Prevalence and genotype-specific distribution of Human papillomavirus in Burundi according to HIV status and urban or rural residence and its implications for control.

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Abstract

Background

Human papillomaviruses are the most important causative agents for invasive cervical cancer development. HPV type-specific vaccination and HPV cervical cancer screening methods are being widely recommended to control the disease but the epidemiology of the circulating HPV types may vary locally. The circulating HPV-strains have never been assessed in Burundi. This study determined the prevalence and genotype-specific distribution of HPV in four different strata in Burundi: HIV-infected or non-infected and women living in rural or urban areas. Implications for HPV diagnosis and vaccine implementation was discussed.

Methods

Four cross-sectional surveys were conducted in Burundi (2013 in a rural area and 2016 in urban area) among rural and urban HIV-infected and uninfected women. Cytology and HPV genotyping was performed to screen women for cervical cancer lesions. Risk factors for HPV infection and cervical cancer lesions were determined using logistic regression model.

Results

HPV prevalence was very high in urban area with significant differences between HIV-positive and negative women ($p<0.0001$). In fact, 45.7% of HIV-positive participants were infected with any HPV type and all were infected with at least one HR/pHR-HPV type. Among the HIV-negative participants, 13.4% were HPV-infected, of whom, only 4 women (2.7%) were infected with HR/pHR-HPV types. In rural, HPV infection did not significantly differ between HIV-positive and negative women (30.0% and 31.3% respectively; $p=0.80$).

In urban, multiple infections with HR/pHR-HPV types were detected in 13.9% and 2.7% among HIV-positive and negative women respectively ($p<0.0001$), whereas in rural, multiple

infections with HR/pHR-HPV types were detected in 4.7% and 3.3% of HIV positive and negative women respectively (p=0.56).

The most prevalent HR/pHR-HPV types in HIV-positive urban women were HPV 52, 51 and 56. In the HIV-negative urban women, the most prevalent HR/pHR-HPV types were HPV 66, 67 and 18. In HIV-positive rural women, the most prevalent HR/pHR-HPV types were HPV 66, 16 and 18. In the HIV-negative rural women, the most prevalent HR/pHR-HPV types were HPV 16, 66 and 18. Independent risk factors associated with cervical lesions were HPV and HIV infections.

Conclusions

There is a high burden of HR/pHR-HPV infections, in particular among HIV-infected urban women. The study points out the need to introduce a comprehensive cervical cancer control program adapted to the context. This study shows that the nonavalent vaccine covers most of the HR/pHR-HPV infections in rural and urban areas among HIV-infected and uninfected women.

Keywords: Human papillomavirus, genotype distribution, cervix, HIV, HPV vaccine, Burundi

Background

Since about three decades, human papillomaviruses (HPVs) have been firmly proven to be the most important etiologic agents for the development of invasive cervical cancer (ICC) [1-3]. Worldwide, HPVs are known to be one of the most common Sexually Transmitted Infections (STIs) and HPV prevalence peaks soon after sexual debut during adolescence and decreases thereafter with increasing age [4;5].

HPVs are small double-stranded DNA viruses, with a large epithelial tropism. Basal epithelial cells are infected with HPVs, causing benign and malignant lesions of the skin and the ano-genital mucosae and the upper aero-digestive tract [6]. Studies on HPV epidemiology and their mechanistic evidence have led to their classification into three groups: (1) HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are known as carcinogenic, also named high-risk (HR)-HPV types; (2) HPV types 26, 53, 66, 67, 68, 70, 73 and 82, are classified as probably/possibly high-risk carcinogenic (pHR)-HPV types and (3) other HPV types such as HPV 6 and 11 are classified as low-risk (LR)-HPV types [7]. However, there is a growing literature providing evidence that the classified (pHR)-risk HPV types may also have to be considered as (HR)-HPV types [7-9].

Risk factors for HPV infection have been documented and include infection with other STIs (including HIV), high number of lifetime sexual partners, early sexual debut and host susceptibility [6;10]. Non-sexual transmission routes have also been documented but account for a small minority of HPV infections. They include perinatal transmission and, possibly, transmission by medical procedures and fomites [6].

Both HIV and HPV are STIs and infection with either one of the two viruses may facilitate transmission of the other [14;15]. Furthermore, HPV infections are more persistent in HIV-infected women compared to HIV-negative women, and as a consequence, cervical lesions

are more frequent in HIV-positive women than in HIV-negative women [16;17]. Thus, HIV-infected women are at higher risk of developing ICC compared to HIV-uninfected women [11-13]. Prevention of ICC in sub-Saharan Africa (SSA) through screening with the conventional Pap smear method or other alternative techniques such as visual inspection with Acetic Acid/Lugol's iodine is challenging and barely rolled out on public health scale [18;19]. New control strategies such as HPV screening with rapid molecular HPV tests and HPV vaccination are emerging and may be promising complementary tools for the Low and Middle Income Countries (LMICs) [20].

Currently, there are three prophylactic HPV vaccines licensed: (1) Cervarix® (GlaxoSmithKline, Brentford, UK); a bivalent vaccine targeting HPV16/18; (2) Gardasil® (Merck Inc, NY, USA), a quadrivalent vaccine targeting HPV6/11/16/18 and (3) Gardasil-9® (Merck Inc., NY, USA), a nonavalent vaccine targeting HPV 6/11/16/18/31/33/45/52/58 [21].

In Burundi, ICC represents the most common female cancer, accounting for approximately 39% of all female cancers [22]. It is responsible, each year, for an estimated 1 421 new cases and 1 080 deaths in Burundi, representing an annually age-standardised incidence and mortality rates of 49.3 and 39.3/100,000 women respectively [22]. Burundi does not have any cervical cancer screening programme because of a variety of factors including lack of adequate infrastructure, insufficiently qualified staff and insufficient investment in resources for pap-smears, biopsies and colposcopy [23;24].

Since end of 2016, a demonstration project on HPV vaccination started which was limited to two districts where girls aged 9–13 years old are being vaccinated with Cervarix®. It is expected to expand at national level in a second phase after evaluation of this demonstration project [25]. However, the impact of HPV vaccination will be only fully realized several decades after a vaccination programme is instituted. Further, immunization can be ineffective

due to insufficient coverage, missed follow-up doses, strain incompatibility and cost. Indeed, it remains challenging to guarantee sufficient coverage and ensure all girls of appropriate age to be vaccinated. Apart from report on cross-protection [26], the bivalent vaccine chosen by Burundi offers protection against the two most prevalent HR-HPV types 16 and 18. Both genotypes are expected to be responsible for 70% of all cancer cases, implying that at least 30% of all cervical cancer cases might not be covered by this prophylactic vaccination.

To date, information about the epidemiology of the circulating HPV strains in Burundi has not yet been ever documented. In the current context of type-specific HPV vaccination and of HPV-based cervical cancer screening, information about the prevalence and the circulating HPV types and their relative contribution to ICC is of great importance to assist in planning for vaccine, cervical cancer screening implementation as well as to monitor the potential impact on circulating HPV types after vaccination.

Hence, this study aimed to document the prevalence and genotype-specific distribution of cervical HPV types in both HIV-infected and uninfected Burundian women living in urban (Bujumbura) or in rural setting (Kirundo).

Materials and methods

Study design, setting and population

Four cross-sectional surveys were conducted: two were conducted from May to July 2013 in Kirundo, a rural health district in the northern part of Burundi. Another two were conducted from March to May 2016 in Bujumbura, the capital city of Burundi.

In the rural area, participants were women attending Kirundo District Hospital and ANSS (Association Nationale de Soutien aux Séropositifs et malades du SIDA)-Kirundo antenna. The province of Kirundo is divided into 4 health district zones but has only 2 district hospitals

(Kirundo and Mukenke). Kirundo district hospital is located at the provincial town of Kirundo and is the biggest hospital in the province. Patients are referred from health centres in Kirundo district and the neighbouring Busoni and Vumbi health districts. Patients can also come directly to the hospital to consult a General Practitioner (GP). ANSS-Kirundo is a local NGO, champion in HIV-care in Kirundo and has the longest active list of HIV-patients coming from the 4 health districts and few from the neighbouring Ngozi province. It is located near Kirundo district hospital.

In the urban area of Bujumbura, participants were women attending an HIV clinic, located in the University Teaching Hospital of Bujumbura, which follows up around 3500 HIV-positive patients. Participants were also women attending a reputed family planning centre, ABUBEF (Association Burundaise pour le Bien-Etre Familial), in Bujumbura with clients from all neighbourhoods and all social classes.

The study was approved by the Burundian National Ethics Committee. A written informed consent to participate in the survey (translated in the local language) was obtained and signed by all participants before being enrolled in the study. Information collected was kept confidential by the use of codes.

Recruitment procedures

General information about the study objectives were given to all participants every morning in the waiting room. During the consultation in the outpatient department, a GP gave clear and detailed information on the study objective and procedures, and proposed the women to participate in the study. Women who signed an informed consent were assisted by the GP to complete a short risk factor questionnaire and blood was collected for HIV testing. Women who declared being HIV-negative or with an unknown HIV status received a pre-test counselling before HIV-testing. A post-test counselling was done before giving back the result.

Those who tested HIV-positive were referred to any HIV clinic of their choice for follow up. Women known to be HIV-positive, followed up at the University HIV clinic or ANSS were not retested for HIV.

After filling the questionnaire, a gynaecologic examination was conducted. Cervical samples were collected using a cytobrush® (Cervex-Brush combi, Rovers Medical Devices B.V., The Netherlands) and placed into liquid-based cytology (LBC) medium (ThinPrep, Hologic). Vials were stored at room temperature until shipment. Specimens were sent to the Department of Virology at Sefako Makgatho Health Science University (SMU) in Pretoria, South Africa and to the Laboratory of Molecular Pathology, AML, Sonic Healthcare, Antwerp, Belgium for HPV genotyping and cytology reading.

