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# Prevalence and distribution of high-risk Human Papillomavirus Genotypes in Cervical cancer tissue biopsies at Bugando Medical Centre, Northwestern Tanzania

**Oscar Ottoman**

droscarmuhini1988@gmail.com

Bugando Medical Centre (BMC)

**Edrick Elias**

Bugando Medical Centre (BMC)

**Idd Shabani**

Bugando Medical Centre (BMC)

**Subira Matiku**

Bugando Medical Centre (BMC)

**Cosmas Mbulwa**

Bugando Medical Centre (BMC)

**Edwin Liwa**

Bugando Medical Centre (BMC)

**Fidelis David**

Bugando Medical Centre (BMC)

**Michael James**

Catholic University of Health and Allied Sciences (CUHAS)

**Caroline Anold Minja**

Catholic University of Health and Allied Sciences (CUHAS)

**Crispin Mukerebe**

National Institute of Medical Research

**Johnson Paul Nyansuku**

Bugando Medical Centre (BMC)

**Titus Leeyio**

Catholic University of Health and Allied Sciences (CUHAS)

**Benson R. Kidenya**

Catholic University of Health and Allied Sciences (CUHAS)

**Peter F. Rambau**

Bugando Medical Centre (BMC)

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**Prevalence and distribution of high-risk Human Papillomavirus Genotypes in Cervical cancer tissue biopsies at Bugando Medical Centre, Northwestern Tanzania**

Oscar Ottoman[1,2*], Edrick Elias[1,2], Idd Shabani[1,2], Subira Matiku[1,2], Cosmas Mbulwa[1,2], Edwin Liwa[1,2], Fidelis David[1], Michael James[3], Caroline Anold Minja[3], Crispin Mukerebe[4], Johnson Paul Nyansuku[1,5] Titus Leeyio[5] Benson R. Kidenya[3], Peter F. Rambau[1,2]

1. *Histopathology and Morbid Anatomy Department, Bugando Medical Centre (BMC), Mwanza, Tanzania.*
2. *Department of Pathology, Catholic University of Health and Allied Sciences (CUHAS), Mwanza, Tanzania.*
3. *Department of Biochemistry and Molecular Biology, Catholic University of Health and Allied Sciences (CUHAS), Mwanza, Tanzania.*
4. *Laboratory Department, National Institute of Medical Research, Mwanza campus, Mwanza, Tanzania.*
5. *Department of Biostatistics and epidemiology, School of public health, Catholic University of Health and Allied Sciences (CUHAS), Mwanza, Tanzania*

*Corresponding author: droscarmuhini1988@gmail.com

**ABSTRACT**

**Background**

Cervical cancer remains a major public health problem in sub-Saharan Africa, where the distribution of high-risk human papillomavirus (hrHPV) genotypes varies geographically. Data on hrHPV genotypes in cervical cancer tissue from northwestern Tanzania are limited. This study determined the prevalence and distribution of hrHPV genotypes in fresh cervical cancer tissue biopsies at Bugando Medical Centre, Mwanza, Tanzania.

**Methods**

We conducted a hospital-based study among 309 women with histologically confirmed cervical cancer between January 2024 and January 2025. We tested fresh cervical tissue biopsies for hrHPV DNA using the Anyplex™ II HPV28 real-time PCR assay. We analysed clinical, demographic, and histopathological characteristics using chi-square and logistic regression. Genotype-level analyses accounted for clustering of multiple detections within women.

**Results**

We detected hrHPV DNA in 277/309 (89.6%) women. Among hrHPV-positive women, 69.0% had multiple-genotype infections. Of 581 genotype detections, HPV16 was most frequent (36.7%), followed by HPV18 (21.9%), HPV45 (7.2%), HPV33 (6.5%), HPV35 (6.0%), HPV31 (5.7%) and HPV59 (5.5%). HrHPV positivity was higher in squamous cell carcinoma than adenocarcinoma (91.7% vs 83.7%; p=0.044), but histological subtype was not independently associated with hrHPV positivity. Among women with known HIV status, HIV-positive status was associated with higher odds of HPV16/18 detection compared with other hrHPV genotypes (OR 1.50, 95% CI 1.09–2.05; p=0.013).

**Keywords:** High-risk Human Papillomavirus, Cervical cancer, Genotype distribution, Clinicopathological factors

## Introduction

Cervical cancer remains one of the most common cancers and a leading cause of cancer-related death among women worldwide, with the burden concentrated in low- and middle-income countries. Persistent infection with oncogenic high-risk human papillomavirus (hrHPV) is a major etiological factor in the development of cervical cancer, with HPV DNA detected in the vast majority of invasive cervical cancer cases. (1). Globally, Sub-Saharan Africa (SSA) has the highest age-standardised incidence rates, with 67.9 and 65.5 cases per 100,000 woman-years reported in Malawi and Zambia, respectively. (2) Cervical cancer is the second most common cancer and the leading cause of cancer death among women in SSA. (3). The region's overall age-standardised incidence and mortality rates are 31.7 and 22.5 per 100,000 women, respectively, approximately three to seven times those of more developed countries and are highest in Eastern Africa (34.5 and 25.3 per 100,000, respectively) (4). The disease burden is particularly heavy in Guinea, Zambia, Tanzania, Malawi, and Mozambique, where incidence rates exceed 50 per 100,000 women. Moreover, incidence trends across SSA have risen in most registries over the past two decades, indicating an increasing rather than a declining burden. (4).

Tanzania exemplifies this burden, as cervical cancer is the most frequently diagnosed cancer among women in the country and the most common cancer among women aged 15–44 years. (5). Age-standardised incidence rates exceed 60 per 100,000 women. (6). More than 10,000 women are diagnosed with cervical cancer each year, with over 6,500 deaths. (7). The dual HIV and cervical cancer epidemics in the country further heighten this burden, as HIV infection significantly increases both the risk of HPV acquisition and the rate of progression to invasive disease. The distribution of high-risk HPV genotypes that cause cervical cancer varies geographically. In a large meta-analysis of African women, the prevalence of HPV16, HPV18, and HPV45 in invasive cervical cancer was estimated at 49.7%, 18.0%, and 10.0%, respectively. (8). Notably, HPV45 is substantially more common in African invasive cancers (11.0%) than in other regions, where it ranges from 3.0% in Eastern Asia to 6.1% in South/Central America. (9). Overall, the adjusted HPV prevalence in African invasive cervical cancers is approximately 86.5%. A multi-country study in Ghana, Nigeria, and South Africa reported HPV positivity in 90.4% of histologically confirmed cases, with HPV16 (51.2%), HPV18 (17.2%), HPV35 (8.7%), and HPV45 (7.4%) the most frequent single infections. (10).

