

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

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20

21 **Abstract**

22 **Background**

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

30 **Methods**

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

35 **Results**

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

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

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

46 The most prevalent HR/pHR-HPV types in HIV-positive urban women were HPV 52, 51 and 56.

47 In the HIV-negative urban women, the most prevalent HR/pHR-HPV types were HPV 66, 67

48 and 18. In HIV-positive rural women, the most prevalent HR/pHR-HPV types were HPV 66, 16

49 and 18. In the HIV-negative rural women, the most prevalent HR/pHR-HPV types were HPV

50 16, 66 and 18. Independent risk factors associated with cervical lesions were HPV and HIV

51 infections.

## 52 **Conclusions**

53 There is a high burden of HR/pHR-HPV infections, in particular among HIV-infected urban

54 women. The study points out the need to introduce a comprehensive cervical cancer control

55 program adapted to the context. This study shows that the nonavalent vaccine covers most

56 of the HR/pHR-HPV infections in rural and urban areas among HIV-infected and uninfected

57 women.

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

## 59 **Background**

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

65 HPVs are small double-stranded DNA viruses, with a large epithelial tropism. Basal epithelial  
66 cells are infected with HPVs, causing benign and malignant lesions of the skin and the ano-  
67 genital mucosae and the upper aero-digestive tract [6]. Studies on HPV epidemiology and  
68 their mechanistic evidence have led to their classification into three groups: (1) HPV types 16,  
69 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are known as carcinogenic, also named high-risk  
70 (HR)-HPV types; (2) HPV types 26, 53, 66, 67, 68, 70, 73 and 82, are classified as  
71 probably/possibly high-risk carcinogenic (pHR)-HPV types and (3) other HPV types such as  
72 HPV 6 and 11 are classified as low-risk (LR)-HPV types [7]. However, there is a growing  
73 literature providing evidence that the classified (pHR)-risk HPV types may also have to be  
74 considered as (HR)-HPV types [7-9].

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

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

83 are more frequent in HIV-positive women than in HIV-negative women [16;17]. Thus, HIV-  
84 infected women are at higher risk of developing ICC compared to HIV-uninfected women [11-  
85 13]. Prevention of ICC in sub-Saharan Africa (SSA) through screening with the conventional  
86 Pap smear method or other alternative techniques such as visual inspection with Acetic  
87 Acid/Lugol's iodine is challenging and barely rolled out on public health scale [18;19]. New  
88 control strategies such as HPV screening with rapid molecular HPV tests and HPV vaccination  
89 are emerging and may be promising complementary tools for the Low and Middle Income  
90 Countries (LMICs) [20].

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

95 In Burundi, ICC represents the most common female cancer, accounting for approximately  
96 39% of all female cancers [22]. It is responsible, each year, for an estimated 1 421 new cases  
97 and 1 080 deaths in Burundi, representing an annually age-standardised incidence and  
98 mortality rates of 49.3 and 39.3/100,000 women respectively [22]. Burundi does not have any  
99 cervical cancer screening programme because of a variety of factors including lack of  
100 adequate infrastructure, insufficiently qualified staff and insufficient investment in resources  
101 for pap-smears, biopsies and colposcopy [23;24].

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

107 due to insufficient coverage, missed follow-up doses, strain incompatibility and cost. Indeed,  
108 it remains challenging to guarantee sufficient coverage and ensure all girls of appropriate age  
109 to be vaccinated. Apart from report on cross-protection [26], the bivalent vaccine chosen by  
110 Burundi offers protection against the two most prevalent HR-HPV types 16 and 18. Both  
111 genotypes are expected to be responsible for 70% of all cancer cases, implying that at least  
112 30% of all cervical cancer cases might not be covered by this prophylactic vaccination.

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

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

## 122 **Materials and methods**

### 123 **Study design, setting and population**

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

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

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

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

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

#### 146 **Recruitment procedures**

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

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

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

#### 164 **Inclusion and exclusion criteria**

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

#### 168 **Sample size determination**

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

#### 174 **HPV testing and liquid based cytology processing**

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



178 For the 300 samples from the urban area, thin-layer slides were prepared with the ThinPrep®  
179 5000 Processor with Autoloader System (Hologic Inc, Marlborough, US) and stained with the  
180 Papanicolaou stain on the Tissue-Tek® Prisma and Film Automated Slide Stainer and  
181 Coverslipper (Sakura Finetek Europe B.V., Netherlands). After scanning of the slides with the  
182 ThinPrep® Imaging System, cytology reading was performed by image-guided screening, with  
183 prior knowledge of HPV infection status. Cytological diagnoses were reported according to  
184 the Bethesda 2001 terminology system as 1) normal, 2) atypical squamous cells of  
185 undetermined significance; atypical squamous cells cannot exclude high-grade lesion; atypical  
186 glandular cells; low-grade squamous intraepithelial lesions (ASCUS/ASC-H/AGC/LSIL), 3) high-  
187 grade squamous intraepithelial lesions (HSIL), or 4) invasive cancer. After the LBC  
188 preparations are made, 800µL of the remaining cell suspension was used for DNA extraction.  
189 Cytology reading was performed only on the 300 urban samples due to budget constraints.