Inclusion and exclusion criteria

Any woman (or girl) aged between 17-65 years, declaring having had vaginal sexual intercourse and agreeing to participate could be included in the study. Exclusion criteria were pregnancy, menstrual period, vaginal discharge and hysterectomy.

Sample size determination

We estimated HPV prevalence in HIV-negative and HIV-positive women to be 21% and 36.3% respectively [16;22;27]. With a power of 80% for a confidence level of 95%, the minimum sample size required was 149 subjects per stratum. The calculation was done using the EpiInfo7 software. We therefore decided to investigate 150 HIV positive and 150 HIV negative women in both the rural and urban settings.

HPV testing and liquid based cytology processing

We initially planned that all samples would be tested at SMU, South Africa. At the time of sample collection of urban specimens, we realized that the laboratory was overloaded and therefore have decided to send urban samples at AML, Antwerp in Belgium.

For the 300 samples from the urban area, thin-layer slides were prepared with the ThinPrep® 5000 Processor with Autoloader System (Hologic Inc, Marlborough, US) and stained with the Papanicolaou stain on the Tissue-Tek® Prisma and Film Automated Slide Stainer and Coverslipper (Sakura Finetek Europe B.V., Netherlands). After scanning of the slides with the ThinPrep® Imaging System, cytology reading was performed by image-guided screening, with prior knowledge of HPV infection status. Cytological diagnoses were reported according to the Bethesda 2001 terminology system as 1) normal, 2) atypical squamous cells of undetermined significance; atypical squamous cells cannot exclude high-grade lesion; atypical glandular cells; low-grade squamous intraepithelial lesions (ASCUS/ASC-H/AGC/LSIL), 3) high-grade squamous intraepithelial lesions (HSIL), or 4) invasive cancer. After the LBC preparations are made, 800µL of the remaining cell suspension was used for DNA extraction. Cytology reading was performed only on the 300 urban samples due to budget constraints.

HPV type-specific detection

For the urban samples, DNA isolation from liquid-based cytology was performed on the Medium Throughput Automation (MTA) (Hologic Inc) with the Genfind® DNA extraction kit. Subsequently, the DNA is amplified using a series of real-time qPCR reactions in the LightCycler 480 (Roche) as previously described by Micalessi et al [28]. Briefly, the RIATOL qPCR HPV genotyping assay is a clinically validated, laboratory developed test, which amplifies 18 HPV types: HPV 6E6, 11E6, 16E7, 18E7, 31E6, 33E6, 35E6, 39E7, 45E7, 51E7, 52E7, 53E6, 56E7, 58E7, 59E7, 66E6, 67 L1 and 68E7. Real-time quantitative PCR for β-globin was always performed and was used as a proxy for the quality of sampling.

The 300 specimens from rural area were tested at the Department of Virology, SMU, Pretoria, RSA. HPV DNA was extracted using AmpliLute liquid media Extraction kit following manufacturer's instructions (Roche Molecular Systems, California, USA). HPV DNA was

detected following conventional nested HPV. PCR was performed with MY09/MY11 (450 bp) and GP5+/GP6+ (150 bp). First round PCR was carried out in a final reaction of 50 µl containing 1 X buffer II (Bioline, Luckenwalde, Germany), 1.5 mM MgCl2 (Bioline, Luckenwalde, Germany), 200 µM of each deoxynucleoside triphosphate (dNTP), 0.2 µM of MY09/MY11 primers and 0.2 µM PCO3 and PCO4 primers (110 bp) (Inqaba Biotechnological Industry, Pretoria, South Africa), 1 unit of Bio Taq DNA polymerase (Bioline, Hilden, Germany), and 10 µl of DNA template. The cycling conditions included an initial denaturation step for 2 minutes at 94˚C followed by 40 cycles at 94˚C for denaturation, 30 seconds at 55˚C for annealing, 1 minute at 72˚C for elongation and a final elongation for 5 minutes at 72°C. The PCR product detected using gel electrophoresis (2% w/v agarose in Tris-acetate EDTA) and visualised using ethidium bromide. Bands of the appropriate size were identified by comparison with a DNA molecular weight marker and the gel was viewed using a Gel DOC system (Syngene, Europe). HPV genotyping was performed using Linear array genotyping test that identifies 37 HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56,58, 59, 61, 62, 64, 66, 67, 68, 69, 70,71, 72 73, 81, 82, 83, 84, 89, IS39) as previously described by Coutlee et al. [29].

Briefly, 50µl of amplicon was added to 50µl of a working master mix containing MgCl2, Amplitaq® GOLD DNA polymerase, Uracil-N-glycosilase, deoxynucleotides, PGMY and β globin primers. The PCR amplification was performed using the gold-plated 96-well Gene Amp PCR System 9700 (Applied Biosystems, Foster City, California, USA) according to manufacturer instructions. Positive reactions appeared as blue lines and were interpreted using the LA HPV GA reference guide.

224 **Statistical analysis**

225 Proportions were compared using Pearson's Chi-square test (or Fisher's exact test when
226 appropriate). Ttest was used to compare means of normally distributed variables (or Kruskal-
227 Wallis test when appropriate). Bivariate analysis was performed to generate odds ratios (ORs)
228 with their 95% confidence interval (CI) to analyse the relationship between each
229 sociodemographic variable and cervical cancer lesions or with HPV infection status. Variables
230 with a bivariate p-value <0.20 were entered in a multivariate logistic regression model to
231 determine adjusted odds ratios (AORs) with their 95% CIs. Analysis was conducted using
232 EpiInfo 3.5.4 software and α-error margin of 5% was considered significant.

233

234 **RESULTS**

235 **Participants and their socio-demographic characteristics**

236 In rural area, we enrolled 150 HIV-negative and 150 HIV-positive women. The mean age of
237 the participants was 39.9 years (SD=8.3) and 36.4 years (SD=7.9) for HIV-positive and HIV-
238 negative women respectively (p=0.0002). Profession categories and marital status differed
239 between HIV-positive and negative women (p<0.0001 for both). Farmers represented 71.3%
240 and 50% in HIV-positive and negative women respectively. The other HIV-negative women
241 were ''employees/shopkeepers'' (47.3%) or ''housewife/Non-working/student/other''
242 (2.7%). A hundred thirty-one HIV-positive women (87.3%) was married compared to seventy-
243 eight (52%) in the HIV-positive group. In the HIV-positive group, 38% were "widowed or
244 divorced" compared to 10% in HIV-negatives. HIV-positive women started earlier sexual
245 intercourse, got married earlier, got pregnant earlier and had a higher median number of
246 sexual partners compared to their HIV-negatives counterparts (all p<0.001) (Table 1).

247 In urban, a total of 151 HIV-positive and 149 HIV-negative participants were enrolled. The
248 mean age of our participants was 41.1 years (SD=9.7) and 39.7 years (SD=8.7) for HIV-positive
249 and HIV-negative women (p=0.19). Profession categories and marital status differed between
250 HIV-positive and negative women (p<0.0001 for both). In the HIV-positive group, majority
251 (42.4%) was in the category ''housewife/Non-working/student/other''. In the HIV-negative
252 group, 77.2% of participants were employees (in public or private) or shopkeepers. In the HIV-
253 negative group, 91.9% were married versus 53% in the HIV-positive group. A high proportion
254 of HIV-positive women were widowed/divorced (36.4%) whereas only 4% who belonged to
255 this category were in the HIV-negative group. HIV-positive women started earlier sexual
256 intercourse, got married earlier, got pregnant earlier and had a higher median number of
257 sexual partners compared to their HIV-negative counterparts (all p<0.001) (Table 1).
258

259 **Table 1 : Baseline socio-demographic characteristics of the participants by study area**
260 **and HIV status, among 600 women, Burundi, 2016**

Variable	Urban			Rural		
	HIV+ (151)	HIV- (149)	P	HIV+ (150)	HIV- (150)	P
	N (%)	N (%)		N (%)	N (%)	
Age group (years)						
17-25	8 (5.3)	8 (5.4)	0.21	6 (4)	10 (6.7)	0.0017
26-35	44 (29.1)	39 (26.2)		42 (28)	63 (42)	
36-45	48 (31.8)	64 (43)		61 (40.7)	60 (40)	
≥46	51 (33.8)	38 (25.5)		41 (27.3)	17 (11.3)	
Mean age(years)+SD	41.1 (9.7)	39.7 (8.7)	0.19	39.9 (8.3)	36.4 (7.9)	0.0002
Profession						
Farmer	31 (20.5)	12 (8.1)	<0.0001	107 (71.3)	75 (50)	<0.0001
Housewife/Non-working/Student/Other	64 (42.4)	22 (14.8)		9 (6)	4 (2.7)	
Public or Private employee/Shopkeeper	56 (37.1)	115 (77.2)		34 (22.7)	71 (47.3)	
Marital status						
Married	80 (53)	137 (91.9)	<0.0001	78 (52)	131 (87.3)	<0.0001
Widowed/Divorced	55 (36.4)	6 (4)		57 (38)	15 (10)	
Single	16 (10.6)	6 (4)		15 (10)	4 (2.7)	
Mean age of menarche (SD)	14.5 (1.7)	14.5 (1.8)	0.71	14.6 (1.7)	14.7 (1.7)	0.71
Median age at sexual intercourse debut(IQR)	18 (16-19)	22 (18-26)	<0.001	18 (16-20)	19 (17-23)	<0.001
Median age of marriage(IQR)	20 (17-25)	26 (23-29)	<0.001*	19 (17-22)	22 (18-26)	<0.001[i]
Median age at 1st pregnancy(IQR)	20 (17-23)	26 (21-29)	<0.001**	19 (17-21)	22 (19-26)	<0.001[ii]
Median gestity(IQR)	4 (2-6)	4 (3-5)	0.99	4 (3-6)	4 (3-6)	0.72
Median parity(IQR)	3 (2-5)	3 (2-5)	0.65	4 (2-6)	4 (2-5)	0.31
Number of lifetime sex partners	N=150	N=147		N=136	N=147	
1	34 (22.7)	72 (49)	-	24 (17.6)	83 (56.5)	-
2+	116 (77.3)	75 (51)	<0.0001	112 (82.4)	64 (43.5)	<0.0001
Median (IQR)	3 (2-5)	2 (1-3)	<0.0001	3 (2-4)	1 (1-2)	<0.0001
Smoking						
Yes	4 (2.6)	2 (1.3)	0.34	23 (15.3)	14 (9.3)	0.11
alcohol consumption						
Yes	48 (31.8)	71 (47.7)	0.005	70 (46.7)	79 (52.7)	0.29
Cytological result						
Normal	115 (76.2%)	142 (95.3%)	0.0005			
ASC-H	2 (1.3%)	0 (0%)				
ASCUS	13 (8.6%)	2 (1.3%)				
ICC	1 (0.7%)	0 (0%)				
HSIL	3 (2%)	2 (1.3%)				
LSIL	16 (10.6%)	3 (2%)				
Not determined	1 (0.7%)	0 (0%)				