However, the relative importance of specific genotypes varies across the continent. In the general population, among sub-Saharan African women, high-risk HPV prevalence ranges from 10.7% in Ghana to 90.8% in Côte d'Ivoire. (11)The genotypes most commonly associated with cervical cancer differ by country, with HPV16 and HPV52 in South Africa and Tanzania, HPV16 and HPV35 in Zimbabwe, and HPV53 and HPV68 in Madagascar. This variation has direct implications for prevention: vaccines targeting only HPV16 and 18 may leave a substantial fraction of cancers in the region unprotected, and genotypes such as HPV35 and HPV45 have been highlighted as targets for optimised vaccine coverage in Tanzania. (12).

In Tanzania, genotyping data are primarily from tertiary centres in the eastern and northern parts of the country. Among women with cervical cancer at the Ocean Road Cancer Institute in Dar es Salaam, a cervical lesion biopsy was taken, DNA was extracted fresh, and HPV DNA was detected in 94% of 100 cases, with HPV16 and/or HPV18 found in 85% (12). A national genotyping study of cervical carcinomas using archived formalin-fixed, paraffin-embedded tissue blocks similarly

found high-risk HPV in 81.1% of tumours, with HPV16 (44.1%), HPV18 (15.9%), HPV35 (8.4%), and HPV45 (5.7%) the most common types (12). In northeastern Tanzania, 89% of invasive cervical cancers at the Kilimanjaro Christian Medical Centre had detectable HPV, with 31 genotypes identified overall and HPV16, HPV35, and HPV45 the most common genotypes in invasive disease (13). For the Lake Zone (northwestern Tanzania), data are more limited: a study of pregnant women in the Mwanza region reported an overall cervical HPV prevalence of 34%, of which 84% of typeable infections were high-risk types, and HPV16 was the most prevalent (18%) (14). More recently, among HIV-infected women attending Bugando Medical Centre, Mwanza, HPV DNA was detected in 54.1% of participants, of whom 65.3% harboured high-risk genotypes, but this was based on exfoliated cervical cells collected by cytobrush as standard tools and techniques used for screening cervical cancer for women with HIV, not tissue (15).

Specimen type influences HPV detection and genotyping accuracy. FFPE tissue is the most commonly used specimen in retrospective studies. Yet, formaldehyde fixation causes DNA fragmentation and DNA-protein cross-linking, and paraffin can reduce both DNA yield and PCR amplification efficiency, making genotyping FFPE specimens technically challenging. (16). Extensive cross-linking and fragmentation further reduce DNA yield. (17). In a direct comparison, fresh cervical tissue yielded significantly higher DNA purity and quantity than FFPE blocks, and HPV was detected in 39% of fresh tissues versus only 13% of FFPE tissues, leading to a recommendation against FFPE blocks for epidemiological HPV detection and typing. (18).

Despite this, no study in the Lake Zone of northwestern Tanzania, the catchment of Bugando Medical Centre, a zonal referral hospital serving approximately eight million people across the regions of Kagera, Geita, Shinyanga, Mara, Simiyu, and Mwanza, has genotyped high-risk HPV in fresh cervical cancer tissue biopsies. Prior regional studies relied on exfoliated cervical cells (15), while the only national-level tissue genotyping used archived FFPE blocks(12). In this context, we determined the prevalence and distribution of high-risk HPV genotypes in fresh cervical cancer tissue biopsies at Bugando Medical Centre, Mwanza. Tanzania introduced a national HPV vaccination programme targeting 14-year-old girls(19), yet second-dose coverage remains around 57% (20), and current vaccines do not cover several genotypes common in Tanzanian

cancers. Reliable fresh biopsy-based genotyping data from our setting are therefore a prerequisite for informing screening strategies, vaccine policy, and future prevention programmes in northwestern Tanzania.

## Materials and methods

### *Study population, study area, and inclusion criteria*

This was a hospital-based study involving 309 confirmed cases of cervical carcinoma. The study was conducted between January 2024 and January 2025, and samples were analysed over one year. Tissue biopsies from patients diagnosed with cervical carcinoma were obtained from the histopathology laboratory at Bugando Medical Centre (BMC), a tertiary referral hospital in Northwestern Tanzania. Demographic, clinical, and associated data were collected for all women who attended the Gynaecology and CTC clinics and underwent cone LEEP biopsies for suspicion of cervical carcinoma following speculum examination and cytological screening (VIA and Pap smear). Laboratory procedures were performed at the Histopathology Laboratory of BMC, the Molecular Biology Laboratory of the Catholic University of Health and Allied Sciences (CUHAS), and the National Institute of Medical Research Laboratory in Mwanza. Tissue samples were collected and divided into two containers for pathological diagnosis: one for 250 mL of 10% Neutral Buffered Formalin and one for HPV DNA analysis, where the cervical samples were kept in a container with 200 mL of 95% ethanol. Two qualified pathologists, blinded to HPV status, evaluated the histological samples and reviewed all specimens; they included only confirmed cervical carcinoma tissue samples and excluded those with inflammatory conditions and non-carcinomatous lesions. The joint CUHAS/BMC Research and Ethics Review Committee (NO. 633/2022) and the Lake Zone Institutional Review Board (NO. MR/53/100/727) granted ethical approval.

