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Human papilloma virus genotype distribution and risk factor analysis amongst reproductive aged women in urban Gambia

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Abbreviations:

API, analytical profile index; AOR, adjusted odds ratio; BLAST, basic local alignment search tool; CIN, cervical intraepithelial lesion; EFSTH, Edward Francis Small Teaching Hospital; FGM, female genital mutilation; HSIL, high squamous intraepithelial lesion; HPV, human papillomavirus; HR-HPV, high risk human papillomavirus; IARC, International agency for research on cancer; KMC, Kanifing municipal council; L1, late gene (1); LR-HPV, low risk human papillomavirus; OR, odds ratio; pHR-HPV, probable high risk human papillomavirus; WCR, West Coast region.

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Abstract

Purpose. Cervical cancer is the most frequently diagnosed female cancer in The Gambia, representing approximately 30% of cases. In 2014, the quadrivalent human papilloma virus (HPV) vaccine was introduced, which offers protection against HPV genotypes 6, 11, 16 and 18. To evaluate the potential effectiveness of this vaccine, genotype distribution and risk factor analysis were assessed.

Methodology. Endocervical samples (n=232) were collected from women aged 20-49 years residing in urban Gambia. A questionnaire was administered to capture socio-demographic and cervical cancer risk factors. HPV detection and genotyping was performed by PCR amplification of the L1 major capsid gene and analysis of sequenced PCR products.

Results/ Key Findings. The prevalence of HPV was 12% (28/232) and the high risk (HR) genotype HPV 52 (5/28) was the most prevalent genotype. HR-HPV sequences had high identity (≥ 90 %) to isolates which originated from America, Europe and Asia but not from Africa. Half (14/28) of participants were co-infected with *Ureaplasma urealyticum/parvum*, which increases the risk of progression to cervical cancer. Female genital mutilation and the use of hormone contraception for >5 years were identified as potential risk factors for HPV infection. Ethnicity-associated differences were also noted; participants of the Fula ethnic group had a higher prevalence of HR-HPV infection (31.3%) compared to the Mandinka (18.8%) and Wollof (12.5%) groups.

Conclusion. These data may have a significant public health impact as the HPV quadrivalent vaccine may be of limited value if the circulating non-HPV 16/18 HR-genotypes are responsible for cytological abnormalities of the cervix.

INTRODUCTION

Human papilloma virus (HPV) infection is the most common sexually transmitted infection in reproductive aged females, and is associated with approximately 80% of cases of cervical cancer [1, 2]. More than 75% of sexually active females will be infected with the virus at some stage in their lives, which in some cases can regress without treatment [3, 4]. However, persistent infection with HPV high risk genotypes over a period of time can lead to cervical intraepithelial neoplasia (CIN), which can progress to cervical cancer [5, 6, 7]. Annually, more than 500,000 new cervical cancer cases and 250,000 deaths are reported, worldwide [8]. Although cervical cancer is a global health problem, more than 80% of these cases occur in Africa where regular cervical cancer screening programmes are not readily available. Cervical cancer survival rates are very low in developing countries due to either late presentation of cases or a lack of adequate treatment services [8, 9].

Approximately 100 HPV genotypes have been identified and 40 of these can infect the genital mucosal tract. According to the International Agency for Research on Cancer (IARC), twelve of these HPV mucosal types; HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are termed high risk (HR–HPV) or oncogenic types [10]. HPV types 26, 53, 66, 67, 68, 70, 73 and 82 are classified as possible or probable high risk (pHR–HPV) [11]. Low risk (LR-HPV) types are associated mostly with condyloma acuminata, genital warts or other benign epithelial lesions. The most common LR-HPV genotypes are HPV-6 and HPV-11 [10, 12]. Although persistent infection with HR-HPV 16 and 18 are responsible for more than 70% of cervical cancer cases, other HR-HPV genotypes have been identified as causative agents for cervical cancer, and other genital and oropharyngeal cancers [12, 13]. While HPV infection is the major risk factor for the development of cervical cancer, several other co-factors are known to increase this risk [14]. These include having multiple sexual partners, the use of hormone contraceptives and smoking. Furthermore, co-infection with other sexually transmitted pathogens may enhance HPV persistence through immunosuppression and tissue damage, which can increase the risk of development of cervical neoplasia and cancer [15].

In an attempt to reduce the burden of HPV infection, three recombinant HPV prophylactic vaccines have been developed: a bivalent vaccine, manufactured by

GlaxoSmithKline that targets HR-HPV 16, 18, a quadrivalent vaccine, marketed by Merck & Co, against HR-HPV 16, 18 and LR-HPV 6 and 11, and more recently Gardasil 9, from Merck & Co, has been licensed, which targets 7 HR-HPV genotypes 16, 18, 31, 33, 45, 52, 58 and LR-HPV, 6 and 11.

Although HPV infection is vaccine-preventable, widespread introduction of the vaccine in resource-limited countries is still in its infancy. In the Gambia, cervical cancer is the most frequently diagnosed cancer, representing approximately 30% (161/545) of all diagnosed cases during the period 1998-2006 [16]. Furthermore, according to The Gambia Health Management Information System, 237 females were diagnosed with cervical cancer in 2016 and 96% of these cases were from the urban region of the country (Banjul, Kanifing Municipal Council (KMC) and West Coast Region (WCR)).

In 2014, The Gambia introduced the quadrivalent HPV vaccine in the WCR, targeting females from 9–13 years. However, the major circulating HR-HPV genotypes are currently unknown in this population; therefore there is a need to collect current data on HPV infection rates and circulating genotypes. The aim of the study was to evaluate the potential value of the quadrivalent vaccine in urban Gambia by investigating the major circulating HR-HPV genotypes in females residing in this area. In addition, the presence of known socio-demographic risk factors for HPV infection was also determined in this population as well as HPV co-infection with selected sexually-transmitted pathogens.

METHODS

Study site and population

This study focused on residents of Banjul, Kanifing Municipal Council and West Coast Region where the majority of cervical cancer cases are reported. Females, aged 20-49 years attending the Edward Francis Small Teaching Hospital (EFSTH) sexual health clinic, for primary health care were enrolled in this study. Informed consent (Figure S1) was obtained and a participant's information sheet (Figure S2) was provided for those who agreed to participate.

Socio-demographic and risk factors data collection

To determine the social and economic implications of HPV in urban Gambia, a questionnaire was administered to each participant to capture socio-demographic and potential risk factors associated with HPV infection (Figure S3).

Sample collection and routine microbiological investigations

Two hundred and thirty-five (235) females were recruited between August 2015 and February 2016. Two endocervical and two high vaginal swabs were collected from each participant, one endocervical and high vaginal swab from each patient was used for routine microbiological investigations and the remaining swabs for PCR amplification. Samples for PCR were placed immediately into specimen transport media (M4RT™, micro-test, Oxoid, Basingstoke, UK), and stored at -70^{o}C until ready for use.

Routine microbiological detection of *Streptococcus agalactiae*, *Candida albicans*, *Neisseria gonorrhoeae,* bacterial vaginosis and *Trichomonas vaginalis* was performed in the Department of Medical Microbiology, EFSTH, using standard operating procedures. For the isolation of *Streptococcus agalactiae*, *Neisseria gonorrhoeae* and *Candida albicans*, high vaginal and endocervical swabs were cultured onto defibrinated sheep blood agar, chocolate agar and Sabouraud agar (Oxoid™, Basingstoke, UK). The blood and chocolate agar plates were incubated overnight at 37°C in an aerobic and a 6% carbon dioxide atmosphere, respectively. Sabouraud plates were incubated aerobically at 28°C for up to 48 hours to isolate *Candida albicans or Candida* species. Colonies of interest were subcultured and incubated overnight to generate pure colonies. These were selected for Gram staining and biochemical identification. *Streptococcus agalactiae* was identified using the Streptex™ rapid latex agglutination test (Thermo Fisher Scientific, Loughborough, UK), *Neisseria gonorrhoeae* was identified using the API NH test (Biomérieux, Basingstoke, UK) and *Candida albicans* was identified by a positive germ tube test. *Trichomonas vaginalis* was detected by vaginal wet mount microscopy for the detection of motile trichomonads. Bacterial vaginosis was diagnosed using Amsel's clinical criteria, by the presence of any three of the following: 1) a homogeneous white vaginal discharge; 2) a vaginal pH of ≥ 4.6; 3) the release of a 'fishy' amine odour when 10% potassium hydroxide was added to a

vaginal fluid sample; 4) the presence of more than 20% clue cells as observed by microscopy [17].