261 *N=276; **N=292; ***N=297; [i]N=282; [ii]:N=294
262

HPV prevalence

In rural, all participants had valid data on HPV genotyping. The HPV prevalence (any) was 30% (45/150) and 31.3% (47/150) among the HIV-positive and negative women respectively (p=0.80). Multiple HPV infection was 11.3% and 14% among the HIV-positive and negative women respectively (p=0.49). The HR/pHR-HPV prevalence was 18.7% and 17.3% in HIV-positive and negative respectively (p=0.76). Multiple HR/pHR-HPV infections was 4.7% versus 3.3% among HIV-positive and negative women respectively (p=0.56).

In the HIV-positive group, infection with any HR-HPV, pHR-HPV and LR-HPV type was 14.7%, 7.3% and 19.3% respectively. In HIV-negative women, infection with any HR-HPV, pHR-HPV and LR-HPV type was 12%, 6.7% and 24.7% respectively (Table 2). Infection with HPV 16 or 18 was 8% versus 6.7% among HIV-positive and negative women respectively (p=0.50). The prevalence of the HR-HPV types included in the Gardasil-9 vaccine was 14% versus 11.3% among HIV-positive and negative women respectively (p=0.49). The most frequent HR-HPV types in the HIV-positive group were HPV 16 (4%), 18 (4%), followed by HPV 33 (3.3%) and HPV 58 (2%). The most frequent pHR-HPV types were HPV 66 (4.7%) and HPV 70 (3.3%). Among the LR-HPV types, the most frequently isolated were HPV 11 (15.3%) and HPV 6 (2%).

In the HIV-negative group, the most frequent HR-HPV types isolated were HPV 16 (4.7%), followed by HPV 18 (2%). The most frequent pHR-HPV types were HPV 66 (4%) and HPV 67 (1.3%). The most frequent LR-HPV types were HPV 11 (20%) and HPV 6 (4%).

Table 2 also presents HPV prevalence results for the urban area. The overall HPV prevalence (any HPV) was 45.7% and 13.4% among HIV-positive and HIV-negative women (p<0.0001). HPV prevalence is presented by cytological results and by HIV-status. Multiple infection with any HPV type was 14.6% and 2.7% among HIV-positive and negative women respectively

286 (p<0.0001). The overall prevalence of HR/pHR-HPV infection was 45.7% and 12.8% with a
287 multiple infection of 13.9% and 2.7% among HIV-positive and negative women respectively
288 (p<0.0001 for both variables). In the HIV-positive group, HR/pHR-HPV prevalence was 28.7%
289 (33/115), 100% (29/29) and 100% (6/6) among women with normal cytology, ASCUS/LSIL and
290 HSIL/ICC/ASC-H respectively.
291 In the HIV-negative group, HR/pHR-HPV prevalence was 8.4% (12/142), 100% (5/5) and 100%
292 (2/2) in women with normal cytology, ASCUS/LSIL and HSIL/ASC-H/ICC respectively.
293 Most prevalent HR/pHR-HPV types, in HIV-positive women, were HPV 52, 18, 51 and 58
294 among women with normal cytology; HPV 56, 51 and 52 in women with ASCUS/LSIL and
295 HPV16 and 18 in women with HSIL/ASC H/ICC. In the HIV-negative women, the most prevalent
296 HR/pHR-HPV types were HPV 67, 39, 45 and 66 in women with normal cytology; HPV 58 and
297 66 in women with ASCUS/LSIL and HPV 16 and 18 in women with HSIL/ASC-H/ICC.
298

299 **Table 2 : Prevalence of human papillomavirus by HIV status in rural and urban areas among 600 women in Burundi**

Type de HPV	Rural			Urban								
				HIV positive women				HIV negative women				
	HIV positive (N=150)	HIV negative (N=150)		Normal (N=115)	ASCUS/LSIL (N=29)	HSIL/ASC-H/ ICC (N=6)	Total (N=151)	Normal (N=142)	ASCUS/LSIL (N=5)	HSIL/ASC-H/ ICC (N=2)	Total (N=149)	
	N (%)	N (%)	p	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	p[a]
Positive	45 (30.0)	47 (31.3)	0.80	33 (28.7)	29 (100)	6 (100)	69* (45.7)	13 (9.2)	5 (100)	2 (100)	20 (13.4)	<0.0001
Multiple	17 (11.3)	21 (14.0)	0.49	8 (7)	11 (37.9)	2 (33.3)	22 (14.6)	2 (1.4)	2 (40)	0 (0)	4 (2.7)	<0.0001
HR/pHR-HPV (any)	28 (18.7)	26 (17.3)	0.76	33 (28.7)	29 (100)	6 (100)	69* (45.7)	12 (8.4)	5 (100)	2 (100)	19 (12.8)	<0.0001
Multiple HR/pHR-HPV	7 (4.7)	5 (3.3)	0.56	8 (7)	10 (34.5)	2 (33.3)	21* (13.9)	2 (1.4)	2 (40)	0 (0)	4 (2.7)	<0.0001
HPV 16-18	12 (8.0)	9 (6.0)	0.50	6 (5.2)	3 (10.3)	4 (66.7)	14* (9.3)	1 (0.7)	1 (20)	2 (100)	4 (2.7)	0.016
HPV 16-18-31-33-45-52-58	21 (14.0)	17 (11.3)	0.49	18 (15.7)	14 (48.3)	5 (83.3)	38* (25.2)	3 (2.1)	4 (80)	2 (100)	9 (6)	<0.0001
35-39-51-53-56-59-66-67-68	8 (5.3)	11 (7.3)	0.50	18 (15.7)	22 (75.9)	2 (33.3)	43* (28.5)	9 (6.3)	2 (40)	0 (0)	11 (7.4)	<0.0001
High risk												
16	6 (4.0)	7 (4.7)		2(1.7)	1 (3.4)	3 (50)	7 (4.6)	0 (0)	0 (0)	1 (50)	1 (0.7)	
18	6 (4.0)	3 (2.0)		4 (3.5)	3 (10.3)	1 (16.7)	8 (5.3)	1 (0.7)	1 (20)	1 (50)	3 (2)	
31	2 (1.3)	1 (0.7)		3 (2.6)	1 (3.4)	1 (16.7)	6 (4)	0 (0)	1 (20)	0 (0)	1 (0.7)	
33	5 (3.3)	1 (0.7)		1 (0.9)	0 (0)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	
35	0 (0.0)	2 (1.3)		2 (1.7)	3 (10.3)	1 (16.7)	6 (4)	0 (0)	0 (0)	0 (0)	0 (0)	
39	0 (0.0)	0 (0.0)		2 (1.7)	1 (3.4)	0 (0)	3 (2)	2 (1.4)	0 (0)	0 (0)	2 (1.3)	
45	0 (0.0)	2 (1.3)		2 (1.7)	1 (3.4)	0 (0)	4 (2.6)	2 (1.4)	1 (20)	0 (0)	3 (2)	
51	1 (0.7)	0 (0.0)		4 (3.5)	6 (20.7)	0 (0)	12 (7.9)	1 (0.7)	0 (0)	0 (0)	1 (0.7)	
52	0 (0.0)	2 (1.3)		7 (6.1)	6 (20.7)	0 (0)	13 (8.6)	0 (0)	0 (0)	0 (0)	0 (0)	
56	1 (0.7)	0 (0.0)		3 (2.6)	7 (24.1)	0 (0)	10 (6.6)	0 (0)	0 (0)	0 (0)	0 (0)	
58	3 (2.0)	2 (1.3)		4 (3.5)	4 (13.8)	1 (16.7)	9 (6)	0 (0)	2 (40)	0 (0)	2 (1.3)	
59	0 (0.0)	0 (0.0)		2 (1.7)	0 (0)	0 (0)	2 (1.3)	1 (0.7)	0 (0)	0 (0)	1 (0.7)	
Any HR	22 (14.7)	18 (12.0)		28 (24.3)	26 (89.6)	6 (100)	61* (40.4)	5 (3.5)	4 (80)	2 (100)	11 (7.4)	
Possibly high risk												
26	1 (0.7)	1 (0.7)										
53	0 (0.0)	1 (0.7)		1 (0.9)	4 (13.8)	0 (0)	5 (3.3)	2 (1.4)	0 (0)	0 (0)	2 (1.3)	
66	7 (4.7)	6 (4.0)		1 (0.9)	2 (6.9)	0 (0)	4 (2.6)	2 (1.4)	2 (40)	0 (0)	4 (2.7)	
67	1 (0.7)	2 (1.3)		3 (2.6)	1 (3.4)	0 (0)	4 (2.6)	4 (2.8)	0 (0)	0 (0)	4 (2.7)	
68	0 (0.0)	0 (0.0)		2 (1.7)	3 (10.3)	0 (0)	6 (4)	0 (0)	0 (0)	0 (0)	0 (0)	
70	5 (3.3)	1 (0.7)										
Any pHR	11 (7.3)	10 (6.7)		6 (5.2)	8 (27.6)	0 (0%)	15* (9.9)	7 (4.9)	2 (40)	0 (0)	9 (6.0)	
Low risk												
6	3 (2.0)	6 (4.0)		0 (0)	0 (0)	0 (0)	0 (0)	1 (0.7)	1 (20)	0 (0)	2 (1.3)	
11	23 (15.3)	30 (20.0)		0 (0)	1 (3.4)	0 (0)	1 (0.7)	0 (0)	0 (0)	0 (0)	0 (0)	
40	2 (1.3)	1 (0.7)										
54	1 (0.7)	3 (2.0)										
55	1 (0.7)	1 (0.7)										
81	2 (1.3)	0 (0.0)										
83	0 (0.0)	1 (0.7)										
84	1 (0.7)	0 (0.0)										
Any LR	29 (19.3)	37 (24.7)		0 (0)	1 (3.4)	0 (0)	1 (0.7)	1 (0.7)	1 (20)	0 (0)	2 (1.3)	