***Laboratory procedures***

***Histopathological procedures***

The confirmed tissue blocks of cervical cancer patients were cut into thin sections of 2–3 µ using a Rotary Microtome. The tissues were then stained with routine Hematoxylin and Eosin (H&E). Deparaffinization was performed in a hot-air oven at 60 °C, and the slides were then placed in two xylene containers for 10 minutes each. This was followed by rehydration, during which the tissue slides were dipped in a series of absolute ethanol (99.8%) and 95% ethanol for 10 minutes, then in clean tap water for 5 minutes. The tissue slides were immersed in Hematoxylin for 10 minutes, then blued in clean running tap water for 5 minutes. The section was immersed in eosin stain solution for 5 minutes, then dehydrated in 95% and 99.8% ethanol for 10 minutes each. Next, we immersed the tissue slides in xylene 10 times in two containers to clear the ethanol. (21) Lastly, we mounted the slides in Dibutyl phthalate Polystyrene Xylene (DPX) for histological evaluation by two registered pathologists blinded to the HPV status of the tissue samples. We used an Olympus CX21 light microscope to review cervical tissue diagnoses and subtypes and to establish histological prognostic factors. We obtained all images of Histomorphological prognostic factors using a digital microscope camera with a 10MP CMOS sensor and a resolution of 3648×2736.

**DNA extraction**

We extracted genomic DNA from confirmed cervical cancer tissue samples stored in 95% ethanol using the QIAamp DNA Mini Kit, following a modified manufacturer's protocol designed to maximise yield from complex tissues. (22). Briefly, mechanically dissociate tissue fragments (up to 25 mg) into small sections to increase surface area for enzymatic digestion. Initiate lysis by adding 180 µL of Buffer ATL and 20 µL of Proteinase K, then incubate overnight at 56 °C. (23). This extended incubation period ensures complete tissue breakdown and the release of high-quality genomic DNA (24).

After lysis, treat the samples with 200 µL of Buffer AL and incubate at 70 °C for 10 minutes to improve DNA-binding efficiency. (22). After adding 200 µL of absolute ethanol (96–100%), transfer the mixture to a QIAamp Mini spin column. The column's silica-gel membrane enables selective DNA binding under high-salt conditions, while subsequent wash steps remove contaminants. (24). We washed the column with 500 µL of Buffer AW1 and 500 µL of Buffer AW2, then centrifuged at 20,000 × g to remove residual ethanol carryover, which can inhibit downstream PCR. (22). DNA was then eluted in 200 µL of Buffer AE after a 1-minute room temperature incubation to ensure optimal recovery (22). We assessed DNA quantity and concentration using a Qubit fluorometer, followed by quality analysis by 2% agarose gel electrophoresis. (25,26).

**PCR amplification**

We identified high-risk HPV genotypes using the Real-Time PCR Anyplex™ II HPV28 PCR System on a Bio-Rad CFX96 Dx real-time PCR platform (manufactured in Singapore; serial number 787BR19205). The assay is designed to detect human papillomavirus DNA, including 19 high-risk HPV genotypes (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73, and 82) and nine low-risk genotypes (6, 11, 40, 42, 43, 44, 54, 61, and 70). In this study, the system was used to detect high-risk HPV genotypes (27).

The kit included three known positive control tubes (5 µL each) containing HPV28 PC1, PC2, and PC3. We also used 5 µL of RNase-free water as a negative control for all 85 cases tested for high-risk HPV genotypes. For PCR reaction preparation, we used Anyplex™ II HPV Master Mix. This mix includes multiplex PCR primers targeting 28 HPV types, a universal internal control (UIC) to monitor PCR performance, and real-time PCR enzyme mixes for amplification and detection. PCR thermal cycling conditions were as follows: initial denaturation at 95°C for 15 minutes; PCR amplification (50 cycles) with denaturation at 95°C for 30 seconds, annealing & extension at 60°C for 1 minute, and a final extension at 60°C for 30 seconds. We performed detection and interpretation using Tagging Oligonucleotide Cleavage & Extension (TOCE) technology for genotyping. We analysed the data using a closed, automated system in the Seegene Viewer

Software, which reports the presence or absence of 28 high-risk genotypes for each tissue sample (see Figure 1). The internal control had to be detected to ensure result validity (27,28).




**Figure 1:** Summary of one cervical fresh tissue biopsy sample tested for high-risk HPV positivity for genotypes 16 and 18, indicated in cycle (graph 3) when analysed using a closed automated system of Seegene Viewer Software for HPV identification.

**Data management and analysis**

We analysed data using Stata version 17. We summarised categorical variables as frequencies and percentages and assessed associations with HrHPV status using Pearson's chi-square or Fisher's exact test, as appropriate. Univariable logistic regression was performed for selected clinically relevant factors, including age, HIV status, parity, lifetime sexual partners, histological subtype, and pathological grade. The multivariable model included age, HIV status, histological subtype,

and pathological grade, selected based on clinical relevance, potential confounding, and univariable findings. We treated age as a continuous variable and combined pathological grades II and III because of sparse observations. We report crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs). For genotype-level analyses, we clustered standard errors at the participant level to account for multiple genotype detections within women. We set statistical significance at $p < 0.05$.

## RESULTS

### Clinical and demographic characteristics of study participants according to high-risk HPV status

A total of 309 women with histologically confirmed cervical cancer were included in the analysis, of whom 277 (89.6%) tested positive for high-risk human papillomavirus (HrHPV) (Figure 2). The HrHPV positivity rate was high across all age groups, ranging from 83.3% among women aged 29 years or younger to 91.5% among those aged 30 to 40 years. No significant association was found between age group and HrHPV status (Fisher's exact p=0.792) (Table 1). Additionally, factors such as marital status, educational attainment, smoking status, alcohol consumption, HIV status, parity, previous cervical cancer screening, contraceptive use, HPV vaccination history, and the number of lifetime sexual partners were not significantly associated with HrHPV status (all $p > 0.05$) (Table 1).

Histological subtype was the only characteristic significantly associated with HrHPV status. HrHPV positivity was higher among women with squamous cell carcinoma than among those with adenocarcinoma (91.7% vs 83.8%; p=0.044) (See Figure 4). HrHPV positivity also increased with pathological grades, from 89.2% in Grade I to 97.2% in Grade III, although this difference was not statistically significant (Fisher's exact p=0.315) (Table 1).