DNA extraction

DNA was extracted from the clinical specimens using QIAamp DNA mini extraction kit (Qiagen, Crawley, UK) following the manufacturer's instructions. To quality control the extraction process, sterile water was used as a negative control. A 5 µl volume of DNA was used in subsequent PCR reactions. Endocervical swabs were used for HPV PCR while both endocervical and high vaginal swabs were used for *Ureaplasma parvum/urealyticum* PCR.

Polymerase Chain Reaction

All PCR amplifications were performed in a 25 µL volume containing 5 µM of each primer, 1x Taq PCR master mix containing 2.5 units of Taq DNA polymerase, 0.2 mM deoxynucleotide trisphosphates, and 1.5mM $MgCl_2$ (Qiagen, Crawley, UK) and 5µl of DNA template. Amplified products were resolved by electrophoresis using 2% (w/v) agarose gels.

Histocompatibility Leucocyte Antigen PCR

To assess the quality of the DNA extracts from clinical specimens prior to HPV PCR testing, the presence (or absence) of human cellular DNA was determined using a PCR assay targeting the histocompatibility leucocyte antigen (HLA) gene. HLA-PCR was carried out using the forward primer 5'GTGGTGTAAACTTGTACCA-3' and reverse primer 5'-GTAGCAGCGGTAGAGTT-3', which amplified a 230 base-pair (bp) region. Thermal cycling was performed as described elsewhere [18]. A positive HLA-PCR test was determined by the observation of a visible PCR product of the expected size following gel electrophoresis and ethidium bromide staining.

HPV Late gene L1 consensus PCR

HLA-PCR positive samples were subjected to PCR that amplifies a 450-bp region of the HPV late gene 1 (L1) using the PGMY09/11 consensus primers. The L1 consensus PGMY09/11 primer pool consists of 5 upstream and 13 downstream oligonucleotides [18, 19]. A W.H.O. International standard HPV16 DNA positive control (NIBSC, Hertfordshire, UK), negative control (molecular grade water) and a

DNA extraction negative control were included in each PCR run. Thermal cycling was carried out as described elsewhere [18].

Ureaplasma parvum/urealyticum PCR

Conserved primers for two species of *Ureaplasma* (*U. urealyticum* and *U. parvum*) UU-1402 Forward 5'- TGCTGGTGGTACAGGTATGAA-3'and UU-1779 Reverse 5'-GAGCATGTCCACCACCA -3', were used, which target a 378 bp region of the urease gene [20]. Positive (Genekam Biotechnology, Duisburg, Germany), negative (molecular grade water) and DNA extraction negative controls were included in each PCR reaction. The thermal cycling consisted of an initial denaturation at 95^{o}C for 3 minutes, followed by 35 cycles of denaturation at 95^{o}C for 30 seconds, annealing at 58^{o}C for 30 seconds, elongation at 72^{o}C for 30 seconds and a final extension step at 72^{o}C for 5 minutes.

HPV genotyping by DNA sequencing

PCR amplicons were purified using a PCR purification kit (Sigma Aldrich, Haverhill, UK) and then sequenced using the Sanger chain termination method. Raw sequence data is provided in Table S1. An NCBI BLAST (http:// www.ncbi.nlm.nih.gov/BLAST/) search was performed for each sequenced product to allocate the HPV genotype [18].

Statistical analysis

Data analysis on HPV prevalence and risk characteristics was carried out using Epi Info™ version 7 (CDC, Atlanta, USA). Descriptive statistics such as frequency distributions and percentages were used to describe HPV prevalence and other related characteristics of the study population. Bivariate and multivariate analyses were carried out on the strength of risk factors association with HPV infection using odds ratio (OR), adjusted odds ratio (AOR), confidence interval of 95% (CI) and a *P* value of ≤0.05 was used to determine statistical significance.

RESULTS

Of the 235 participants recruited, 3 (1.3%) had samples where inadequate cellular DNA had been collected so were excluded from further analysis.

Socio-demographic and HPV risk characteristics of participants

A total of 232 females aged 20-49 years were included in the study with a mean age of 31.8 years (± 7.5 SD). Thirty percent of the participants were involved in petty trading as means of economic subsistence, whilst 26% of the participants identified as housewives. Eighty percent of participants were married and 48.3% of participants had at least 12 years of education. Three participants (1.3%) reported to be sexually inexperienced although they did not have an intact hymen and 6 (2.6%) participants reported to have their sexual debut at the age of <14 years as a result of early marriage. Sixty seven percent (67%) of participants had their sexual debut at the age of ≥18 years and approximately 40% reported of having ≥2 life time sexual partners. Sixty percent (60%) of participants reported their partners having other sexual partners and more than 80% reported never using a condom during sexual intercourse. Seventy eight percent (78%) reported using hormone contraceptives. Approximately 63% of participants underwent female genital mutilation (FGM). Table 1 shows the socio-demographic and risk factor characteristics of participants, expressed in absolute values and percentages.

Multivariate analysis of HPV Risk Factors

Female genital mutilation (FGM), low annual income, fewer than 12 years of education and partners having other sexual partners were risk factors for HPV infection but not associated significantly with the infection (*P>0.05*). Hormone contraceptive use for >5 years was found to be a risk factor and was associated significantly with HPV infection (AOR 4.2, *P=0.03*) (Table 2). Participants who had their sexual debut at the age of ≥18 years were twice as likely to be infected with HPV (AOR 2.2, *P=0.17*). Being married was found to be a protective factor against HPV infection ; however, stratification analysis (not shown) indicated that married females who had sex in the preceding 12 months without using condoms were at increased risk of HPV infection (AOR 2.1, *P>0.05*). Table 2 shows the risk factor characteristics associated with HPV infection in this study.

HPV Prevalence and genotype distribution

Of the 232 participants with adequate cellular DNA, HPV DNA was detected in 28. The overall HPV prevalence was found to be 12.1% with 9 different HR/ pHR and 7 different LR genotypes identified. Twelve (42.9%) women were infected with a HR-HPV genotype, 4 (15.4%) with pHR carcinogenic types and 12 (42.9%) with LR types. The most prevalent HR-HPV type detected was HPV 52 (17.9%), followed by HPV 51 and 58, each at 7.1% and the most prevalent pHR-HPV was HPV 66 (7.1%). HPV 61 was the most common LR-HPV genotype, accounting for 14.3% of all genotypes.

HPV genotypes were allocated according to the IARC genotype classification using raw sequence data (Table S1) [10]. HPV genotypes with homology differences less than 2% to the closest known genotypes were identified as HPV variant types (99 - 100%) and those between 2% and 10% were identified as subtypes (90 - 98%). All HR/pHR-HPV genotypes identified in this study showed 98 -100 % identity to DNA sequences deposited in the GenBank database except one which was nominally allocated as a subtype of HPV genotype 35, although it showed only 82% identity to a known HPV 35 type (Table 3). The putative HPV 35 sequence was submitted to GenBank (accession number MH844101). Furthermore, none of the HPV sequences identified were homologous to HPV sequences isolated in Africa.

HPV prevalence by age and ethnic group

The HPV age-specific prevalence curve of participants showed a peak in the 21-25 age group (32.1%), followed by a steady decline in the ages between 26 and 40 years, and a sharp decline was seen in later years. However, HR-HPV prevalence was higher in the 26-30 age group (41.7%) and pHR-HPV types was higher in the 21-25 age group (75%) (Table 4). HPV infection was not detected in the 20 year age group. Ethnicity-associated differences were also noted, HR/ pHR-HPV prevalence was higher in the Fulas (31.3%), followed by Mandinkas (18.8%) and a lower prevalence seen in the Wollofs (12.5%). However, LR-HPV genotypes were identified mostly in the Mandinka ethnic group, accounting for more than 40% of the LR types. The prevalence of both HR and LR-HPV genotypes was lower in the Wollofs compared to the other two major ethnic groups. The study also revealed a higher overall HPV prevalence in the Fula ethnic group (32.1%) and this group were

found to be more than twice at risk of HPV infection than the other two major ethnic groups (AOR 2.1, 95% CI 1.0, 4.9) (Table 2).