300 *Including 1 participant with undetermined cytological result. [a] comparison of total HIV+ and total HIV- in urban

Figure 1 (part A) presents the age-specific prevalence of HR/pHR-HPV by HIV status and by study area. It appears that HIV-positive urban women are highly infected with HR/pHR-HPV types than their counterparts in rural across all age groups. Rural HIV-negative women have slightly higher prevalence compared to their counterparts in urban in all age groups, except in the younger women who were not infected with any HR/pHR-HPV type.

Overall, among these HPV infections, HR-HPV types are the most predominant. In urban, HR/pHR-HPV infections decreased with increasing age, with a second peak in the age group ≥46 years, both in HIV-positive and negative women. In rural, the decrease in HR/pHR-HPV prevalence was slow compared to urban across all age groups.

Figure 1 (part) B presents the age-specific prevalence of abnormal cytological results in urban area. Further, it also presents the HR/pHR-HPV infections among these women with abnormal cytological results and it clearly appears that HR-HPV infections are the most predominant among women with abnormal cytological results. Cytological abnormalities also seem to decrease with increasing age, with a second peak in age group ≥46 years as it was also for HPV prevalence.

Cytology results were available for all urban women except one HIV-positive woman with an undetermined cytological result. Cytological abnormalities were more frequent in HIV-positive women compared to HIV-negative women (p=0.0005).

In the HIV-positive group, among 150 women with a valid result on LBC, 23.3% (35/150) had an abnormal cytological result, including 8.7% (13/150) with ASCUS, 10.6% (16/150) with LSIL, 1.3% (2 patients) with ASC-H, 2% with HSIL and 1 patient with ICC. In the HIV-negative group, among 149 women with valid results on LBC, 4.7% (7/149) had abnormal cytological results

323 including 2 patients (1.3%) with ASCUS, 3 patients (2%) with LSIL and 2 patients (1.3%) with
324 HSIL.
325
326 **Figure 1: Age-specific prevalence of HPV (A) and of abnormal cytological results (B) stratified**
327 **by HIV status in rural and urban women, Burundi.**
328 In the bivariate analysis, abnormal cytology was significantly associated with HR/pHR-HPV
329 infection (OR=199; 95% CI:26.7-1483), age (older women i.e. age groups 36-45 years and ≥46
330 years being protected compared to younger women aged 17-25 years), number of lifetime
331 sexual partners (having had two or more lifetime sexual partners was associated with a higher
332 risk of abnormal cytology, OR=2.57; 95% CI: 1.14-5.80 compared to having had only 1 sexual
333 partner), HIV infection status (being HIV-infected was associated with 6 times higher risk of
334 having abnormal cytology than those who are HIV-uninfected, OR=6.17 95% CI: 2.64-14.42)
335 and profession (being an employee or a shopkeeper was a protective factor of having
336 abnormal cytology compared to farmers, OR=0.30 95%CI: 0.13-0.69). After having adjusted
337 for age, marital status, HIV infection, profession and number of lifetime sexual partners,
338 HR/pHR-HPV infection remained the only stronger significant predictor for abnormal cytology
339 (OR=162.54; 95%CI: 20.9-1261.4).
340
341

342 **Table 3: Predictors of abnormal cytological results (ASCUS +) in urban women.**

Variable	**Crude OR (95%CI)**	**P**	**Adjusted OR (95%CI)**	**p**
HR/pHR-HPV infection				
No	-			
Yes	199.1 (26.7-1482.9)	<0.0001	162.54 (20.94-1261.42)	<0.0001
Age group (years)				
17-25	-		-	
26-35	0.34 (0.11-1.10)	0.07	0.29 (0.05-1.66)	0.17
36-45	0.16 (0.05-0.54)	0.003	0.24 (0.04-1.44)	0.12
≥46	0.26 (0.08-0.85)	0.03	0.19 (0.03-1.18)	0.07
HIV infection				
Yes	6.17 (2.64-14.42)	<0.0001	1.69 (0.54-5.29)	0.37
No	-		-	
Number of lifetime sexual partners (N=297)				
1	-		-	
≥2	2.57 (1.14-5.80)	0.02	1.69 (0.59-4.89)	0.33
Profession				
Farmer	-		-	
Housewife/Non-working/Student/other	0.42 (0.17-1.05)	0.06	0.23 (0.06-0.93)	0.04
Public or Private employee/Shopkeeper	0.30 (0.13-0.69)	0.005	0.41 (0.11-1.50)	0.18
Marital status				
Married	-		-	
Widowed/Divorced	1.83 (0.88-3.87)	0.11	0.94 (0.31-2.88)	0.92
Single	3.74 (0.88-15.83)	0.07	1.52 (0.20-11.27)	0.68
age sexual debut				
<17	-			
≥17	0.66 (0.33-1.34)	0.25		
Age of marriage (N=276)				
<17	-			
≥17	0.97 (0.27-3.44)	0.96		
age at first pregnancy (N=292)				
<17	-			
≥17	0.60 (0.23-1.57)	0.30		
Gestity				
<6	-			
≥6	1.78 (0.89-3.57)	0.10		
Parity				
<6	-		-	
≥6	0.80 (0.30-2.18)	0.67	0.66 (0.16-2.84)	0.58
Alcohol consumption				
Yes	-			
No	1.36 (0.68-2.71)	0.38		

343

344

345 Table 4 presents the predictors of HPV infection in urban and rural. In urban, abnormal
346 cytology and HIV infection were the most important contributor to a positive HPV diagnosis
347 (OR=148.72; 95%CI: 19-1164.6 and 4.06 95%CI: 1.78-9.22 respectively), whereas in rural,
348 there was no significant contributor identified, after having controlled for other covariates
349 (age, lifetime number of sex partners, profession, parity and marital status).

350 Figure 2 presents the distribution of HR/pHR-HPV among HPV-positive participants by HIV
351 status. In the HIV-positive rural women, infection with HPV 16 or 18 represented 32.1% of all
352 HR/pHR-HPV infections. In the HIV-negative rural women, infection with HPV 16 or 18
353 represented 30.8% of all HR/pHR-HPV infections. Infections with HPV 16/18/31/33/45/52/58
354 represented 60.7% and 53.9% of the circulating HR/pHR-HPV infections among HIV-positive
355 and negative rural women respectively.
356 In urban women, HPV 16/18 infections represented 8.7% and 15.8% of all HR/pHR-HPV
357 infections in HIV-positive and negative women respectively.
358 Infections with the HPV types included in the Nonavalent vaccine (i.e. HPV
359 16/18/31/33/45/52/58) represented 31.9% and 42.1% of all HR/pHR-HPV infections in HIV-
360 positive and negative urban women respectively.

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Figure 2: Distribution of HR/pHR-HPV types among HPV + women by study area and HIV status

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Table 4: Predictors of the HPV (any) infection by study area (rural and urban) in women, Burundi.