**Table 1. Clinical and demographic characteristics of study participants according to high-risk HPV status**

| Characteristic | Category | Overall N | HrHPV | | P-value |
|---|---|---|---|---|---|
| | | | Positive n (%) | Negative n (%) | |
| **Age group** | ≤29 years | 6 | 5 (83.3) | 1 (16.7) | 0.792[a] |
| | 30–40 years | 59 | 54 (91.5) | 5 (8.5) | |
| | 41–60 years | 173 | 155 (89.6) | 18 (10.4) | |
| | ≥61 years | 71 | 63 (88.7) | 8 (11.3) | |
| **Marital status** | Single | 12 | 11 (91.7) | 1 (8.3) | 0.396[a] |
| | Married | 223 | 196 (87.9) | 27 (12.1) | |
| | Divorced | 17 | 17 (100.0) | 0 (0.0) | |
| | Widow | 57 | 53 (93.0) | 4 (7.0) | |
| **Education level** | None | 102 | 92 (90.2) | 10 (9.8) | 0.867[a] |
| | Primary | 183 | 164 (89.6) | 19 (10.4) | |
| | Secondary | 22 | 19 (86.4) | 3 (13.6) | |
| | College | 2 | 2 (100.0) | 0 (0.0) | |
| **Smoking status** | Never smoker | 300 | 269 (89.7) | 31 (10.3) | 1.000[a] |
| | Ever smoker | 9 | 8 (88.9) | 1 (11.1) | |
| **Alcohol consumption** | Never drinker | 251 | 223 (88.8) | 28 (11.2) | 0.337[b] |
| | Ever drinker | 58 | 54 (93.1) | 4 (6.9) | |
| **HIV status** | Negative | 151 | 136 (90.1) | 15 (9.9) | 0.831[b] |
| | Positive | 102 | 90 (88.2) | 12 (11.8) | |
| | Unknown | 56 | 51 (91.1) | 5 (8.9) | |
| **Parity** | 0–5 children | 105 | 91 (86.7) | 14 (13.3) | 0.218[b] |
| | ≥6 children | 204 | 186 (91.2) | 18 (8.8) | |
| **History of cervical cancer screening** | Yes | 29 | 26 (89.7) | 3 (10.3) | 1.000[a] |
| | No | 274 | 245 (89.4) | 29 (10.6) | |
| | Don't know | 6 | 6 (100.0) | 0 (0.0) | |
| **History of contraceptive use** | Yes | 81 | 74 (91.36) | 7 (8.64) | 0.430[a] |
| | No | 223 | 199 (89.2) | 24 (10.8) | |
| | Don't know | 5 | 4 (80.0) | 1 (20.0) | |
| **History of HPV vaccination** | Yes | 4 | 4 (100.0) | 0 (0.0) | 1.000[a] |
| | No | 293 | 262 (89.4) | 31 (10.6) | |
| | Don't know | 12 | 11 (91.7) | 1 (8.3) | |

| | | | | | |
|---|---|---|---|---|---|
| **Number of lifetime sexual partners** | 0–1 | 268 | 240 (89.6) | 28 (10.4) | 0.892[b] |
| | >1 | 41 | 37 (90.2) | 4 (9.8) | |
| **Histological subtype** | Squamous carcinoma | 229 | 210 (91.7) | 19 (8.3) | **0.044[b]** |
| | Adenocarcinoma | 80 | 67 (83.7) | 13 (16.3) | |
| **Pathological grade** | Grade I | 125 | 111 (89.2) | 14 (10.8) | 0.315[a] |
| | Grade II | 148 | 139 (93.9) | 9 (6.1) | |
| | Grade III | 36 | 35 (97.2) | 1 (2.8) | |

**Notes:** Percentages are calculated within each characteristic category. [a] Fisher's exact test. [b] Pearson's chi-square test.