HPV and co-infection with other sexually-transmitted pathogens

Of the 28 females positive for HPV, 14 (50%) were co-infected with *Ureaplasma parvum/urealyticum*, 5 (18%) with *Candida albicans*, 3 (10.7%) with *Streptococcus agalactiae*, 1 (3.6%) with *Trichomonas vaginalis* and 4 (14.8%) were diagnosed with bacterial vaginosis. Of those positive for *Ureaplasma parvum/urealyticum*, 11 (79%) were infected in both the vagina and cervix, 2 (14%) in the cervix only and 1 (7%) in the vagina only. In addition, of the 14 HPV-positive females that were co-infected with *Ureaplasma*, 7 (50%) were additionally co-infected with either *Candida albicans*, *Trichomonas vaginalis* or were diagnosed with bacterial vaginosis.

DISCUSSION

The introduction of any HPV vaccine prevention strategy requires consideration of the major circulating HR-HPV genotypes in the population. HPV genotyping is very important in primary cervical cancer screening as persistent infections with HR genotypes can progress to cervical cancer, especially in females aged 30 years or older. The Gambia is a small country in West Africa with a population of less than 2 million [21]. Females between the ages 15-65 years represent 52% of the population and most are at risk of being diagnosed with cervical cancer. The quadrivalent vaccine has been introduced in the urban region of the Gambia and this is the first report of HPV genotype distribution where most cervical cancer cases are reported.

Overall HPV prevalence was found to be 12.1%, which is slightly lower than the 13% prevalence reported for rural Gambia, 18% in nearby Dakar, Senegal and 40.8% in Egypt [3, 22, 23]. Of the 28 HPV positive samples in this study, 12 (42.9%) were HR-HPV genotypes and 4 (14.3%) were pHR-HPV genotypes. This is somewhat greater than the HR-HPV prevalence reported for Dakar, Senegal (17.4%) and for south-western Nigeria (19.6%) [24, 25], where both studies targeted women from 18–80 years old. The differences in prevalence seen in these studies could be attributed to the different age groups targeted or, perhaps more importantly could be due to variability of HPV genotypes in different geographical locations. The higher HR-HPV

prevalence seen in this study could also be reflecting selection bias, since cervical samples were collected from individuals who chose to attend a sexual health clinic.

HPV 52 was the most common high risk genotype identified, accounting for 31.3% of the total HR/ pHR-HPV genotypes and 17.9% of all genotypes. HPV 61 was the most frequent LR genotype identified with an overall prevalence of 14.3% and accounting for 33.3% of all LR-HPV. In contrast to earlier work in rural Gambia, where HR-HPV 16 and LR-HPV 42 were the most common genotypes identified, this study showed that 89% of HPV genotypes identified do not match those included in the quadrivalent vaccine [22]. Similarly, work carried out in an urban region of Senegal, the only country to neighbour The Gambia also found that HPV 52 was the most common genotype [25]. The same observation was also seen in studies carried out in Kenya and Tanzania [2, 4, 26].This augments the findings of Bruni *et al* [1] that HPV 52 is a major genotype in Africa.

HPV 16 and 18 are the predominant circulating genotypes found in Southern Africa, Europe and America. However, HPV 16 was found to be the fifth most common HR/ pHR genotype with a prevalence of 6.3%. HR-HPV 18 was detected in none of the samples. Although the burden of cervical cancer is higher in Africa compared to Europe and America, HPV 16 and 18 seems to lose its predominance as the major circulating genotype in some parts of Africa. Studies in Africa have shown that other HR genotypes such as HPV 31, 35 and 58 are major circulating genotypes [27-30], indicating that the HPV bivalent and quadrivalent vaccine may not be as effective in Africa as previously thought [31-33]. Considering the high burden of cervical cancer cases and the lower prevalence of HPV 16 and 18 in Africa, it could be that other HR-HPV genotypes may be responsible for the high burden of the disease. In addition, a study in Asia found that HR-HPV 52 and 58 genotypes (3.8% and 5.6% respectively) were associated with a number of cases of invasive cervical cancer and high squamous intraepithelial lesions (HSIL) [34]. This finding further asserts the importance of determining the major circulating genotypes in a population before introduction of the HPV vaccine and is an important step in effective HPV infection prevention strategies.

It was shown here that 10.7% of participants were infected with HPV genotypes targeted by the quadrivalent vaccine and 35.7% were positive for HPV genotypes

included in the Gardasil 9 vaccine. This data may have important public health implications as the HPV quadrivalent vaccine may be of limited value for The Gambia if the circulating non-HPV 16, 18 HR-genotypes are responsible for cervical cytological abnormalities and progression to cervical cancer.

DNA sequence analysis has shown that none of the HR/pHR-HPV genotypes detected were homologous to isolates from Africa found in the GenBank database, but rather isolates from America, Asia and Europe. This indicates that it is possible that these HR/pHR-HPV genotypes were imported into The Gambia (Table 3). This highlights a key difference with an earlier study in rural areas which found that many of the HR-HPV sequences were homologous to isolates from Africa [22]. Contributing factors may be linked to the fact that the urban area is a popular tourist destination therefore the lifestyle and sexual behaviour of the participants may be different. The isolate nominally allocated to HPV 35 may be a previously unrecognised type as the partial L1 gene sequence differed by more than 10% to the closest match, a HPV 35 genotype isolated in Ecuador (Table 3). Further work is required to determine this.

Infection with HPV is common in young females; however most of these infections are transient and regress within 12 months, with only a small percentage developing persistent infection [3, 35]. The high HPV prevalence peak seen in the 21-25 age group follows population norms of sexual initiation as 77.8% of the participants had their sexual debut at the age of ≥18 years. A sharp decline in prevalence was observed in those greater than 40 years old, which is consistent with viral transience. A similar finding was also observed in Abuja, Nigeria [27]. Studies carried out in Africa and Asia have reported a biphasic or a flat shaped HPV age-specific curve in older ages [23, 36]. However, 41.7% of the HR-HPV genotypes were found in the 26-30 age group, which highlights the importance of early and regular HPV and cervical cancer screening. The study data also revealed that 93% of participants had never had cervical cancer screening (Table 1). This may be due to either lack of awareness about cervical cancer or accessibility to screening, or both. The Gambia Histopathology Laboratory is situated at EFSTH, Banjul and it is currently the only laboratory offering cytology in the country. In addition, there are no decentralised

national cervical cancer screening programmes therefore making access to screening a significant problem.

Whilst HPV infection plays a vital role in cervical cancer development, other socio and risk co-factors appear to contribute to the increased risk of disease progression. Bosch *et al* [37] have also showed that females who used hormone contraceptives for more than 5 years are at increased risk for developing cervical cancer and this work supports this assertion (Table 2). However, association studies on HPV positive females and long term use of hormone contraceptives have failed to reach consensus [38]. There is a potential association between HPV infection and prolonged use of hormone contraceptives in the development of cervical cancer, which needs addressing with a larger study population.

It was found that 98.9% of participants whose partners have other sexual partners are at increased risk of being infected with HPV (AOR 3.5; *P=0.30*) but not associated significantly with HPV infection (Table 2). Married participants who had sexual intercourse in the last 12 months without using condoms were found to be twice at risk of acquiring HPV infection when compared with unmarried participants (AOR 2.1, *P>0.05*). This interaction may be linked to polygamy, which is a common practice in The Gambia and Africa and has implications of increased frequency of sexual activity with more than one partner. In The Gambia, 39% of females live in a polygamous union with one or more co–wife [39], which increases the risk of acquiring and transmitting HPV. Furthermore, 91.7% (22/24) of participants who were HPV-infected reported not using a condom during sexual intercourse in the preceding 12 months. The majority (80.6%) of the respondents were married and are less likely to report using condom during sexual intercourse than unmarried women. Another contributing factor could also be poor negotiating power with their partners on condom use during sexual intercourse, especially those in polygamous relationship. However, using condoms in the preceding 12 months was found to be a protective factor against HPV infection (AOR 0.7, 95% CI 0.2-35).