Variable	Urban area				Rural area			
	Crude OR (95% CI)	**P**	**Adjusted OR (95% CI)**	**P**	**Crude OR (95% CI)**	**P**	**Adjusted OR (95% CI)**	**p**
Age group (years)								
17-25	Ref		Ref		Ref			
26-35	0.48 (0.16-1.42)	0.19	0.63 (0.11-3.65)	0.61	0.83 (0.28-2.48)	0.74		
36-45	0.26 (0.09-0.76)	0.01	0.36 (0.06-2.15)	0.26	0.73 (0.25-2.17)	0.57		
≥46	0.53 (0.18-1.56)	0.25	0.77 (0.13-4.52)	0.78	0.53 (0.16-1.72)	0.29		
HIV infection								
Yes	5.43 (3.07-9.60)	<0.0001	4.06 (1.78-9.22)	0.0008	0.94 (0.57-1.53)	0.8		
No	Ref				Ref			
Number of lifetime sexual partners	N=297				N=283			
1	Ref					Ref		
≥2	2.38 (1.34-4.20)	0.003	1.35 (0.61-2.99)	0.46	1.14 (0.68-1.93)	0.61		
Profession								
Farmer	Ref					Ref		
Housewife/Non-working/Student/other	0.71 (0.34-1.50)	0.37	1.04 (0.36-3.04)	0.94	0.84 (0.22-3.17)	0.79	0.83 (0.21-3.20)	0.78
Public or Private employee/Shopkeeper	0.37 (0.19-0.75)	0.006	1.32 (0.46-3.80)	0.60	1.79 (1.07-2.98)	0.03	1.60 (0.94-2.71)	0.08
Marital status								
Married	Ref				Ref			
Widowed/Divorced	2.61 (1.47-4.74)	0.001	1.31 (0.57-3.00)	0.53	1.11 (0.62-1.98)	0.72		
Single	3.88 (1.01-14.96)	0.05	1.46 (0.23-9.18)	0.69	1.38 (0.52-3.68)	0.52		
age sexual debut								
<17								
≥17	0.63 (0.36-1.08)	0.09	1.09 (0.43-2.74)	0.86	1.05 (0.58-1.90)	0.87		
Age of marriage (N=276)								
<17								
≥17	1.01 (0.41-2.52)	0.98			1.40 (0.54-3.65)	0.49		
age at first pregnancy (N=292)								
<17								
≥17	0.59 (0.27-1.29)	0.18	0.97 (0.28-3.33)	0.96	0.84 (0.40-1.77)	0.65		
Gestity								
<6								
≥6	1.33 (0.76-2.32)	0.32			0.55 (0.31-0.96)	0.04	0.75 (0.28-2.01)	0.56
Parity								
<6	Ref							
≥6	0.94 (0.46-1.93)	0.87			0.53 (0.28-0.99)	0.05	0.74 (0.24-2.26)	0.6
Alcohol consumption								
Yes	0.75 (0.45-1.25)	0.27			0.72 (0.43-1.17)	0.18	0.71 (0.43-1.19)	0.19
No								
Cytology result								
Abnormal	193.96 (26.05-1444.13)	<0.0001	148.72 (18.99-1164.60)	<0.0001				
Normal	Ref							

Discussion

This study describes, to our knowledge, for the first time in Burundi the HPV prevalence and type-distribution according to HIV status in rural and urban areas. This may put in perspective diagnostic screening and vaccination program using type specific tools. Moreover, it presents HPV prevalence and type-distribution according to cytological findings in the urban area.

These data are of young adult women, never screened for cervical lesions and before any national screening and/or vaccination programme has been rolled out.

According to Bruni L. et al. based on an extensive meta-analysis, the age distribution of cervical HPV infection in Africa is a bimodal curve, with a first peak at younger ages just after sexual debut, a lower prevalence plateau at middle ages, and a variable rebound at older ages(≥ 45 years) [30;31]. Our results from rural and urban follow a similar pattern, show high HPV prevalence in younger women (less than 35 years) with a relatively slow decrease with increasing age. The small differences with the pattern described by Bruni L. et al. and in other studies [32;33] could be due to small numbers in our analysis. Differences in age-specific HPV prevalence has also been reported in a study done in Kenya where HPV prevalence was similarly high across all age groups [34]. The authors hypothesised that the sexual behaviour of these women and/or their partners might not change with age to the same extent as in Western countries.

Our results show a high overall HPV prevalence ranging from of 13.4% to 45.7% in urban HIV-negative and positive women respectively ($p<0.0001$). Our data show significant differences in HR/pHR-HPV prevalence between HIV-positive and negative women in urban (45.7% and 12.8% respectively, $p<0.0001$). This pattern has been described in other studies [4;16;33;36]. Studies done in Africa among HIV-positive women have reported a HR/pHR-HPV prevalence ranging from 27% to 52% [4;32;36;37]. Two studies done in Rwanda by Ngabo et al. and

394 Mukanyangezi et al. found HR/pHR-HPV prevalence in HIV-negative women of 8.1% and 20%,
395 which is in line with our results [36;37]. In Tanzania, Dartell et al. reported a similar HR-HPV
396 prevalence of 17.2% among HIV-negative women [32].

397 The overall HPV prevalence was 30 to 31.3% in rural women with no difference according to
398 HIV status (p=0.80). This high HPV prevalence is in line with other studies conducted in East
399 Africa [30;31;35]. However, HR/pHR-HPV prevalence of rural HIV-positive and negative
400 women were not significantly different (18.7% and 17.3% respectively, p=0.76). This was an
401 unexpected result and our hypothesis is that, HPV infection being related to sexual behaviour,
402 this would imply that these HIV-positive rural women (probably also their partners) changed
403 their sexual behaviour because of the illness and counselling received from HIV clinic health
404 workers where they are followed up for their HIV treatment and did not get re-infected with
405 new HPVs. This argument is reinforced by the observed consistently higher HPV prevalence
406 of urban HIV-positive women across all age groups and suggests differences in sexual
407 behaviour among HIV-positive rural and urban women.
408 Moreover, a selection bias cannot be ruled out as the participants were not randomly
409 selected; all women were enrolled from the health facilities and thus may not represent the
410 rural population. Therefore, more information in robust studies is needed to fully understand
411 the pattern in this area. In the literature, lack of significant differences in HPV prevalence
412 among HIV-positive and negative women has also been reported in a study done in a rural
413 community in Zimbabwe [38].

414 As we expected, stronger independent predictors for abnormal cytology results were a
415 positive HR/pHR-HPV infection and HIV infection (table 3). Indeed, persistent HR-HPV
416 infection has been shown to be a necessary cause for the development of cervical lesions and

417 HIV-infected women being at higher risk of developing cervical lesions. With the WHO last
418 recommendations to start HAART among HIV-infected persons irrespective to their immunity
419 level, we can only speculate that HAART will lead to a more rapid clearance of HR/pHR-HPV
420 types among HIV-positive women, and as a consequence a reduction in incidence of cytology
421 and histology diagnosed cervical lesions, as this has been reported by Kelly et al. [39] in their
422 recent meta-analysis.

423 Stratified by cytological results, our findings show a HPV prevalence of 28.7% and 9.2% in HIV-
424 positive and negative women respectively with normal cytology, which is in line with data
425 from East Africa showing HPV prevalence in women with normal cytology ranging from 3.2%
426 to 41.4% [27;30;40;41].
427 HR/pHR-HPV prevalence in HIV-positive women with ASCUS/LSIL was 100% in our results and
428 is higher than the prevalence found in a meta-analysis by Clifford M. et al [16]; which was
429 69.4%. We think that this difference could be due to large sample size in their study compared
430 to ours. Another reason could be differences in the sensitivity of the HPV detection methods
431 used in these studies as it has been reported [42]. Larger meta-analyses including studies on
432 HR/pHR-HPV prevalence in the East-African region also showed similar results compared to
433 ours among women with HSIL/ICC which ranged from 76.6 % to 96.6 %, and clearly showed
434 an increase in HR/pHR-HPV prevalence as the severity of the cytological lesions increases
435 [27;43].

436 HPV strains and implications in the choice of HPV vaccines
437 Regarding the most isolated HPV strains among HIV-positive urban women with normal
438 cytology, our findings are in agreement with results found in two robust meta-analyses
439 presenting type-specific HPV prevalence in HIV-positive women with normal cytology in Africa

despite some differences in ranking [16;41]. Consistent results with the literature are also found among women with LSIL/ASCUS. In women with high grade lesions (despite few numbers in our study, N=4), type-specific HPVs are concordant with results from other studies showing that the HPV 16 is by far the most prevalent HPV type [43;44].

In the HIV-negative group, this high frequency of HPV 67 in our results has not been investigated in larger meta-analyses.

In the meta-analysis by Guan et al. [27], African women with LSIL/ASCUS were HPV 58 positive in important proportions, and women with HSIL/ICC were predominantly infected by HPV 16 & 18, which is in agreement with our findings.

Our data from rural area cannot be disaggregated by cytological results but results are roughly similar to results found in Rwanda and Kenya [27;35]. Stratified by HIV status, the most frequent HPV types in our results are overall the same as results from a study done in Tanzania [32]. Our results highlight a high prevalence of the much known carcinogenic HPV types (HPV 16, 18, 52, 51, 56, 33 and 58) and very likely to persist in this unscreened population, which translates into high rates of cervical precursor lesions, making this population a high priority for public health interventions, regardless of their HIV-status. This is reinforced by the fact that this population has never been screened for cervical cancer lesions and clearly shows the need for implementation of cervical cancer screening intervention in Burundian women population.

In rural, the results in figure 2 imply that the Cervarix® or Gardasil® vaccines would target 32.1% and 30.8% of the HR/pHR-HPV infections among HIV-positive and negative women respectively. The nonavalent vaccine, Gardasil-9®, would target 60.7% and 53.9% of the circulating HR/pHR-HPV infections among HIV-positive and negative women respectively.

In urban women, Gardasil® or Cervarix® vaccine would target 8.7% and 15.8% of all HR/pHR-HPV infections in HIV-positive and negative women respectively and Gardasil-9® would prevent 31.9% and 42.1% of all HR/pHR-HPV infections in HIV-positive and negative women respectively. Overall, our results showed that Gardasil-9 vaccine covers most HR/pHR-HPV infections. If we look at the urban results, cervarix® would target 2/3rd and all HSIL/ASC-H/ICC among HIV-positives and negatives respectively; whereas Gardasil-9® would target 7 on 8 and 100% of all HSIL/ASC-H/ICC among HIV-positives and negatives respectively (Table 2).

We are aware, however, of the described cross-protection [45] of HPV vaccines and studies on HPV strain distributions in invasive cervical cancer cases are also needed. The results of these studies plus the related cost-effectiveness studies are necessary elements to provide Burundian health authorities a sound evidence to choose the appropriate HPV screening and/or vaccination programmes.