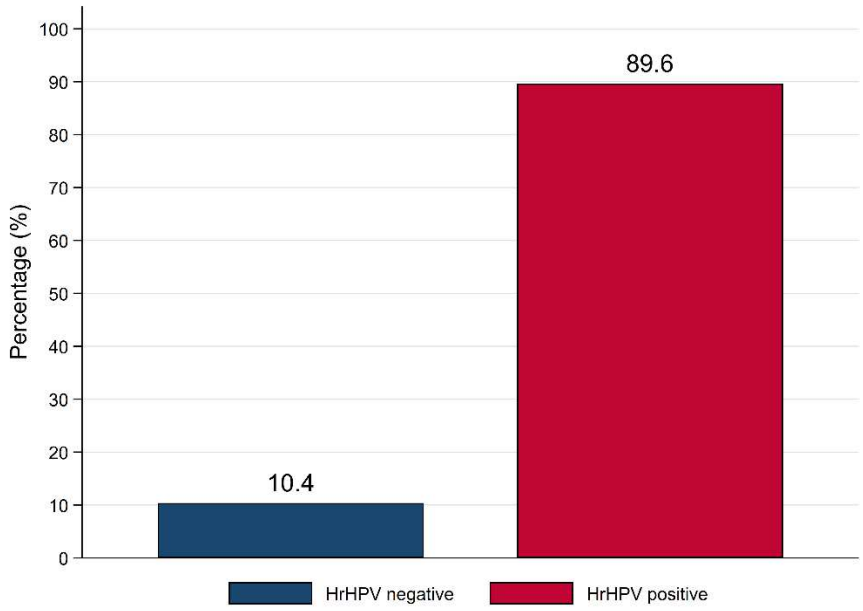



**Figure 2:** Prevalence of high-risk human papillomavirus among women with cervical cancer (n=309)

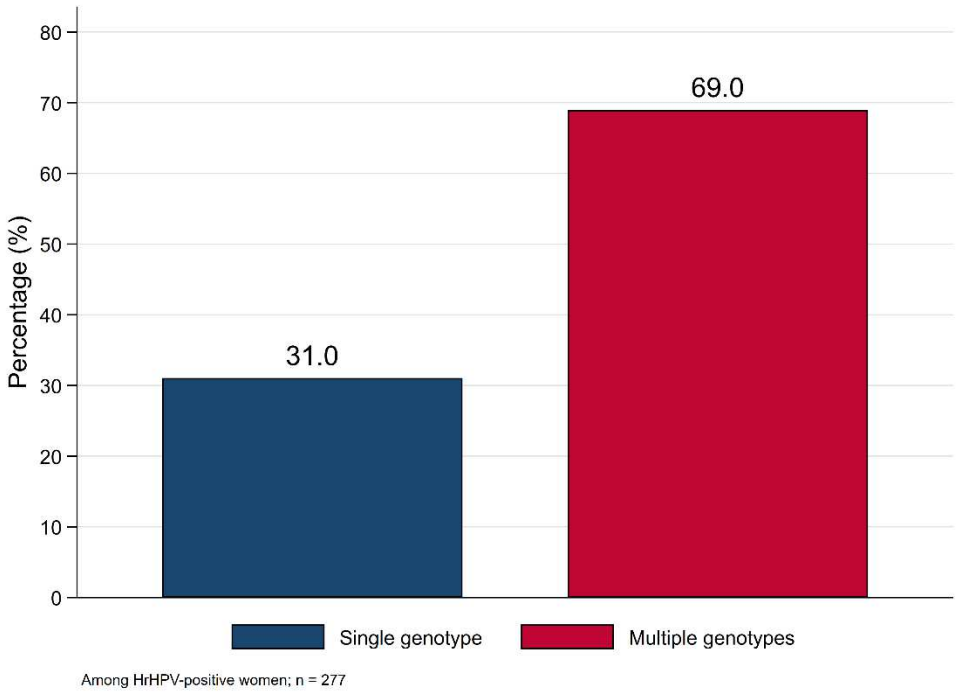



**Figure 3**: Pattern of high-risk HPV infection among HR-HPV-positive women with cervical cancer

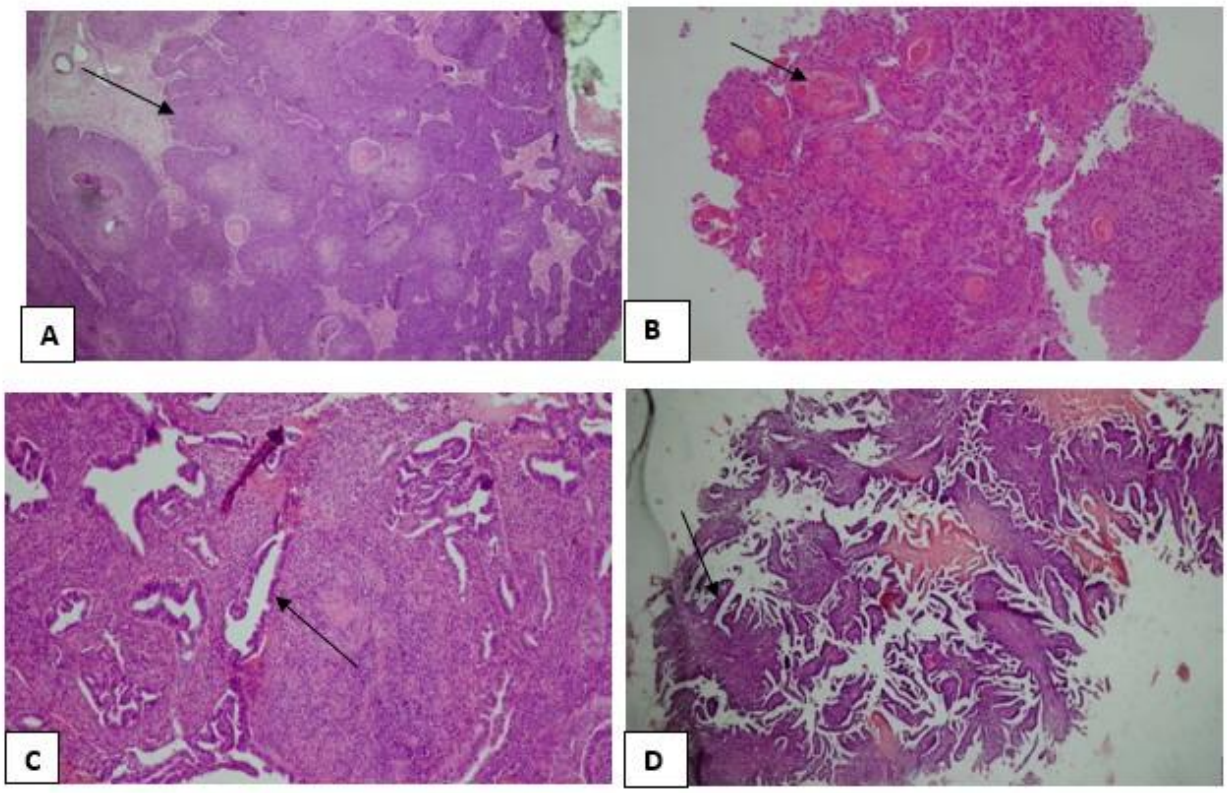



**Figure 4:** Hematoxylin and Eosin stains of cervical cancer tissue fragments. A-Non-keratinising squamous cell carcinoma with basaloid features, with nuclear peripheral palisading indicated by a black arrow. B-Keratinising squamous cell carcinoma with multiple keratin pearls, indicated by black arrows. C-Invasive adenocarcinoma, not otherwise specified (NOS). D-Villoglandular adenocarcinoma pointed to by a black arrow (x10 HPF)

**Pattern and Distribution of High-Risk HPV Genotypes**

In this study, we identified 581 hrHPV genotype detections among the 277 hrHPV-positive women, reflecting both single- and multiple-genotype infections (Figure 3). HPV16 was the most frequently detected genotype, accounting for 36.7% of all genotype detections, followed by HPV18 (21.9%) (Figure 5). Other detected genotypes included HPV45 (7.2%), HPV33 (6.5%), HPV35 (6.0%), HPV31 (5.7%), and HPV59 (5.5%). HPV58 (2.6%), HPV52 (2.4%), HPV39 (2.4%), HPV51 (2.1%), and HPV56 (1.0%) were comparatively less frequent (Figure 5).