#### 190 HPV type-specific detection

191 For the urban samples, DNA isolation from liquid-based cytology was performed on the  
192 Medium Throughput Automation (MTA) (Hologic Inc) with the Genfind® DNA extraction kit.  
193 Subsequently, the DNA is amplified using a series of real-time qPCR reactions in the  
194 LightCycler 480 (Roche) as previously described by Micalesse et al [28]. Briefly, the RIATOL  
195 qPCR HPV genotyping assay is a clinically validated, laboratory developed test, which amplifies  
196 18 HPV types: HPV 6E6, 11E6, 16E7, 18E7, 31E6, 33E6, 35E6, 39E7, 45E7, 51E7, 52E7, 53E6,  
197 56E7, 58E7, 59E7, 66E6, 67 L1 and 68E7. Real-time quantitative PCR for β-globin was always  
198 performed and was used as a proxy for the quality of sampling.

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

202 detected following conventional nested HPV. PCR was performed with MY09/MY11 (450 bp)  
203 and GP5+/GP6+ (150 bp). First round PCR was carried out in a final reaction of 50 µl containing  
204 1 X buffer II (Bioline, Luckenwalde, Germany), 1.5 mM MgCl<sub>2</sub> (Bioline, Luckenwalde,  
205 Germany), 200 µM of each deoxynucleoside triphosphate (dNTP), 0.2 µM of MY09/MY11  
206 primers and 0.2 µM PCO3 and PCO4 primers (110 bp) (Inqaba Biotechnological Industry,  
207 Pretoria, South Africa), 1 unit of Bio Taq DNA polymerase (Bioline, Hilden, Germany), and 10  
208 µl of DNA template. The cycling conditions included an initial denaturation step for 2 minutes  
209 at 94°C followed by 40 cycles at 94°C for denaturation, 30 seconds at 55°C for annealing, 1  
210 minute at 72°C for elongation and a final elongation for 5 minutes at 72°C. The PCR product  
211 detected using gel electrophoresis (2% w/v agarose in Tris-acetate EDTA) and visualised using  
212 ethidium bromide. Bands of the appropriate size were identified by comparison with a DNA  
213 molecular weight marker and the gel was viewed using a Gel DOC system (Syngene, Europe).  
214 HPV genotyping was performed using Linear array genotyping test that identifies 37 HPV  
215 genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56,58, 59, 61, 62, 64,  
216 66, 67, 68, 69, 70,71, 72 73, 81, 82, 83, 84, 89, IS39) as previously described by Coutlee et al.  
217 [29].  
218 Briefly, 50µl of amplicon was added to 50µl of a working master mix containing MgCl<sub>2</sub>,  
219 Amplitaq® GOLD DNA polymerase, Uracil-N-glycosilase, deoxynucleotides, PGMY and β globin  
220 primers. The PCR amplification was performed using the gold-plated 96-well Gene Amp PCR  
221 System 9700 (Applied Biosystems, Foster City, California, USA) according to manufacturer  
222 instructions. Positive reactions appeared as blue lines and were interpreted using the LA HPV  
223 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  $\alpha$ -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 (%)	
<b>Age group (years)</b>						
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
<b>Profession</b>						
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)	
<b>Marital status</b>						
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)	
<b>Mean age of menarche (SD)</b>	14.5 (1.7)	14.5 (1.8)	0.71	14.6 (1.7)	14.7 (1.7)	0.71
<b>Median age at sexual intercourse debut(IQR)</b>	18 (16-19)	22 (18-26)	<0.001	18 (16-20)	19 (17-23)	<0.001
<b>Median age of marriage(IQR)</b>	20 (17-25)	26 (23-29)	<0.001*	19 (17-22)	22 (18-26)	<0.001 <sup>i</sup>
<b>Median age at 1<sup>st</sup> pregnancy(IQR)</b>	20 (17-23)	26 (21-29)	<0.001**	19 (17-21)	22 (19-26)	<0.001 <sup>ii</sup>
<b>Median gestity(IQR)</b>	4 (2-6)	4 (3-5)	0.99	4 (3-6)	4 (3-6)	0.72
<b>Median parity(IQR)</b>	3 (2-5)	3 (2-5)	0.65	4 (2-6)	4 (2-5)	0.31
<b>Number of lifetime sex partners</b>	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
<b>Smoking</b>						
Yes	4 (2.6)	2 (1.3)	0.34	23 (15.3)	14 (9.3)	0.11
<b>alcohol consumption</b>						
Yes	48 (31.8)	71 (47.7)	0.005	70 (46.7)	79 (52.7)	0.29
<b>Cytological result</b>						
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; <sup>i</sup>N=282; <sup>ii</sup>:N=294