Study limitations

Our study population choose 4 similar strata according to HIV status and urban/rural and participants were not randomly selected. Thus, our overall study results do not represent the general female population of Burundi. This was a deliberate choice as we wanted to avoid that urban women and HIV infected women would be underrepresented. According to the 2010 Burundi Demographic Health Survey, 91% of Burundian women live in rural areas where HIV prevalence is estimated at 1.2% in women aged 15-49 years old, we assume that the results of the HIV negative women in Kirundo may be most representative result for the Burundian situation. This limitation turns indeed out to be a strength as we provide comprehensive baseline estimates of HPV prevalence and type-specific distribution in HIV-positive and negative women in rural and urban Burundi. And we observed the limited additional risk of rural HIV-infected women (who are relatively few) and the increased risk of

the urban HIV-positive women. Another limitation is related to the use of two different HPV tests for rural and urban data which might have different sensitivity. Also, cytology results were only available for urban population with relatively few numbers across different cytological strata. Thus, caution is required for the inference of our results to the general population.

Conclusions

In Burundi there is a high burden of HPV infection, in particular HR/pHR-HPV infections which mimics other similar settings. We observe that HIV-infected women in Bujumbura are a specific risk group to be targeted in contrast to the rural area where all women seem to have similar distributions of HPV circulating strains. This study strengthens the urgent need to introduce a comprehensive cervical cancer control program adapted to the context. This most probably may include a HPV-vaccination programme though cannot be the sole component due to the actual burden, the coverage and efficacy and lag time of the impact. Complementary studies in invasive cervical cancer cases are needed to further elucidate the most appropriate control tools.

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References

(1) Munoz N, Bosch FX, de Sanjose S., Tafur L, Izarzugaza I, Gili M, et al. The causal link between human papillomavirus and invasive cervical cancer: a population-based case-control study in Colombia and Spain. Int J Cancer 1992 Nov 11;52(5):743-9.

(2) Munoz N, Bosch FX, de Sanjose S., Viladiu P, Tormo J, Moreo P, et al. [Human papilloma virus in the etiology of cervicouterine cancer]. Bol Oficina Sanit Panam 1993 Oct;115(4):301-9.

(3) Munoz N, Bosch FX, de Sanjose S., Shah KV. The role of HPV in the etiology of cervical cancer. Mutat Res 1994 Mar 1;305(2):293-301.

(4) Mbulawa ZZA, van SC, Hu NC, Meiring TL, Barnabas S, Dabee S, et al. High human papillomavirus (HPV) prevalence in South African adolescents and young women encourages expanded HPV vaccination campaigns. PLoS One 2018;13(1):e0190166.

(5) Schiffman M, Castle PE. The promise of global cervical-cancer prevention. N Engl J Med 2005 Nov 17;353(20):2101-4.

(6) IARC. Working group on the evaluation of carcinogenic risks to humans. World Health Organization, editor. [100B], 255-314. 2012. Lyon France, IARC. Ref Type: Serial (Book,Monograph)

(7) Halec G, Schmitt M, Dondog B, Sharkhuu E, Wentzensen N, Gheit T, et al. Biological activity of probable/possible high-risk human papillomavirus types in cervical cancer. Int J Cancer 2013 Jan 1;132(1):63-71.

(8) Halec G, Alemany L, Lloveras B, Schmitt M, Alejo M, Bosch FX, et al. Pathogenic role of the eight probably/possibly carcinogenic HPV types 26, 53, 66, 67, 68, 70, 73 and 82 in cervical cancer. J Pathol 2014 Dec;234(4):441-51.

(9) Munoz N, Bosch FX, de SS, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003 Feb 6;348(6):518-27.

(10) Gravitt PE. Evidence and impact of human papillomavirus latency. Open Virol J 2012;6:198-203.

(11) Clifford GM, Polesel J, Rickenbach M, Dal ML, Keiser O, Kofler A, et al. Cancer risk in the Swiss HIV Cohort Study: associations with immunodeficiency, smoking, and highly active antiretroviral therapy. J Natl Cancer Inst 2005 Mar 16;97(6):425-32.

(12) Frisch M, Biggar RJ, Goedert JJ. Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. J Natl Cancer Inst 2000 Sep 20;92(18):1500-10.

(13) Reddy D, Njala J, Stocker P, Schooley A, Flores M, Tseng CH, et al. High-risk human papillomavirus in HIV-infected women undergoing cervical cancer screening in Lilongwe, Malawi: a pilot study. Int J STD AIDS 2014 Jun 13.

(14) Smith-McCune KK, Shiboski S, Chirenje MZ, Magure T, Tuveson J, Ma Y, et al. Type-specific cervico-vaginal human papillomavirus infection increases risk of HIV acquisition independent of other sexually transmitted infections. PLoS One 2010 Apr 8;5(4):e10094.

(15) Strickler HD, Burk RD, Fazzari M, Anastos K, Minkoff H, Massad LS, et al. Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus-positive women. J Natl Cancer Inst 2005 Apr 20;97(8):577-86.

(16) Clifford GM, Goncalves MA, Franceschi S. Human papillomavirus types among women infected with HIV: a meta-analysis. AIDS 2006 Nov 28;20(18):2337-44.

(17) Minkoff H, Feldman J, Dehovitz J, Landesman S, Burk R. A longitudinal study of human papillomavirus carriage in human immunodeficiency virus-infected and human immunodeficiency virus-uninfected women. Am J Obstet Gynecol 1998 May;178(5):982-6.

(18) Denny L, Quinn M, Sankaranarayanan R. Chapter 8: screening for cervical cancer in developing countries. Vaccine 2006;24(suppl):S3-71-S3-77.

(19) Maseko FC, Chirwa ML, Muula AS. Health systems challenges in cervical cancer prevention program in Malawi. Glob Health Action 2015;8:26282.

(20) Qiao YL, Sellors JW, Eder PS, Bao YP, Lim JM, Zhao FH, et al. A new HPV-DNA test for cervical-cancer screening in developing regions: a cross-sectional study of clinical accuracy in rural China. Lancet Oncol 2008 Oct;9(10):929-36.

(21) de Martel C., Plummer M, Vignat J, Franceschi S. Worldwide burden of cancer attributable to HPV by site, country and HPV type. Int J Cancer 2017 Aug 15;141(4):664-70.

(22) Bruni L, Barrionuevo-Rosas L, Serrano B, Brotons M, Cosano R, Muñoz J, et al. Human Papillomavirus and Related Diseases in Burundi. Summary Report 2016-02-26. Barcelona: ICO Information Centre on HPV and Cancer (HPV Information Centre); 2016.

(23) Nelson AM, Milner DA, Rebbeck TR, Iliyasu Y. Oncologic Care and Pathology Resources in Africa: Survey and Recommendations. J Clin Oncol 2016 Jan 1;34(1):20-6.

(24) Ndizeye Z, Vanden Broeck D, Vermandere H, Bogers JP, Van Geertruyden JP. Knowledge and practices of general practitioners at district hospitals towards cervical cancer prevention in Burundi, 2015: a cross-sectional study. Global Health 2018 Jan 16;14(1):4.

(25) Ministère de la Santé Publique et de la Lutte contre le SIDA. Plan d'introduction du vaccin contre le virus du papillome humain. Bujumbura: Ministère de la Santé Publique et de la Lutte contre le SIDA; 2013 Sep.

(26) Lu B, Kumar A, Castellsague X, Giuliano AR. Efficacy and safety of prophylactic vaccines against cervical HPV infection and diseases among women: a systematic review & meta-analysis. BMC Infect Dis 2011 Jan 12;11:13.

(27) Guan P, Howell-Jones R, Li N, Bruni L, de SS, Franceschi S, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer 2012 Nov 15;131(10):2349-59.

(28) Micalessi IM, Boulet GA, Bogers JJ, Benoy IH, Depuydt CE. High-throughput detection, genotyping and quantification of the human papillomavirus using real-time PCR. Clin Chem Lab Med 2011 Dec 20;50(4):655-61.

(29) Coutlee F, Rouleau D, Petignat P, Ghattas G, Kornegay JR, Schlag P, et al. Enhanced detection and typing of human papillomavirus (HPV) DNA in anogenital samples with PGMY primers and the Linear array HPV genotyping test. J Clin Microbiol 2006 Jun;44(6):1998-2006.

(30) Bruni L, Diaz M, Castellsague X, Ferrer E, Bosch FX, de Sanjose S. Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. J Infect Dis 2010 Dec 15;202(12):1789-99.

(31) Forman D, de MC, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, et al. Global burden of human papillomavirus and related diseases. Vaccine 2012 Nov 20;30 Suppl 5:F12-F23.

(32) Dartell M, Rasch V, Kahesa C, Mwaiselage J, Ngoma T, Junge J, et al. Human papillomavirus prevalence and type distribution in 3603 HIV-positive and HIV-negative women in the general population of Tanzania: the PROTECT study. Sex Transm Dis 2012 Mar;39(3):201-8.

(33) McDonald AC, Tergas AI, Kuhn L, Denny L, Wright TC, Jr. Distribution of Human Papillomavirus Genotypes among HIV-Positive and HIV-Negative Women in Cape Town, South Africa. Front Oncol 2014;4:48.

(34) De VH, Parisi MR, Karani A, Mandaliya K, Muchiri L, Vaccarella S, et al. The prevalence of human papillomavirus infection in Mombasa, Kenya. Cancer Causes Control 2010 Dec;21(12):2309-13.

(35) de Sanjose S., Diaz M, Castellsague X, Clifford G, Bruni L, Munoz N, et al. Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. Lancet Infect Dis 2007 Jul;7(7):453-9.