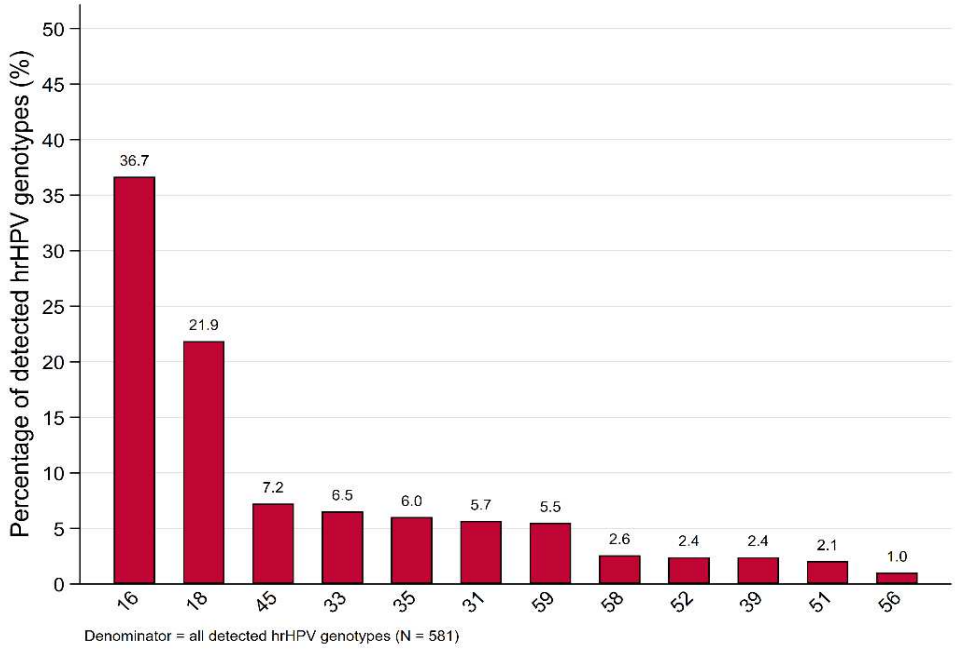



**Figure 5:** Distribution of high-risk HPV genotype detections among HrHPV-positive women with cervical cancer

**Association of High-Risk HPV Genotype Distribution with HIV Status and Histological Subtype**

Among the 473 hrHPV genotype detections from women with known HIV status, 281 were HPV16/18 and 192 were other hrHPV genotypes. Of the HPV16/18 detections, 164 (58.4%) occurred among HIV-negative women and 117 (41.6%) among HIV-positive women, whereas 130 (67.7%) of the other hrHPV genotype detections occurred among HIV-negative women and 62 (32.3%) among HIV-positive women (Table 2). After accounting for clustering of multiple genotype detections within individual women, HIV-positive status was significantly associated with higher odds of a detected genotype being HPV16/18 rather than another hrHPV genotype (OR = 1.50, 95% CI: 1.09–2.05; p = 0.013) (Table 2).

By histological subtype, 340 of the 581 hrHPV genotype detections were HPV16/18, while 241 were other hrHPV genotypes. Among HPV16/18 detections, 265 (77.9%) occurred in women with squamous cell carcinoma and 75 (22.1%) in women with adenocarcinoma. A similar distribution was observed for other hrHPV genotypes, with 181 (75.1%) occurring in squamous cell carcinoma and 60 (24.9%) in adenocarcinoma (Table 3). After accounting for clustering of genotype detections within individual women, there was no significant association between histological subtype and HPV genotype category (OR = 0.85, 95% CI: 0.63–1.16; p = 0.315). Thus, while HPV16/18 distribution differed significantly by HIV status, there was no evidence of a corresponding difference according to cervical cancer histological subtype (Table 3).

**Table 2. Distribution of high-risk HPV genotype categories according to HIV status among women with cervical cancer**

| HPV genotype category | Overall, n | HIV | | Cluster-adjusted OR (95% CI) | P-value |
|---|---|---|---|---|---|
| | | negative, n (%) | positive, n (%) | | |
| **HPV16/18** | 281 | 164 (58.4) | 117 (41.6) | 1.50 (1.09–2.05) | **0.013** |
| **Other hrHPV genotypes** | 192 | 130 (67.7) | 62 (32.3) | **Reference** | |

**Table 3. Distribution of high-risk HPV genotype categories according to histological subtype among women with cervical cancer**

| HPV genotype category | Overall, n | Squamous carcinoma, n (%) | Adenocarcinoma, n (%) | Cluster-adjusted OR (95% CI) | P-value |
|---|---|---|---|---|---|
| **HPV16/18** | 340 | 265 (77.9) | 75 (22.1) | 0.85 (0.63–1.16) | **0.315** |
| **Other hrHPV genotypes** | 241 | 181 (75.1) | 60 (24.9) | Reference | |

**Factors associated with high-risk HPV positivity among women with cervical cancer**

In the univariable analysis, histological subtype was significantly associated with HrHPV positivity. Women with adenocarcinoma had 53% lower odds of HrHPV positivity than women with squamous carcinoma (cOR = 0.47, 95% CI: 0.22–0.99; p = 0.048). Women with Grade II/III disease had higher odds of HrHPV positivity than those with Grade I disease (cOR = 2.09, 95% CI: 0.85–5.14; p = 0.109), although this association was not significant. Age, HIV status, parity, and lifetime number of sexual partners were not significantly associated with HrHPV positivity in the univariable analyses (Table 4).

In the multivariable analysis, none of the factors included in the model was independently associated with HrHPV positivity. Each additional year of age was associated with a small reduction in the odds of HrHPV positivity (aOR = 0.98, 95% CI: 0.95–1.02; p = 0.284), while HIV-positive women had lower adjusted odds of HrHPV positivity than HIV-negative women (aOR = 0.68, 95% CI: 0.25–1.84; p = 0.443); neither association was statistically significant. Similarly, adenocarcinoma was not independently associated with HrHPV positivity after adjustment (aOR = 1.34, 95% CI: 0.35–5.11; p = 0.664). Women with Grade II/III disease had more than twice the adjusted odds of HR-HPV positivity compared with women with Grade I disease (aOR = 2.29, 95% CI: 0.85–6.21; p = 0.103) (Table 4).