As also reported by Wall *et al* [22], this study found that HPV infection was higher in the Fula ethnic group and this group were significantly more susceptible to HPV infection (AOR 2.1; *P=0.15*) (Table 2). Similarly, Sighoko *et a*l [16] also noted an ethnicity variation in their study on cervical cancer in The Gambia. They found the

Fula ethnic group were more at risk of being diagnosed with cervical cancer compared to the other ethnic groups. The differences seen in the prevalence of HPV infection in the different ethnic groups may be linked to possible genetic factors as previously reported [16, 22, 40], or FGM being a predisposing factor. FGM is a common cultural practice amongst certain ethnic groups of The Gambia and more than 50% of females have undergone FGM before the age of 5 years. However, 75% of females aged 15-49 years had undergone FGM in the Gambia with slightly higher burden of 79% seen in the rural area compare to 72% in the urban area [39]. Data on FGM showed that all the Fula (9/9) females who were infected with HPV underwent FGM. In The Gambia, Wollof females are least likely to have had FGM and were found to be at reduced risk for HPV infection (AOR 0.5; *P=0.35*). In contrast to male circumcision, which is thought to be a protective factor against HPV infection in males and their female partners [41, 42], this study showed that participants that have undergone FGM were twice likely to be at risk of being diagnosed of HPV infection (AOR 2.1; *P*=0.12), however FGM was not found to be associated significantly with HPV infection. Similarly, studies of Senegalese and Malian females also found FGM to be a risk factor for HPV infection [43, 44]. FGM is practised in many African countries especially in north-eastern Africa where HPV and cervical cancer burdens are high. The association between FGM and HPV infection could be a result of genital tissue damage leading to chronic inflammation making these females more susceptible to infection. Furthermore, since most females with FGM are susceptible to recurrent genital infections, this can result in an impaired immune response and therefore can lead to an inability to clear HPV infection.

It was shown that 50% of participants infected with HPV were co-infected with *Ureaplasma parvum*/*urealyticum*. Others have also found a high prevalence of *Ureaplasma* in females with high grade squamous intraepithelial lesions (HSIL) compared to those with normal cytology [45, 46]. Although *Ureaplasma parvum*/*urealyticum* infections are known to be sexually-transmitted, they are often not diagnosed and treated. These microorganisms can cause chronic pelvic inflammatory disease and infertility if left untreated. In addition, *Ureaplasma* can damage the vaginal epithelium and causes cervical mucus degradation thus potentially facilitating HPV progression to cervical cancer [47]. This work adds to the

body of evidence that *Ureaplasma* infection is an important co-factor in HPV infection and highlights the importance of testing for *Ureaplasma parvum*/*urealyticum* and providing appropriate treatment to HPV-infected females, especially those with abnormal cytological results.

In conclusion, there is an apparent difference between the major, circulating HR-HPV genotypes in urban and rural areas of The Gambia; however, both studies underscore the need for a multivalent vaccine that targets all major HR-HPV genotypes in the general population. Although, the quadrivalent vaccine has been piloted in The Gambia, this study raises important public health issues with HPV vaccination programmes in developing countries. The introduction of accessible HPV DNA testing and cytology screening would be beneficial to Gambian women in cervical cancer prevention. In this work, participants were not screened for cervical cancer and future studies to investigate HPV genotype distribution from cervical cancer specimens would be necessary for enhanced cervical cancer intervention strategies in The Gambia.

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Ethical considerations

Ethical considerations were reviewed and approved by The Gambia Government and Medical Research Council Joint Ethics Committee, Gambia and the University of Westminster Research Ethics Committee, London.

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Table 1. Univariate analysis of socio-demographic and risk factors of study participants (n= 232)

Characteristics	Number	Percentage (%)
Residence		
Banjul	104	44.8
KMC /WCR	128	55.2
Age group (years)		
20	7	3.0
21-25	53	22.8
26-30	57	24.6
31 -35	46	19.8
36-40	33	14.2
>40	36	15.5
Ethnic group		
Mandinka	57	24.6
Fula	49	21.1
Wollof	42	18.1
Jola	20	8.6
Serere	30	12.9
Others	34	14.7
Education level		
None	37	16.0
Primary	38	16.4
Secondary	99	42.7
College	13	5.6
Quaranic studies	45	19.4
Occupation		
House wife	61	26.3
Petty trading	71	30.6
Business	37	16.0
Civil servant	24	10.3
Others	39	16.8
Annual Income		
<D75,000 (USD 1,563)	180	77.6
>D75,000 (USD 1,563)	52	22.4
Marital status		
Married	187	80.6
Single	45	19.4
Age of sexual debut*		
<18 years	75	32.8
≥18 years	154	67.2
Life time sexual partner(s)*		
1	138	60.5
≥2	91	39.7
Partner(s) have other sex partners**		
Yes	84	60.8
No	52	37.7
Don't know	2	1.4
Condom use (last 12 months)		
Yes	22	10.4
No	189	89.6

FGM		
Yes	145	62.5
No	87	37.5
Past screening for cervical cancer		
Yes	14	6.1
No	218	93.7
Family member diagnosed with cervical cancer		
Yes	5	2.2
No	227	97.8
Hormone contraceptive use		
Yes	181	78.0
Never	50	21.6
Yes, but stopped	1	0.4

*3 Participants reported never having a sexual relationship

**Only participants that reported having 1 lifetime sexual partner were asked this question

KMC/WCR - Kanifing Municipal Council / West Coast Region

Table 2. Risk factor characteristics associated with HPV infection

Characteristics	HPV DNA results		Adjusted OR (95% CI)	*P*-value
	Positive (28)	Negative		
Age group				
<21	0	7		
21-25	9	44	1.5 (0.6, 3.6)	0.52
26-30	7	50		
31-35	5	41		
36-40	5	28		
>41	2	34		
Ethnic Group[#]				
Fula	9	40	2.1 (1.0, 4.9)	0.15
Mandinka	8	49	1.2 (0.4, 2.7)	0.94
Wollof	3	39	0.5 (0.1, 1.7)	0.35
Age of sexual debut				
≥18 years	23	131	2.2 (0.8, 6.2)	0.17
<18 years	5	70		
Marital status				
Married	23	164	0.9 (0.3, 2.5)	0.91
Single	5	40		
FGM				
Yes	21	124	2.1 (0.9, 5.7)	0.12
No	7	80		
Education				
<12 years	11	109	2.0 (0.9, 4.5)	0.16
≥ 12years	17	95		
Lifetime sexual partner(s)				
≥2	14	77	1.8 (0.8, 4.1)	0.23
1	14	124		
Partner(s) have other sex partners				
Yes	13	99	3.5 (0.4, 28)	0.30*
No	1	27		
Hormone contraceptive use				
>5 years	13	61	4.2 (1.3, 13.6)	0.03
<5years	10	97		
Low income				
<D75,000 (USD1563)	20	160	1.7 (0.5, 5.5)	0.51
>D75,000 (USD1563)	8	44		
Condom use in last 12 months				
Yes	2	20	0.7 (0.2, 3.3)	0.95*
No	23	166		

[#] Major ethnic group, *Fisher's exact test used due to sample size

661 Mantel Haenszel odds ratio- adjusted for age

662 **Table 3** Comparison of HR/pHR HPV DNA sequences from this study with isolates
663 deposited in the GenBank database

HPV genotype	GenBank Accession Number	Isolate Number	Origin	Percentage similarity to Gambian samples
16	KY549284	C484604r11164343NP	Netherlands	98
35	KU050113	ECU-08	Ecuador	82
51	KF707619	R60	Switzerland	98
	KJ676061	R72	Switzerland	99
52	KF707618	CRO 1F6	Croatia	99
	EU077215	23	Canada	100
	EU077215	23	Canada	100
	EU077215	23	Canada	100
	KY077858	KOR_M10- 4515	South Korea	99
53	KU951263	CN10	China	99
56	KU298919	110A.56	Brazil	99
58	HM63967	ww100HK_973	Hong Kong	99
	HQ537776	Rw644	New York, USA	99
66	KU298927	83A.66	Brazil	98
	KU298928	118A.66	Brazil	98
73	KU298936	58c.73	Brazil	99

664

Table 4. HPV genotype distribution amongst the different age groups

	Age group (years)				
	21-25	26 -30	31 - 35	36 - 40	41 - 49
HPV genotype	6	**51**	**16**	6	42
	35	**52**	**51**	**52**	54
	52	**52**	**58**	61	
	53*	**52**	61	61	
	54	**58**	62	83	
	56	61			
	66*	66			
	73*				
	89				

Genotypes: Bold; high risk HPV, *; probable high risk HPV and non-bold; low risk HPV. None of the 20 years old age group were infected with HPV.