(36) Mukanyangezi MF, Sengpiel V, Manzi O, Tobin G, Rulisa S, Bienvenu E, et al. Screening for human papillomavirus, cervical cytological abnormalities and associated risk

factors in HIV-positive and HIV-negative women in Rwanda. HIV Med 2018 Feb;19(2):152-66.

(37) Ngabo F, Franceschi S, Baussano I, Umulisa MC, Snijders PJ, Uyterlinde AM, et al. Human papillomavirus infection in Rwanda at the moment of implementation of a national HPV vaccination programme. BMC Infect Dis 2016;16:225.

(38) Baay MF, Kjetland EF, Ndhlovu PD, Deschoolmeester V, Mduluza T, Gomo E, et al. Human papillomavirus in a rural community in Zimbabwe: the impact of HIV co-infection on HPV genotype distribution. J Med Virol 2004 Jul;73(3):481-5.

(39) Kelly H, Weiss HA, Benavente Y, de SS, Mayaud P. Association of antiretroviral therapy with high-risk human papillomavirus, cervical intraepithelial neoplasia, and invasive cervical cancer in women living with HIV: a systematic review and meta-analysis. Lancet HIV 2018 Jan;5(1):e45-e58.

(40) De Vuyst H., Alemany L, Lacey C, Chibwesha CJ, Sahasrabuddhe V, Banura C, et al. The burden of human papillomavirus infections and related diseases in sub-saharan Africa. Vaccine 2013 Dec 29;31 Suppl 5:F32-F46.

(41) Clifford GM, Gallus S, Herrero R, Munoz N, Snijders PJ, Vaccarella S, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet 2005 Sep 17;366(9490):991-8.

(42) Rebolj M, Bonde J, Preisler S, Ejegod D, Rygaard C, Lynge E. Differential Detection of Human Papillomavirus Genotypes and Cervical Intraepithelial Neoplasia by Four Commercial Assays. J Clin Microbiol 2016 Nov;54(11):2669-75.

(43) Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, et al. Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 2007 Aug 1;121(3):621-32.

(44) de Sanjose S., Quint WG, Alemany L, Geraets DT, Klaustermeier JE, Lloveras B, et al. Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. Lancet Oncol 2010 Nov;11(11):1048-56.

(45) Wheeler CM, Castellsague X, Garland SM, Szarewski A, Paavonen J, Naud P, et al. Cross-protective efficacy of HPV-16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by non-vaccine oncogenic HPV types: 4-year end-of-study analysis of the randomised, double-blind PATRICIA trial. Lancet Oncol 2012 Jan;13(1):100-10.

List of abbreviations

ABUBEF: Association Burundaise pour le Bien-Etre Familial

AGC: Atypical Glandular Cells

ANSS: Association Nationale de Soutien aux Séropositifs et malades du SIDA

ASC-H: Atypical Squamous Cells cannot exclude High-grade lesion

ASCUS: Atypical Squamous Cells of Undetermined Significance

CI: Confidence Interval

CIN: Cervical Intraepithelial Neoplasia

DNA: DesoxyriboNucleic Acid

HIV: Human Immunodeficiency Virus

HPV: Human Papillomavirus

HR/pHR-HPV: High-risk/possible high-risk human papillomavirus

HSIL: High-grade Squamous Intraepithelial Lesions

ICC: Invasive Cervical cancer

LMICs: Low and Middle Income Countries

LR-HPV: Low-risk human papillomavirus

LSIL: Low-grade Squamous Intraepithelial Lesions

OR: Odds ratio

PCR: Polymerase Chain Reaction

S.D: Standard Deviation

SSA: Sub-Saharan Africa

STI: Sexually Transmitted Infection

VIA: Visual Inspection with 5% Acetic acid

VILI: Visual Inspection with Lugol's Iodine

678 WHO: World Health Organization

679 **Declarations**

680 Availability of data and material

681 The dataset used and/or analysed during the current study are available from the

682 corresponding author on reasonable request.