**Table 4. Univariable and multivariable logistic regression for factors associated with HR-HPV positivity among women with cervical cancer.**

| Factor | Category/unit | Crude OR (95% CI) | P-value | Adjusted OR (95% CI) | P-value |
|---|---|---|---|---|---|
| **Age (years)** | | 1.01 (0.98–1.04) | 0.622 | 0.98 (0.95–1.02) | 0.284 |
| **HIV status** | Negative | 1.00(Ref) | — | 1.00(Ref) | — |
| | Positive | 0.83 (0.37–1.85) | 0.644 | 0.68 (0.25–1.84) | 0.443 |
| **Parity** | 0–5 children | 1.00(Ref) | — | — | — |
| | ≥6 children | 1.59 (0.76–3.34) | 0.221 | — | — |
| **Lifetime sexual partners** | 0–1 partner | 1.00(Ref) | — | — | — |
| | >1 partner | 1.08 (0.36–3.25) | 0.892 | — | — |
| **Histological subtype** | Squamous carcinoma | 1.00(Ref) | — | 1.00(Ref) | — |
| | Adenocarcinoma | **0.47 (0.22–0.99)** | **0.048** | 1.34 (0.35–5.11) | 0.664 |
| **Pathological grade** | Grade I | 1.00(Ref) | — | 1.00(Ref) | — |
| | Grade II/III | 2.09 (0.85–5.14) | 0.109 | 2.29 (0.85–6.21) | 0.103 |

## Discussion

In this single-centre study of women with histologically confirmed cervical cancer in northwestern Tanzania, 89.6% tested positive for high-risk HPV DNA in fresh cervical tissue biopsies. This high prevalence is consistent with the essential oncogenic role of high-risk HPV in invasive cervical cancer, in which HPV DNA is detected in the vast majority of cases. (1). It also aligns closely with other African series in which DNA was extracted from fresh tissue, with high-risk HPV detected at a rate of 89% at Kilimanjaro Christian Medical Centre. (29) and 90.4% in a multi-country study of invasive cervical cancers from Ghana, Nigeria, and South Africa (30). Notably, our prevalence exceeded the 81.1% reported in a recent national Tanzanian genotyping study that relied on archived formalin-fixed paraffin-embedded tissue. (12) and was far higher than the 54.1% detected among HIV-infected women attending Bugando Medical Centre in an earlier study that used exfoliated cytobrush samples rather than fresh tumour tissue (15). These contrasts suggest that specimen type materially influences the molecular detection of HPV in cervical cancer, an issue examined further below.

HPV16 was the most frequently detected genotype, followed by HPV18, HPV45, HPV33, HPV35, HPV31, and HPV59, which were the next most common, while HPV58, HPV52, HPV39, HPV51, and HPV56 were comparatively infrequent. The dominance of HPV16 and HPV18 mirrors the pattern reported in meta-analyses of invasive cervical cancer among African women, in which HPV16 and HPV18 accounted for 49.7% and 18.0% of infections, respectively (8). HPV45, the third most frequent genotype, is also characteristic of the African continent, where it accounts for

11.0% of invasive cancers, considerably more than in Eastern Asia (3.0%) or South/Central America (6.1%) (4).

At the same time, the combined HPV16/18 share of 58.6% in our cohort is lower than the 85% reported in a Dar es Salaam study that likewise used fresh cervical biopsies at the Ocean Road Cancer Institute. (31). This difference is likely explained by the substantial burden of multiple infections captured in our samples, with an average of approximately two genotypes per hrHPV-positive woman, which dilutes the proportional contribution of the two most common types rather than indicating a lower absolute attribution of HPV16/18 to cervical cancer in this region. Non-16/18 genotypes nonetheless contributed substantially in our setting; HPV45, HPV33, HPV35, HPV31, and HPV59 together accounted for 30.9% of all hrHPV identifications. The 6.0% share of HPV35 is broadly consistent with the 8.4% reported in the national Tanzanian series (12) and the 8.7% observed in the multi-country West African study (30), and it reinforces earlier calls for genotype-informed vaccine formulations for Tanzania(12). The 5.5% contribution of HPV59 is notable, as this genotype is not included in any currently licensed vaccine and is infrequently emphasised in sub-Saharan genotype distributions.

Demographic and behavioural characteristics were not associated with hrHPV positivity; however, positivity was high across all age strata. Marital status, education, smoking, alcohol use, parity, prior screening, contraception, vaccination history, and lifetime number of sexual partners showed no significant association. In multivariable analysis, none of the examined factors was independently associated with hrHPV positivity. This is consistent with the disease's natural history. By the time invasive cancer is established, hrHPV is present in the overwhelming majority of tumours, regardless of a woman's age, sexual history, or other characteristics, because those factors act primarily on the earlier stages of HPV acquisition, persistence, and neoplastic progression rather than on hrHPV detectability within established tumours. (1). The uniformly high positivity therefore reinforces that hrHPV testing has a ceiling effect in women with frank invasion, and that demographic risk stratification adds little once disease is present.

The only clinicodemographic characteristic associated with hrHPV status was histological subtype, with higher positivity in squamous cell carcinoma than in adenocarcinoma. In univariable analysis, adenocarcinoma was associated with approximately half the odds of hrHPV positivity compared with squamous cell carcinoma, although this association attenuated after adjustment. The lower hrHPV positivity observed in adenocarcinoma mirrors a well-documented pattern in the international literature, in which pooled analyses have consistently reported lower HPV DNA prevalence in adenocarcinoma than in squamous cell carcinoma, with figures of about 76.5% against 87.3% in the 2003 IARC meta-analysis, and 80.3% against 90.1% in the later analysis, both of which remained significant after adjustment for testing methods and tissue source (1,32). Part of this deficit reflects genuinely HPV-negative glandular tumours, including rare subtypes such as

gastric-type and mesonephric adenocarcinomas that are rarely hrHPV-positive (33,34). The univariable association in our data on adenocarcinoma carrying roughly half the odds of hrHPV positivity attenuated after adjustment, suggesting that other tumour characteristics explain part of the difference. Yet the direction is highly consistent with prior work (32). These findings have practical implications for screening. As primary HPV-based algorithms are increasingly adopted, there is a legitimate concern that hrHPV-negative adenocarcinoma could be missed, since cytology already disproportionately misses glandular lesions and their clinical detection may require different approaches (34,35). While HPV-based screening is highly effective against adenocarcinoma overall, it identifies women at high risk of adenocarcinoma earlier than cytology alone (36). Our results support the view that HPV testing cannot entirely replace morphological diagnosis, particularly for glandular lesions, and that histological evaluation of clinically suspicious cases should not be deferred solely based on an HPV-negative result.