Figure S1. Participant consent form used in this study

PARTICIPANT CONSENT FORM

Project Title: **Human Papilloma Virus co-infection with Sexually Transmitted Pathogens amongst women of reproductive age in urban Gambia**

Statement by subject

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand*

and I

confirm that my choice to participate is entirely voluntarily,

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

understand that I grant access to identifiable data about me to authorised persons described in the information sheet,

am aware that part of my sample will be taken abroad for further analysis

agreed for my sample to be stored for future research

have received time to consider to take part in this study,

agree to take part in this study.

Participant Details

Participant Identification Number: |__|__|__|__|__|__|__|__|__|__|__|__|

Age: …….

Contact number…… ---------- - - - - - - - - - - - - - -

Signature/Thumbprint of volunteer:

Date …….

** Only required if the participant is unable to read or write*

This form has been read by / I have read the above to

_ (Write name of volunteer)

in a language that she understands. I believe that she has understood what I explained and that she has freely agreed to take part in the study.

Signature of field worker: _

Name of field worker: _

Date: |___|___| / |___|___| / |___|___|___|___|

Contact for further information

If you have any problem or query about any aspect of the study at any time, please do not hesitate to contact the researcher or the Hospital Public Relation Officer (PRO) on the contacts given below:

[redacted]

A copy of this consent document has been provided to the participant.

Figure S2. Participation Information sheet used in this study.

PARTICIPANT INFORMATION SHEET

Project Title: **Human Papilloma Virus co-infection with Sexually Transmitted Infection Pathogens amongst women of reproductive age in urban Gambia**

You are kindly invited to take part in a research project, designed by Haddy Bah Camara of Edward Francis Small Teaching Hospital (EFSTH), Banjul, The Gambia in collaboration with the University of Westminster (UoW), London, UK. Mrs. Haddy Bah Camara and the study team will provide you with all the information concerning the project and your participation. Do not hesitate to contact the study team if there is anything you do not understand. You can confirm your participation by signing or thumb-printing the consent form below.

What is the purpose of the study?

The purpose of this study is to determine whether women infected with sexually transmitted bacteria will also be infected with Human Papilloma Virus, which can cause cervical cancer. The study involves collaboration with Edward Francis Small Teaching Hospital, Gambia and University of Westminster, UK

The results from the study will help in making health policies on HPV and STI management in Gambia; provide education to the community and serve as foundation for future researches on HPV in Gambia.

What will you be asked to do?

Participation in this study is voluntary. Swab or blood specimens will be collected from you as part of your routine clinic appointment. A sterile cotton swab will be introduced into your cervix (womb) to collect the sample or a sterile syringe and needle will be introduced into your vein to collect a venous blood sample (where applicable). This will be done by an experienced staff of the clinic in the safest way possible. You have every right to withdraw from the exercise if you are uncomfortable or unwell.

Risks and Discomfort

It is very unlikely that there will be any side effects for taking part in the study. You may experience slight discomfort when swab / blood sample are being taken. However this will be done in the safest way possible.

Why have I been ask to participate?

You have been asked to participate because of your present condition and history of infection /Non existing infection (Family Planning client)

Do I have to take part?

Participation is voluntary. However, if you decide to take part, a copy of this information sheet will be given to you to keep. You are free to withdraw at any time without giving reasons and can request the removal of your sample from the study. Moreover your decision to withdraw will not affect the health care you receive.

What do I have to do?

In order to be recruited for the study, you are kindly requested to answer some few questions below. You can then confirm your participation by signing/ thumb printing the consent form below.

Confidentiality

This is a student research project which may be published. In the course of the project and in the event of subsequent publication, your participation and any other personal details will be kept highly confidential. Your sample will be given a specific research number and anonymized. Access to identifiable data will be held in Gambia only by your respective health provider, who has access to your information. Dr Patrick Kimmitt, Dr Edward Wright and Haddy Bah Camara will only handle anonymized samples with no bearing to your identity.

Expenses and Payments

Participation is entirely voluntary and as such there will be no payment for your participation in the study.

Fig. S3 Questionnaire used in this study to capture socio-demographic and HPV risk factor data

QUESTIONNAIRE

Please allow me 15 minutes of your time to answer the following questions. Your genuine answers will help the health care provider to make corrective decisions in providing quality health care. Your answers will be kept confidential and known only to the care provider. Your identity will not be distributed, published or sold.

1. Health facility

Name……. Type…….

2 Participant Identifier number …….

3 What is your date of birth? : Age in Years (…….)

4. Region……District……

5. What is the purpose of your visit today?

__ Family Planning visit (go to Question. 7)

__ STI visit, (go to Question. 6)

__ HIV management visit (go to Question. 7)

6. . Do you have any of the following symptoms today? (**Tick)**

__Vaginal itching\ discharge with fishy or strong odour

__Pain or burning when urinating

__bleeding between periods

__Pain during sex

__Sores, skin rashes with rough red or reddish brown spots on hands or feet

__Genital warts

__ Lower abdominal pain

__Others (specify) …….

- If YES to any of the questions, find out how long she has/had the symptoms------

SECTION B: Socio economic background

7. What is your ethnicity? (**Tick)**

__ Wollof

__ Mandika

__ Jola

__ Serere

__ Sarahule

__ Fula

__ Others (Specify)

8. What is the highest level of education you have completed? (**Tick)**

__ None

__ Primary level

__ Secondary level

__ College

__ University

__ Quaranic studies

9. What is your current occupation? (**Tick)**

__ Student

__ Petty trader

__ Business woman

__ Civil servant

__ House wife

__ Farmer

__ Others (specify)

10. How many people are currently living in your household, including yourself? ___

a. Of these people, how many are children ≤18 years old? ___

11. Which of these categories best describes your total combined family income for your household for the past 12 months? (**Tick)**

__ <D10, 000

__ D 10,000 - 35,000

__ D35, 000 - 50,000

__ D51, 000 – 65,000

__ D66, 000 – 75,000

__ D 76,000 – 85,000

__ D85, 000 - 100,000

__ > D100, 000

__ Don't Know / Not sure

12. Have you ever smoked cigarettes? (**Tick)**

__ Yes (How many a day?) Specify_

__ No

__ Quit

13. Have you ever been screened for cervical cancer? (**Tick)**

__ Yes

__ No

__ Don’t Know

__Not sure

14. Have any of your family members been diagnosed with cervical cancer? (**Tick)**

__ Yes

__ No

__ Don’t Know

15. This visit, swab samples will be collected from you by a health care provided, but would you have preferred to collect the samples yourself?

__ Yes.(if yes, why?)

- Embarrassment
- Others ……. (Specify)
- Don’t mind either way

__ No

SECTION C: Reproductive and Hormonal factors

B. Menstrual periods

16. At what age did your menstrual periods begin? (**Tick)**

__ 10 years

__ 11 years

__ 12 years

__ 13 years

__ 14 years

__ 15 years

__ 16 years

__ 17 years or older

17. Have you had a menstrual period within the last 12 months? (**Tick)**

__ Yes, I still have a menstrual cycle (go to question18)

__ Yes, but my menstrual cycle stopped within the last year

__ No, my menstrual cycle stopped more than one year ago

__ Don’t know

18. When was your last menstrual period?

|___||___| / |___||___||___||___| OR |___||___|

Month YEAR AGE

__ Don’t know

19. Which of these best describes why your menstrual cycle stopped? (**Tick)**

__ Breastfeeding

__ Birth control or medications

__ Natural menopause

__ Surgery to remove the uterus or ovaries

__ Other (specify) _____

__ Don’t know

Pregnancy

20. Have you ever been pregnant? (**Tick)**

__ No (go to question 24)

__ Yes

__ Don’t know (go to question 24)

21. How old were you when you first became pregnant? (**Tick)**

__ Less than 15 years

__ 15-19 years

__ 20-24 years

__ 25-29 years

__ 30-34 years

__ 35-39 years

__ 40-44 years

__ 45 years or older

__ Don’t know

22. How many times have you been pregnant? Please include stillbirths, miscarriages, abortions, tubal or ectopic pregnancies, and live births. (**Tick)**

__ 1

__ 2

__ 3-4

__ 5-9

__>10

SECTION D. Contraceptive use

23. Did you ever take birth control pills for birth control or to regulate menstrual periods? (**Tick)**

__ No, never (go to question 28)

__ Yes

__ Yes, but stopped taking them now (go to question 25 & 26)

24. How old were you when you first started taking birth control pills? (**Tick)**

__ less than 15 years

_ 15-19 years

__ 20-29 years

__ 30-39 years

__ 40-49 years

25. How old were you when you last took birth control pills? (**Tick)**

__ Still taking birth control pills

__ Less than 15 years

__ 15-19 years

__ 20-29 years

__ 30-39 years

__ 40-49 years

__ 50 years or older

26. How many total years of birth control pills have you used? (**Tick)**

__ less than 5 years

__ 5-9 years

__ 10-14 years

__ 15-19 years

__ 20-24 years

__ 25-29 years

__ 30-34 years

__ 35 years or more

**Could repeat 14-17 for injectable contraceptive or shot, contraceptive hormonal patch, vaginal ring, intrauterine device, other (specify)*

SECTION E. WOMAN'S SEXUAL HISTORY

The next questions are about your sexual history. I realize this is a personal subject, but it is very important to the study. Please take the time to recall the information as accurately as possible. I want to remind you that this is a private interview and that the information you give me will not be linked to your name.