683 Authors' contributions

684 Conceptualisation: ZN, JPVG, DVB

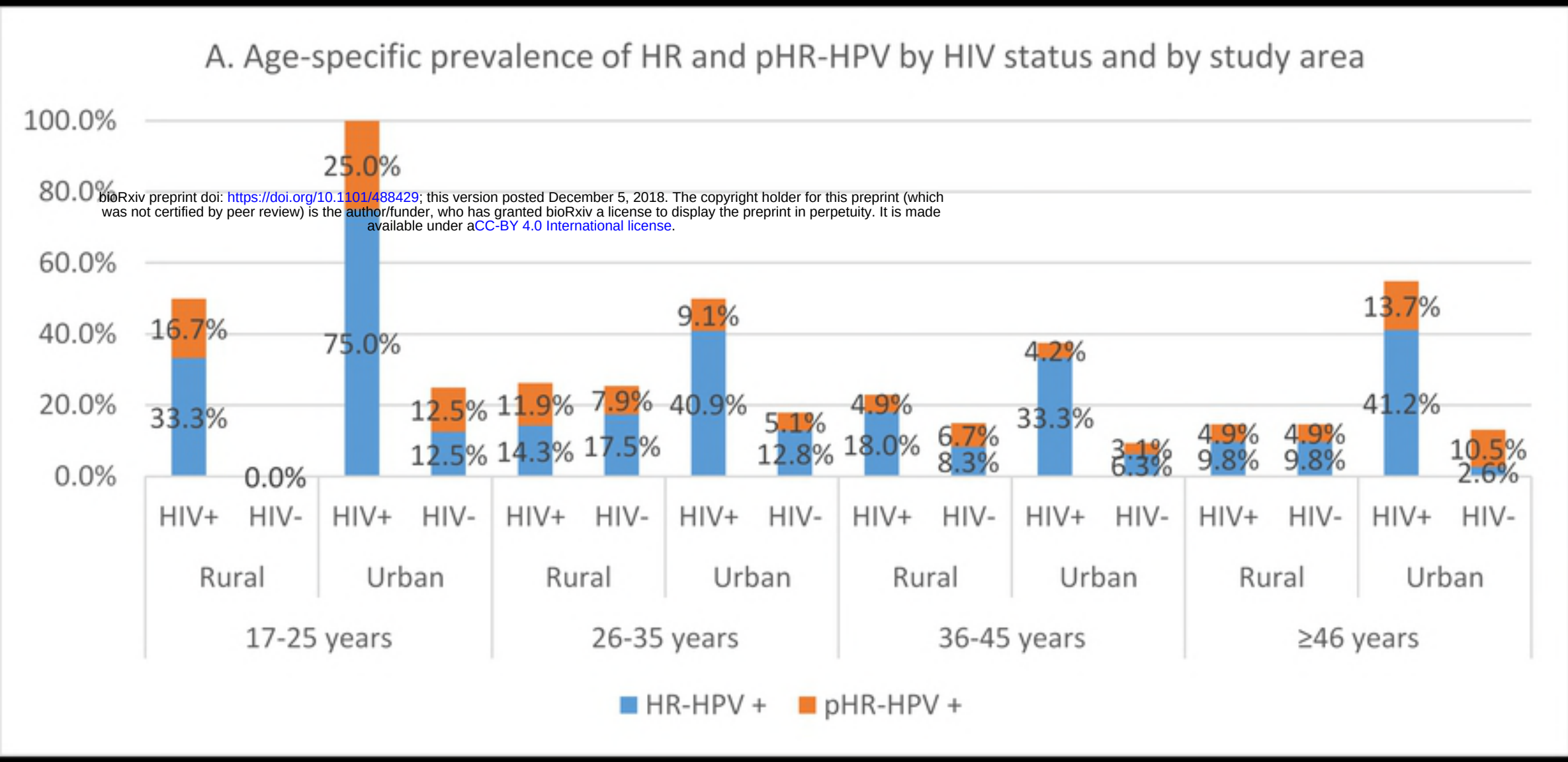
685 Data curation: ZN, JPV

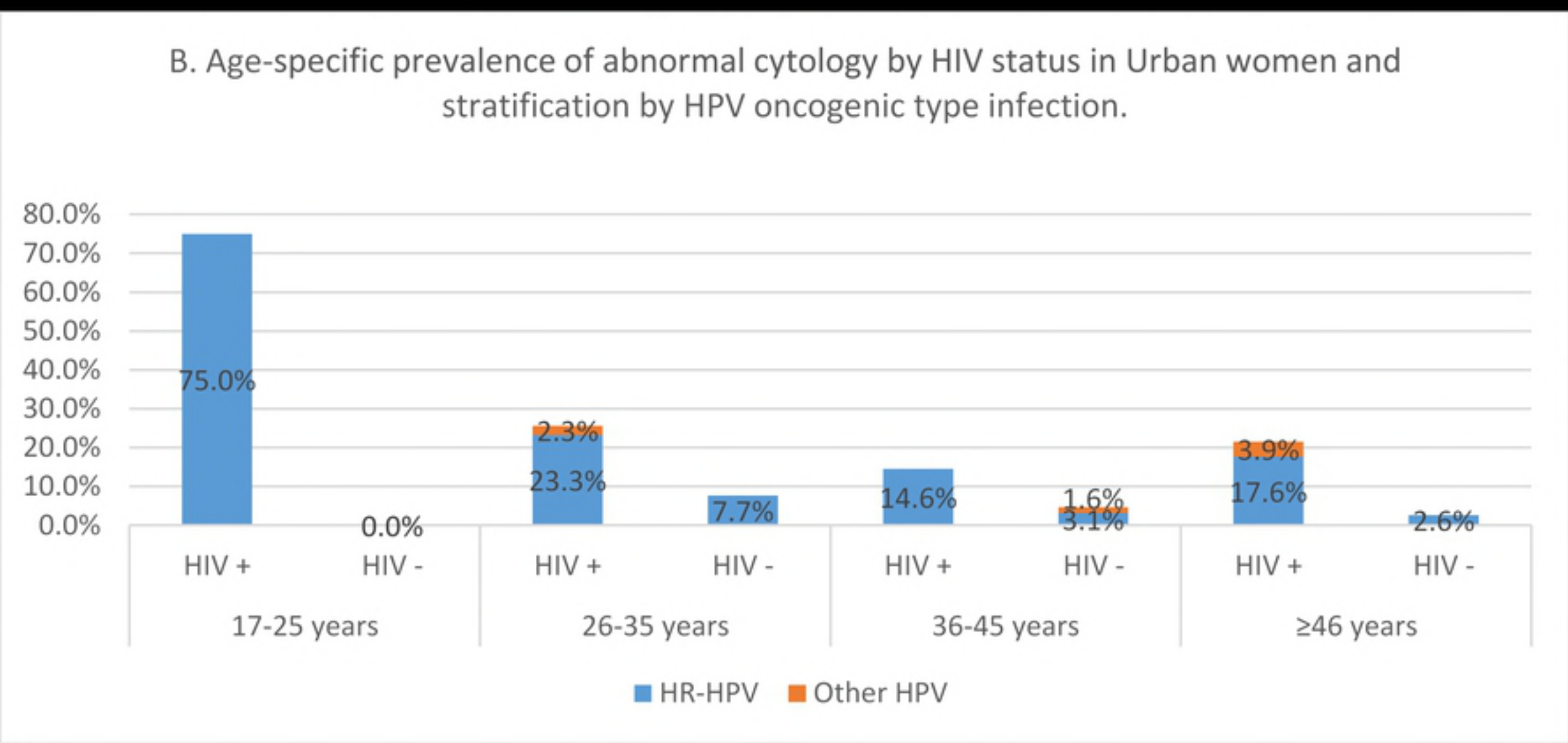
686 Analysis and interpretation of data: ZN, JPVG, DVB

687 Investigation (laboratory part): DVB, LRL, JB, IB

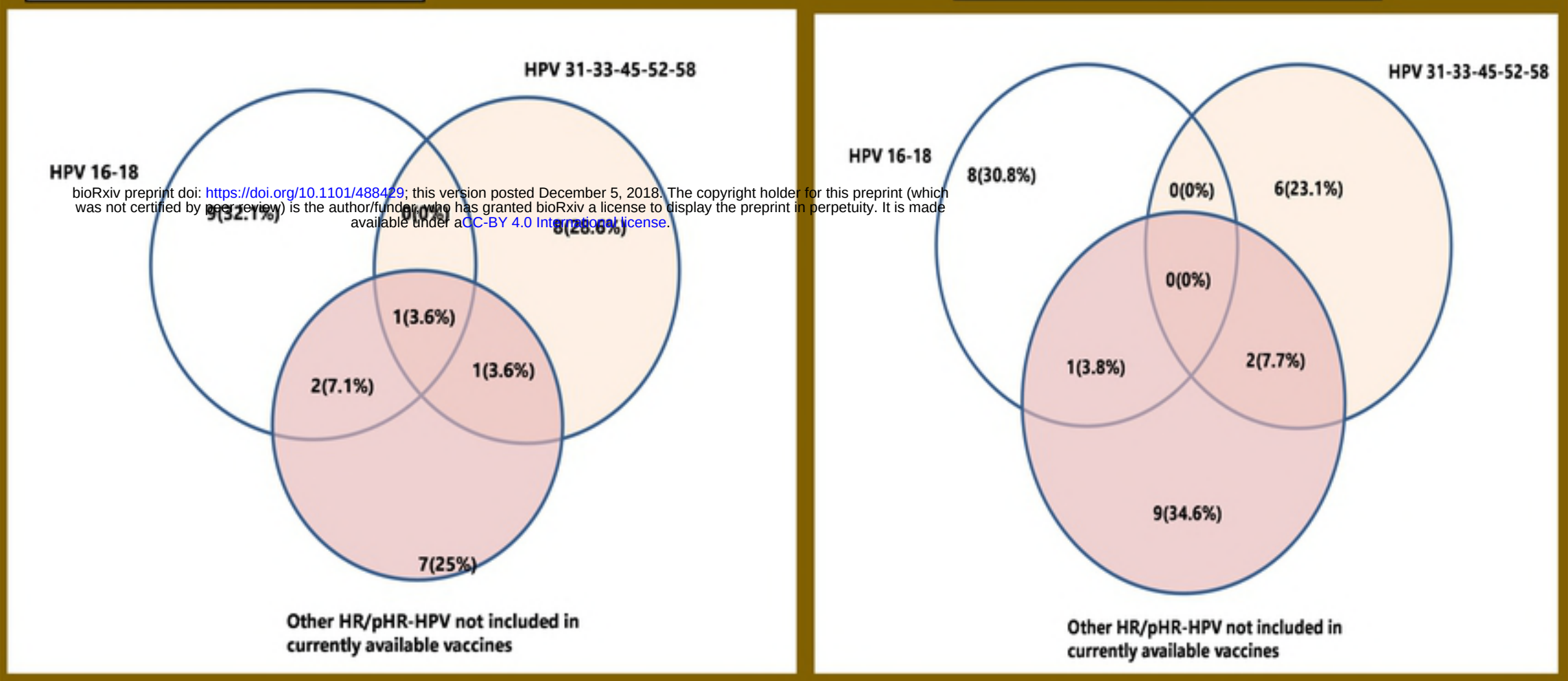
688 Methodology: ZN, JPV, DVB

689 Wrote or reviewed and approved the final manuscript: ZN, JPVG, DVB, LRL, JB, IB





Figure



Figure

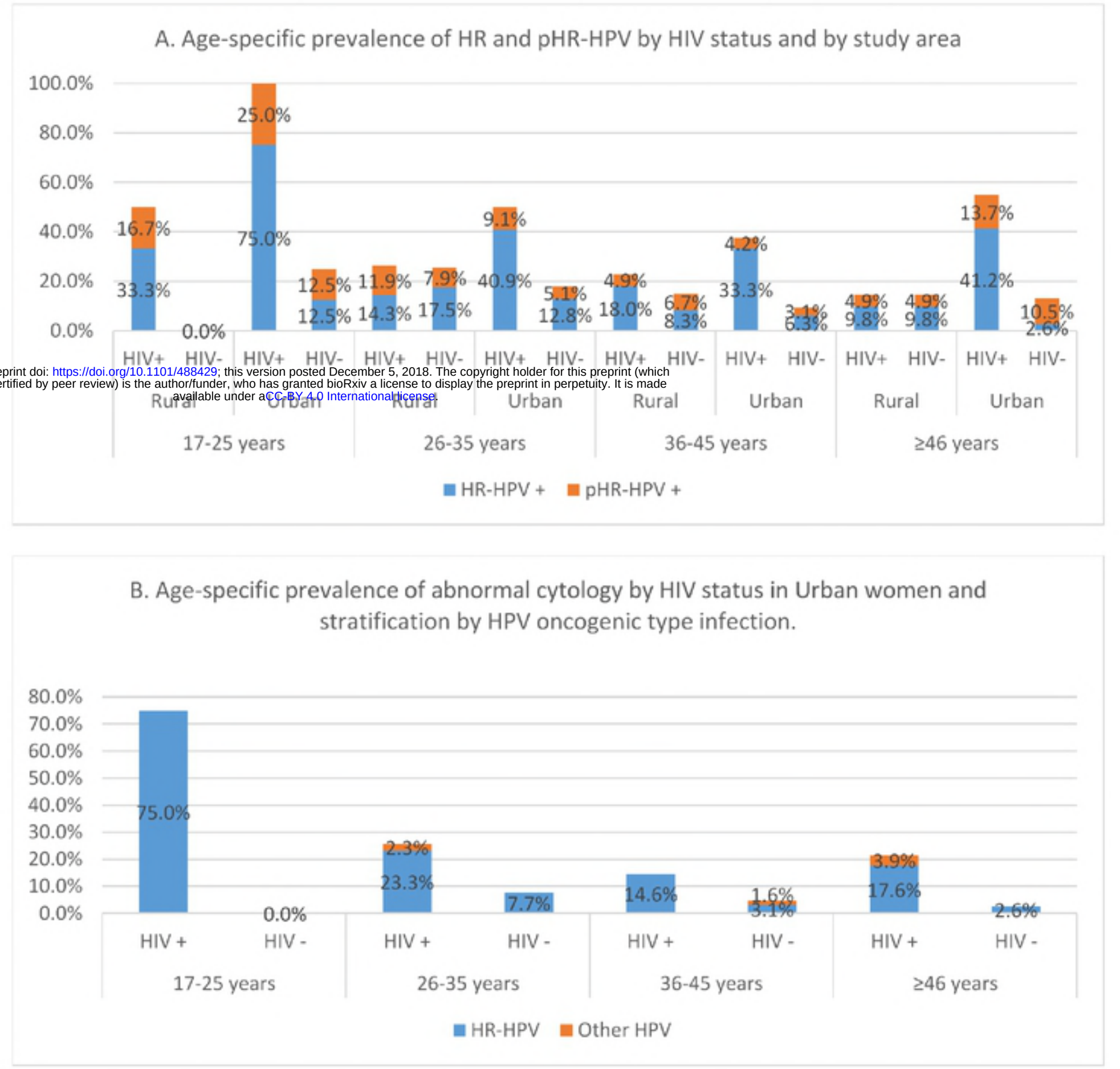


Figure 1: Age-specific prevalence of HPV (A) and of abnormal cytological results (B) stratified by HIV status in rural and urban women, Burundi

Figure