In this study, HIV-positive women were significantly more likely to harbour HPV16/18 than other hrHPV genotypes, even after accounting for within-woman clustering of multiple genotypes, adding to a heterogeneous literature. Some studies of invasive disease report an enrichment of HPV18 among HIV-positive women. One study reported 43.4% versus 24.1% in HIV-positive compared with HIV-negative women with invasive cervical carcinoma in Zimbabwe. It reduced genotype diversity in HIV-positive cancers, with cases shifted towards the few most oncogenic types. (37,38). A study done in South Africa reported similar type distributions by HIV status among HPV-positive women. (39). Our data, together with the dominant HPV16/18/45 profile reported across sub-Saharan Africa regardless of serostatus (40), suggest that HPV16/18 remains particularly oncologically important in HIV-positive women in our setting. The finding also has a practical corollary because HIV-positive women in this population are disproportionately infected with the types covered by the current bivalent, quadrivalent, and nonavalent vaccines; vaccination could plausibly have a high impact on cancer prevention in this group, a hypothesis consistent with pooled evidence from the region. (40).

A principal methodological strength of this study is the use of fresh cervical cancer tissue biopsies for DNA extraction. Formaldehyde fixation causes DNA fragmentation and DNA-protein cross-linking, and paraffin embedding can reduce both DNA yield and PCR amplification efficiency, making genotyping of archival formalin-fixed paraffin-embedded material technically challenging (41,42). A direct comparison reported significantly higher DNA purity and quantity in fresh cervical tissue than in formalin-fixed, paraffin-embedded blocks, with HPV detected in 39% of fresh tissues versus only 13% of paraffin blocks(43); even between frozen tissue and FFPE samples, detection of a given genotype varied by up to twofold (44). Sample preservation and processing may therefore explain a substantial share of the inter-study variability in hrHPV prevalence across sub-Saharan Africa, and the 89.6% positivity achieved here with fresh tissue,

approaching the 94% reported from the fresh-biopsy ORCI series(45), supports fresh tissue as the preferred substrate for HPV genotyping in this setting.

These findings have direct implications for prevention. Tanzania has introduced national HPV vaccination of 14-year-old girls. (19 years) Yet second-dose coverage remains around 57% (20). In our cohort, HPV16 and HPV18 together accounted for 58.6% of genotype detections, and expansion to a nonavalent formulation covering HPV16, 18, 31, 33, 45, 52, and 58 would extend theoretical coverage to approximately 83.0% of all hrHPV identifications. A residual 17.0%, predominantly HPV35, HPV59, HPV39, HPV51, and HPV56, would remain uncovered by currently licensed vaccines, underscoring the importance of genotype surveillance for future vaccine selection.

Several limitations should be acknowledged. This was a single-centre, hospital-based study at a zonal referral hospital. Although Bugando Medical Centre serves a population of approximately 13 million people across the Lake Zone(46), referral bias cannot be ruled out. HIV status was unknown for 56 of 309 participants (18.1%), and the multivariable findings should be interpreted with this caveat. Genotype proportions were calculated per detection rather than per woman; given the high burden of multiple infections, per-woman estimates may differ and are preferable for direct public health translation. Finally, 32 tumours (10.4%) remained hrHPV-negative despite fresh tissue use, which may reflect assay sensitivity, atypical histology, or genuinely HPV-independent carcinogenesis. Future multicentre, biopsy-based studies with per-woman genotype attribution and longitudinal sampling would strengthen the evidence base for the Lake Zone and comparable East African regions.

**Conclusions**

This study found a high prevalence of high-risk HPV among women with histologically confirmed cervical cancer tissue biopsies in northwestern Tanzania, with HPV16 and HPV18 being the most frequently detected genotypes. Other high-risk genotypes, particularly HPV45, HPV33, HPV35, HPV31, and HPV59, also contributed substantially to the genotype profile. HIV-positive women were more likely to harbour HPV16/18 than other high-risk HPV genotypes. At the same time, we found no independent association between high-risk HPV positivity and the demographic, behavioural, or clinicopathological factors assessed. These findings support strengthening HPV vaccination and prevention strategies in Tanzania and highlight the importance of continued HPV genotype surveillance. Multicentre studies using per-woman genotype attribution are warranted to inform locally relevant cervical cancer prevention strategies.

## Declarations

**Abbreviation**

| | |
|---|---|
| BMC: | Bugando Medical Centre |
| CI: | Confidence interval |
| CUHAS: | Catholic University of Health and Allied Sciences |
| DNA: | Deoxyribonucleic acid |
| EDTA: | Ethylenediaminetetraacetic acid |
| FFPE: | Formalin-fixed paraffin-embedded |
| HIV: | Human immunodeficiency virus |
| HPF: | High-power field |
| hrHPV: | High-risk human papillomavirus |
| OR: | Odds ratio |
| PCR: | Polymerase chain reaction |

***Ethics approval and Consent to participate***

The joint CUHAS/BMC Research and Ethics Review Committee (CREC) approved the study (reference number 633/2022), and the Lake Zone Institutional Review Board approved it (reference number MR/53/100/727). We obtained informed consent from all participants in accordance with the Declaration of Helsinki.

**Consent for Publication**

Not Applicable

**Availability of data and materials**

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request, subject to appropriate ethical and institutional restrictions. The study did not generate novel DNA or RNA sequence data requiring deposition in a public nucleotide sequence repository.

**Competing interests**

The authors declare no competing interests.

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**Authors Contribution**

OO, PFR, and BRK conceived, designed, and coordinated the study. EE, SM, IS, CM, MJ, OO, FD, CM, and CAM coordinated and executed the study; OO, TL, JN, and BRK analysed and interpreted the data; OO, CM, and CAM wrote the initial draft of the manuscript, which all authors critically revised. All authors reviewed and approved the final manuscript.

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