27. Are you married? (**Tick)**

__ Married*, (Is this your first married)?

__ Yes (go to question 28)

__ No (How many times have you been married?), Specify__

__ Single

__ Divorced

__ Separated

__Widow

28. Have you been circumcised? (**Tick)**

__ Yes

__ No

__ Don't Know

29. How old were you when you **first** had sexual intercourse with a man?

|__|__|AGE

__NEVER HAD INTERCOURSE

30. Throughout your life, with how many different men have you had sexual intercourse with?

|__|__|
No. of Men

__Don't know

BOX E1
IF NUMBER OF MEN = 1 ……..CONTINUE
IF NUMBER OF MEN = GREATER THAN 1 .....Q.34
IF DON'T KNOW Q.35

---

31. Did your partner have any other sexual partners besides yourself, either before he met you or during the time you were together? (**Tick)**

__ Yes

__ No

__ Don't Know

32. Besides yourself, how many sexual partners would you say he had?

|__|__|

NUMBER OF PARTNERS

__ Don't Know

33. Would you say it was . . . (READ)

__ 2 or 3,

__Between 4 and 6,

__Between 7 and 10

__ More than 10

__ DON'T KNOW

34. Within the last year, have you had sexual intercourse?

__ Yes (go to 34b)

34b. Does your partner (s) use condoms during sexual intercourse?

---Yes

----No

__ No

35. During the last year, what is the total number of men with whom you have had sexual intercourse?

|__|__| (go to question 38)

NUMBER. OF MEN

__DON'T KNOW

36. Would you say it was...? (READ)

__ 1

__ 2

__ between 3 and 5

__ >5

37. Within the last year, were any of these partners new partners, that is, partners with whom you had sexual intercourse for the first time?

__ Yes

__ No

38. With how many new partners did you have sexual intercourse in the last year?

|__|__|

NUMBER OF NEW PARTNERS

__ DON'T KNOW

39. Would you say it was……. (READ)

__ 1

__ 2

__ between 3 and 5

__ >5

Thank you for your participation.

Table S1. Raw sequence data of high and low risk HPV genotypes identified by DNA sequencing and nucleotide BLAST search (L1 gene, 450bp)

HR- High risk genotype; pHR – Presumptive high risk genotype; LR- Low risk genotype

HPV 16 HR

ATaatnatCTTCtaGTGTGCCTCCTGgaggAGGTTGTAAACCAAAATTCCAGTCCTCCAAAAT
AGTGGAATTCATAGAATGTATGTATGTCATAACGTCTGCAGTTAAGGTGATTTTGCACAG
TTGAAAAATAAACTGTAAATCATATTCCTCCCCATGTCGTAGGTACTCTTTAAAGTTAGTA
TTTTTATATGTAGGTTCTGAAGTAGATATGGCAGCACATAATGACATATTTGTACTGCGT
GTAGTATCAACAACAGTAACAAATAGTTGGTTACCCCAACAAATGCCATTATTgTGGCCC
TGCGcaaaa

HPV 35 HR

GCCGGCGCCGGCGACAAACCCAGAAAAACATCCCCCCCTCTGTTCCCTCTGCACACCC
CCCTATAGAAAATTCCCCTTTTTTTGTCCCCCTCCACACCCCCCTCACACAACCATATCT
TTCCTCTTATCTCCCCCAACGCCCTGCGTCAAGGCCAAAATTCCAATCCTCTCCAATCT
CACGCCTTCCTACTATCAATATATGTCTTATCCTCTTCTGTTACTCTTCTTTTACATAACT
GAAAAATAAACTGTACATCATATTCTTCTCCATGCCTTAAATATTCCTTACAATTATCTTTT
TTATATATACGGACACTACAATACTCAACATAACACACAAACATATTTATTCTACGCCTTC
TTTCATCTACCGTAAACAACAGATAATTACTCCTATTTTTCTGTCTTTTAGTGTGATCATA
GACGTGAATCTCTAAATGAAGCTCGTGGGACCGTCTATTGTGGATCTGCGGTTAGAGC
CGGGGCGTGTCATTAAAGGGTGTATCTGGTTAGAGGTCCCC

HPV 51 HR

AGTACAAATTTAACTATTAGTACTGCCACTGnnnnnntTTCCCCAACATTTACTCCAAGTAA
CTTTAAGCAATATATTAGGCATGGGGAAGAGTATGAATTGCAATTTATTTTTCAGTTATG
TAAAATTACTTTAACTACAGAGGTAATGGCTTATTTACACACAATGGACCCTACCATTCT
TGAACAGTGGAATTTTGGATTAACATTACCTCCGTCTGCTAGTTTGGAGGATGCATATAg
GTTTGttCgAAATGCAGCCACCAgCTGTCAAAAGGACACCCCTCCACAGGCTAAGCCAGA
TCCTTTGGCCAAATATAaattTtGGGATGtTGATTTAAAGGAACGGTTTTCgtTAgATTTAGAC
cAatTTGCATTGgGtCGCA

HPV 51 HR

CGgaggtAatGttaatCcAAAATTccactgTTCAagAaTGGTaggATCCAttgngtgtAAAtangCcaTtAC
CtctgtagTtAAagtaaTTTTGCATAactgAAAAaTAAATTgCAATTCatactcTtCCCcAtgcctAATATA
TTgCTtAAagTtacttggagtAAAtGTTGGGGAAACCGCAGCagtggcagTGCTAATagTtAAATTTG
TACTTctGgTAGTATCAACACAGGTAATAAAAAGCTGATTGTTCCAGCAAATGCCATTATT
GTGGCCcTGCGCAgtGCa

HPV 52 HR

1247 AcntcccaaanTATAGTCCTTtaaGGATCTTCCTTTCCTTTaGGTGGTGTGTTTTTTTGACATG
1248 TtATAGCAGTAGAAGTGACAAATCTGTATGTGTCCTCCAAAGATGCAGACGGTGGTGGG
1249 gTAAGGCCAAATTGCCAGTCCTCTAAAATAGTGGCATCCATCTTATGAATGTATGTCATA
1250 ACATCAGCTGTTAATGTAATTTTGCACAATTGAAAAATAAATTGTAAATCAAATTCCTCGC
1251 CATGACGAAGGTATTCCTTAAAATTTTCATTtttatATGTGCTTTCCTTTTTAACCTCAGCAC
1252 ATAAAGTCATGTTAGTGCTACGAGTGGTATCCACAACTGTGACAAACAACTGATTGCCC
1253 CAACATATGCCATTATTGTGGCCCTGCGc

1254

1255 HPV 52 HR

1256 atccaCcTCCcAanCnTATAGTCCTTtaaGGATCTTCCTTTCCTTTaggTGGTGTgTTTTTTTGA
1257 CAAGTTATAGCAGTAGAAGTGACAAATCTGTATGTGTCCTCCAAAGATGCAGACGGTGG
1258 TGGGGTAAGGCCAAATTGCCAGTCCTctAAAATAGTGGCATCCATCTTATGAATATATGT
1259 CATAACATCAGCTGTTAATGTAATTTTGCACAATTGAAAAATAAATTGTAAATCAAATTCC
1260 TCGCCATGACGAAGGTATTCCTTAAAATTTTCATTTTTATATGTGCTTTCCTTTTTAACCT
1261 CAGCACATAAAGTCATGTTAGTGCTACGAGTGGTATCCACAACTGTGACAAACAACTGA
1262 TTGCCCCAACATATGCCATTATTGTggCCcTGCGc

