the effectiveness of HPV vaccination programs in this high-risk group will also be essential for informing national policy.

CHAPTER 6: Conclusion and recommendations

6.1. conclusion

This study provides critical insights into the prevalence, genotype distribution, and risk factors associated with hr-HPV infection among men MSM in Rwanda. The findings revealed a considerable burden of hr-HPV infections, with prevalence rates of 28.3% at the anal site and 39.2% at the penile site. Although statistical analysis did not show a significant difference between these anatomical sites, both were confirmed as important reservoirs for hr-HPV infection. High-oncogenic genotypes, particularly HPV16, were the most predominant across both sites, underscoring their oncogenic potential and their significance in cancer prevention strategies. The detection of multiple high-risk genotypes in a notable proportion of participants further suggests an elevated risk for HPV-related malignancies in this population.

HIV status was found to be a key determinant of hr-HPV infection patterns. HIV-positive participants had significantly higher prevalence of penile hr-HPV infections and were more likely to present with multiple hr-HPV genotypes compared to HIV-negative individuals. These findings highlight the synergistic interaction between HIV and hr-HPV, where immunosuppression in HIV-positive individuals facilitates persistent and diverse hr-HPV infections. Despite these alarming findings, hr-HPV infection among MSM in Rwanda remains largely underrecognized, with limited preventive measures and screening programs currently in place. This highlights the urgent need for targeted interventions to address this public health challenge.

6.2. Recommendations

Based on the findings of this study, several recommendations are proposed to mitigate the burden of HPV among MSM in Rwanda. First, HPV vaccination programs should be expanded to specifically include MSM, with priority given to HIV-positive individuals who are at an increased risk of persistent and multiple HPV infections. Public health awareness campaigns should be strengthened to educate both MSM and healthcare providers about HPV transmission, associated risks, and preventive measures.

Second, routine screening for anal and penile HPV-related lesions should be incorporated into HIV care services to enable early detection and timely treatment of precancerous lesions, thereby reducing the risk of progression to malignancy. Strengthening antiretroviral therapy (ART) coverage and adherence is also crucial to decrease immunosuppression and reduce susceptibility to HPV persistence.

Third, comprehensive care models that integrate HIV and HPV management should be developed to address the dual burden of these infections. In addition, further large-scale research is warranted to confirm these findings across diverse MSM populations in Rwanda and to monitor longitudinal outcomes such as HPV persistence, progression to cancer, and the influence of ART on HPV natural history. Molecular studies focusing on viral load, immune responses, and genotype interactions would provide deeper insights into co-infection dynamics.

National HPV vaccination strategies should consider vaccines that cover a broader spectrum of high-risk HPV types, given the diverse genotype distribution observed in this study. Integrating HPV prevention and control into existing sexual health policies targeting key populations is essential for reducing the long-term burden of HPV-associated diseases in Rwanda.

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