1263

1264 HPV 52 HR

1265 cngTTTTTTTGACAAGTTATAGCAGTAGAAGTGACAAATCTGTATGTGTCCTCCAAAGATG
1266 CAGACGGTGGTGGGGTAAGGCCAAATTGCCAGTCCTCTAAAATAGTGGCATCCATCTT
1267 ATGAATATATGTCATAACATCAGCTGTTAATGTAATTTTGCACAATTGAAAAATAAATTGT
1268 AAATCAAATTCCTCGCCATGACGAAGGTATTCCTTAAAATTTTCATTTTTATATGTGCTTT
1269 CCTTTTTAACCTCAGCACATAAAGTCATGTTAGTGCTACGAGTGGTATCCACAACTGTGA
1270 CAAACAACTGATTGCCCCAACATATGCCATTATTGTGGCCCTGCGcAa

1271

1272 HPV 52 HR

1273 TTTttnGAcAAGttATAGCagtagaagtGACAAAtctGTATGTGTCCTCCAAAGATGCagACGGtgg
1274 TGGGgtAAGGCCAAATTGCCAGTCCTCTAAAATagtGGCATCCATCttATGAATGTATGTCA
1275 TAACATCAGcTGTTAATGTAATTTTGCACAATTGAAAAATAAATTGTAAATCAAATTcctCG
1276 CCATGACGAAGgTATTCCTTAAAATTTTCATTTTTATATGTGCTTTCCTTTTTAACCTCAG
1277 CACATAAAGTCATGTTAGTGCTACGAGTGGTATCCACAACTgtGACAAACAACTGATTGC
1278 CCCAACATATGCCATTATTGTGGCCCTGCGc

1279

1280 HPV 52 HR

1281 gtCcngTTGTGGaTnACcaCTCGTagcACtaaCATGACTTTATGTGCTGAGGttAAAAaGGAAA
1282 GCACATATAAAAATGAAAATTTTAAGGAATACCTTCGTCATGGCGAGGAATTTGATTTAC
1283 AATTTATTTTTCAATTGTGCAAAATTACATTAACAGCTGATGTTATGACATATATTCATAA
1284 GATGGATGCCACTATTTTAGAGGACTGGCAATTTGGCCTTACCCCACCACCGTCTGCAT
1285 CTTTGGAGGACACATACAGATTTGTCACTTCTACTGCTATAACTTGTCAAAAAAACACAC

CACCTAAAGGAAAGGAAGATCCTTTAAAGGACTATATGTTTTGGGAGGTGGATTTAAAA
GAAAAGTTTTCTGCAGATTTAGATCAGTTTccTTTAGGTCGa

HPV 56 HR

tgtagtaganncTACTAGAAGTacTAACATGACTATTAGTACTGCTACAGAACaGTTAAGtAAAT
ATGATGCACGAAAAATTAATCAGTACCTTAGACATGTGGAGGAATATGAATTACAATTTG
TTTTTCAATTATGCAAAATTACTTTGTCTGCAGAGGTTATGGCATATTTACATAATATGAA
TGCTAACCTACTGGAGGACTGGAATATTGGGTTATCCCCGCCAGTGGCCACCAGCCTA
GAAGATAAATATAGATATGTTAGAAGCACAGCTATAACATGTCAACGGGAACAGCCACC
AACAGAAAAACAGGACCCATTAGCTAAATATAAATTTTGGGATGTTAACTTACAGGACAG
TTTTTCTACAGACCTGGATCAATTTCCACTAGGTcg

HPV 58 HR

aTaccaCTCgtagcACtAaTATGACAttATGCACTGAAgtaactAAAGAagATACAtatAAAAATaatA
aTTttAAGGAATATgtAcGtCatgTtGAAGAATATGACTtaCagTTtGTTTTTCAGCTTTGCAAAAT
TACACTAActgCAGAGgtAATGACATATATACATACTATGAATTCAGATATTTTGGAGGaCT
GgcAATTTGGTTTAACACCTCcTCCgtCTGCCaGTTTACAGGACACATATAGATTTGTTACC
TCCCAGGCTATTACTTGCCAAAAAACAGCACCCCCTAAAGAAAAGGAAGATCCATTAAA
TAAATATACTTTTTGGGAGGTTAACTTAAAGGAAAAGTTTTCTGCAGATCTGGATCAGttcc
TTtnGGGACg

HPV 58 HR

gtanttaCTCCAAagTATATTtATTtAaTGGATCTTCCTTTTCTTTaGGGGGTGCtgTTTTTtGGC
AAgtAATAGCCTGGGaggTAACAAATCTATATGTGTCCTgtAAACTGGCAGACGGAGGaGG
TGTtAAACCAAATTGCCAGTCCTCCAAAATATCTGAATTCATAGTATGTATATATGTCATT
ACCTCTGCAGTTAGTGTAATTTTGCAAAGCTGAAAAACAAACTGTAAGTCATATTCTTCA
ACATGACGTACATATTCCTTAAAATTATCATTTTTATATGTACCTTCCTTATTTACTTCAGT
GCATAATGTCATATTAGTGCTACGAGTGGTATCAACCACGGTAACAAATAATTGATTGCC
CCAGCAAATGCCATTGTTATgTCCCTGTGc

HPV 66 pHR

CtnCCcAaacTtataTTTAGccaggnaTCCTGCTTTTCTGCaGGGGgcngctnCCCTctGacaTgtaat
aGCtgtgCTTttaataTACcTataTTTATCCtctAAGctaGTtGCAActggtGGGgATAAGCCAATATTC
CAAtCGtCTAATAAAGtATTattCATATTATGCAAATATGCCatAaCTTCTGCAGTTAAGGTTA
TTTTACAAAGTTGAAACACAAACTGTAGTTCATATTCCTCCACATGGCGAAGGTATTGAT
TGATTTCACGGGCATCATATTTAGTTAATGTGCTTTTAGCTGCATTAATAGTCATGTTGG
TGCTTCTGGTAGTATCCACAACAGtAACAAATACCTGATTACCCCAGCATATGCCATTAT
TATgtCCCTGTGcnca

HPV 66 pHR

aancTtATATTTaGccaGgggatCCTGCTTTTCTGCAGGGGGctgnCCcntCTGACATgtaaTAGC
TGTGCTTttAaTATACCTATaTttaTCCTCtAAGcTaGTTGCAACTggtGGGGATAATCCAatATT
CCAATCGtCTAATAAAGTATTATTCATATTATGCAAATATGCCATAACTTCTGCAGTtAaGG
TTATTTTACAAAGTTGAAACACAAACTGTAGTTCATATTCCTCCACATGGCGAAGGTATT
GATTGATTTCACGGGCATCATATTTAGTTAATGTGCTTTTAGCTGCATTAATAGTCATGTT
GGTGCTTCTGGTAGTATCCACAACAGTAACAAATACCTGATTACCCCAGCATATGCCATt
AtnaTGTCCCTGTGCa

HPV 53 pHR

cAAanTtnaaTttaGATAGTGGGTCcnnCTTTTcaGGaGGggActgcaTCcTTTTGACAGGTTATA
GCTGCACTTTTTACATATCTGTATTTGTCCTCTAAGCTAGTGGCAACAGGAGGCGACAA
ACCTATATTCCAGTCTTCCAGTAAGGTAGAATTCATAGTATGTAAATAGGCCATAACCTC
AGCAGACAGGGATATTTTACATAGTTGAAACACAAATTGTAATTCATATTCCTCTGCATG
CCTAACATACTGTTTAATTTGCTTTGAATTATATGTGGACATagACTGTGTGGTTGCAGAA
AGAGTCATGTTTGTATTCCTGGTGGTATCCACAACAGTTACAAATAACTGATTGTTCCAA
CAGATGCCATTATTATGTCCCTGTGCA

HPV 73 pHR

gtttGATTTACaGtTTGTTTTTCAGTTATGTAAAATTAGTTTAACTACTGAGGTAATGACATAT
ATACATTCTATGAATTCTACTATATTGGAAGAGTGGAATTTTGGTCTTACCCCACCACCG
TCAGGTACTTTAGAGGAAACATATAGATATGTAACATCACAGGCTATTAGTTGCCAACGT
CCTCAACCTCCTAAAGAAACAGATGACCCATATGCCAAGCTATCCTTTTGGGATGTAGA
TCTTAaagaAaAGTTTTCTGCAgAATTAGACCAgTTTCCCTTGgGTCg

HPV 6 LR

ccTTTtCAggAntGggCTTTTGACaGgtaatGgccTGTGACTGcACATACCTATAGGTATCTTCTA
ATGTACCATTTGGGGGAGGCGATAACCCAAAGTTCCAGTCTTCCAAAACAGAGGGATTC
ATTGTGTGAATATAGGCCATTACTTCAGCAGACAATGTAATGCTACATAATTGAAAAATA
AATTGTAAATCATACTCTTCCACATGACGCATGTACTCTTTATAATCAGAATTGGTGTAT
GTGGAAGATGTAGTTACGGATGCACATAATGTCATGTTGGTACTGCGTGTGGTATCTAC
CACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGTCCCTGTGcaaa

HPV 6 LR

ACCTCcccAAaaaCtaaGgTTCTTATAGGGATCTGGCTTTTCCTTTTcaGGAGTGGGCTTTTG
ACAGGTAATGGCCTGTGACTGCACATACCTATAGGTATCTTCTAATGTACCATTTGGGG
GAGGCGATAACCCAAAGTTCCAGtCTTCCAAAACAGAGGGATTCATTgTGTGAATATAGG
CCATTACTTCAGCAGACAATGTAATGcTACATAATTGAAAAATAAATTGTAAATCATACTC
TTCCACATGACGCATGTACTCTTTATAATCAGAATTGGTGTATGTGGAAGATGTAGTTAC

GGATGCACATAATGTCATGTTGGTACTGCGTGtGGTATCTACCACAGTAACAAACAGTTG
ATTACCCCAACAAATACCATTGTTATgtCCCTGTGCatGc

HPV 42 LR

AttntacataCCTATAACTATcTtCTAAAGTTcctGAAGGTGgTGGTGCAACACCAACATTCCaC
TCCTcTaatATGttaGGAttCATATTGtgtATATATGACATTACTTCAACagtnAatgTtATCTTACA
CAATTGaaaTATAAATTgcACATCataTTCTTCAGCAtgtcTTAAATATTCCTTAAAATTATCAG
CTGTatatgTATCACCAGATGTTgCAgtgncACACAAAGTCatgTtAGTACTacGgataCTATCnnn
ncanttAAAAaTAGctgaTTttCccaaca

HPV 54 LR

ATTTTTTTGTTGCCCTCCACACCCCCCCACTATACAAACCTATTTTTTTCTTCCCTACTAC
TTCTCCTCGGGGGGTTATACCATATTTCCTTTCCTCTACAATAGTGCCATTCATTCCATG
AATATATGCCTTATCATCTGCTGTAAGGGTTATGGTACATAACTC

HPV 54 LR

tnagntCacagTCCAAAaGTAAaTTtaCTGTAAGGATCCTCCTTTTCCTTTGCAGGGGcannnTt
CTTTTGACATGCAATGGCCtgtgACTGTACAAACCTATATGTGTCCTCCAAACTACTTgtAG
CTGGGGGGGTTATACCAAAGTTCCAGTCCTCTAGAATAGTGGGATTCATTCCATGAATA
TAGGCCATAACATCTGCTGTAAGGGTTATGGTACATAACTGAAATATAAACTGTAAATCA
TATTCCTCCACATGTCTAATATACTCCCTAAAGTCAGAATTATTAAAGCTATCCTGCGTG
GATGCTGTAGCACACAATGTTAGGTTAGTACTACGGGTGGTATCTACAACTGTTAAAAA
CAATTGATTGCCCCAACAAATACCATTGTTGTGGCCCTGGGc

HPV 61 LR

tacaccTCtggactgCAAAAACCTAtatgtgtCTTctaGACTGGTAGAGGGTGGAGGTACCACACCA
AAGTTCCAGTCATCCAACAAGGCTTTATTCATATTATGTAGGTAGGCCATAATTTCAGGG
GTTAAATGTATTTTACATAACTGAAAAATGAATTGCAAATCAAACTCTTCTGTATGGCGC
AAATATTCCCTAAAGCTTGTGGCTTTATATTCAGATACAGGGGGGGATGTAGCAGtACAa
atGGTTACATtagtacTGcgnnTGGTatggacAacGGTtacAaacant

HPV 61 LR

tacaccTCtggactgCAAAAACCTAtatgtgtCTTctaGACTGGTAGAGGGTGGAGGTACCACACCA
AAGTTCCAGTCATCCAACAAGGCTTTATTCATATTATGTAGGTAGGCCATAATTTCAGGG
GTTAAATGTATTTTACATAACTGAAAAATGAATTGCAAATCAAACTCTTCTGTATGGCGC
AAATATTCCCTAAAGCTTGTGGCTTTATATTCAGATACAGGGGGGGATGTAGCAGtACAa
atGGTTACATtagtacTGcgnnTGGTatggacAacGGTtacAaacant

HPV 61 LR

tGnActGcanAAACCTATaTGTGTCTTCtaaaCtgntanAGGGTGGAGGTACCACACCAAAGTT
CCAGTCATCCaACAAgGCCTTATTCATATTATGTAGGTAGGCCATAATTTCAgGGGTTAA
ATGTATTTTACATAACTGAAAAATAAATTGCAAATCAAACTCTTCTGTATGGcGCAAATAT
TCCCTAAAGCTTGtGGCTTTATATTCAGATACAGGGGGGGATGCAGCAGTACAAATGgnT
aCATTAgtACTGCGGGTGGTATCCACAACGGTTACAAacaAtTCATTAAACCAACAAATACC
ATTGTTGTGgcCcTGg

HPV 62 LR

GttctgtggTGgnTncTACTagAaGTACTAATTTTACTATTTGTACCGCCTCCacTGCTGCAGCA
GAATACAAGGCTACCAACTTTaGGGAATTTTTGCGACACACGGAAGAATTtGATTTGCAA
TTTATATTTCAATTGTGCAAAATACAGTTAACCCCCGAAATCATGGCCTACCTGCATAAT
ATGAACAAGGACCTTTtGGATGACTGGAACTTTGGGGTTTTACCTCCCCCTTCCACTAGT
TTAGATGAGACATATCGCTATTTGCAGTCTCGGGCTATTACATGTCAAAAGGGGGCTGC
TTCCCCgtCCCCCAAGGTGGACCCGTATGCGCAAATGACATTTTGGACTGTGGATCTTA
AGGACAAGTTGTCTACTGATTTGGACCAGTTTCccTTGGgtc

HPV 83 LR

GAtccTtatnaGGGgCaGGGgCGGAagnCCcTTTTGgcaggtAatagCACGGgactGCagaTAGCGA
TaGgTATCATCAAGGctGGtGgAaGgAGGtnntAACACGCCAAAATTCCACTCATCCAATAAA
TGTTCATTCATACTATGTAGGTATGCCATAATTTCAGGGGTAAGATGTATTTTGCAAAGT
TGCAATATAACCTGTAAGTCATATTCCTCGGTGTGGCGGaGGTATTCCTTAAAGTTAGA
GGCTGTGTATTCATTAGCCTGTGTAGCAGCAGCTGAAATAGTAATATTGGTACTGCGGG
TAGTATCCACAACTGTAACAAATAACTCATTAAACCAACAAATGCCATtAttaTGTCCCTGT
Gc

HPV 89 (CP6108) LR

gTTCTAcacGCTTTAaggAaTATTTAAgACACACtgaGgAaTATGACCTACAGTTTATATTCCA
ACTATGTAAGATACACCTAACGCCTGAGATAATGTCCTATTTACACAATATGAATGACAC
ATTGTTAGATGAATGGAACTTTGGTGTCATTCCCCCTCCCTCCACTAGTTTGGATGATAC
CTATCGCTTTCTTACCTCTCGGGCCATTACATGTCAAAAGGGCACTGCTGCCCCAGAAC
CTAAAAAGGATCCATATGATAAGTTATCCTTTTGGGATGTGGATCTTAAGGAACGTTTGT
CCACTGATCTCGACCAGTTTCCCTTGggTCGa