262

## 263 HPV prevalence

264 In rural, all participants had valid data on HPV genotyping. The HPV prevalence (any) was 30%  
265 (45/150) and 31.3% (47/150) among the HIV-positive and negative women respectively  
266 ( $p=0.80$ ). Multiple HPV infection was 11.3% and 14% among the HIV-positive and negative  
267 women respectively ( $p=0.49$ ). The HR/pHR-HPV prevalence was 18.7% and 17.3% in HIV-  
268 positive and negative respectively ( $p=0.76$ ). Multiple HR/pHR-HPV infections was 4.7% versus  
269 3.3% among HIV-positive and negative women respectively ( $p=0.56$ ).

270 In the HIV-positive group, infection with any HR-HPV, pHR-HPV and LR-HPV type was 14.7%,  
271 7.3% and 19.3% respectively. In HIV-negative women, infection with any HR-HPV, pHR-HPV  
272 and LR-HPV type was 12%, 6.7% and 24.7% respectively (Table 2). Infection with HPV 16 or 18  
273 was 8% versus 6.7% among HIV-positive and negative women respectively ( $p=0.50$ ). The  
274 prevalence of the HR-HPV types included in the Gardasil-9 vaccine was 14% versus 11.3%  
275 among HIV-positive and negative women respectively ( $p=0.49$ ). The most frequent HR-HPV  
276 types in the HIV-positive group were HPV 16 (4%), 18 (4%), followed by HPV 33 (3.3%) and  
277 HPV 58 (2%). The most frequent pHR-HPV types were HPV 66 (4.7%) and HPV 70 (3.3%).  
278 Among the LR-HPV types, the most frequently isolated were HPV 11 (15.3%) and HPV 6 (2%).  
279 In the HIV-negative group, the most frequent HR-HPV types isolated were HPV 16 (4.7%),  
280 followed by HPV 18 (2%). The most frequent pHR-HPV types were HPV 66 (4%) and HPV 67  
281 (1.3%). The most frequent LR-HPV types were HPV 11 (20%) and HPV 6 (4%).

282 Table 2 also presents HPV prevalence results for the urban area. The overall HPV prevalence  
283 (any HPV) was 45.7% and 13.4% among HIV-positive and HIV-negative women ( $p<0.0001$ ).  
284 HPV prevalence is presented by cytological results and by HIV-status. Multiple infection with  
285 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								P <sup>a</sup>
	HIV positive (N=150)	HIV negative (N=150)	P	HIV positive women				HIV negative women				
				Normal (N=115)	ASCUS/LSIL (N=29)	HSIL/ASC-H/ ICC (N=6)	Total (N=151)	Normal (N=142)	ASCUS/LSI L (N=5)	HSIL/ASC-H/ ICC (N=2)	Total (N=149)	
N (%)	N (%)		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)		
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
<b>High risk</b>												
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)	1 (0.7)	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)	
<b>Possibly high risk</b>												
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)	
<b>Low risk</b>												
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. <sup>a</sup> comparison of total HIV+ and total HIV- in urban



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

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

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

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

319 In the HIV-positive group, among 150 women with a valid result on LBC, 23.3% (35/150) had  
320 an abnormal cytological result, including 8.7% (13/150) with ASCUS, 10.6% (16/150) with LSIL,  
321 1.3% (2 patients) with ASC-H, 2% with HSIL and 1 patient with ICC. In the HIV-negative group,  
322 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
<b>HR/pHR-HPV infection</b>				
No	-			
Yes	199.1 (26.7-1482.9)	<0.0001	162.54 (20.94-1261.42)	<0.0001
<b>Age group (years)</b>				
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
<b>HIV infection</b>				
Yes	6.17 (2.64-14.42)	<0.0001	1.69 (0.54-5.29)	0.37
No	-		-	
<b>Number of lifetime sexual partners (N=297)</b>				
1	-		-	
≥2	2.57 (1.14-5.80)	0.02	1.69 (0.59-4.89)	0.33
<b>Profession</b>				
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
<b>Marital status</b>				
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
<b>age sexual debut</b>				
<17	-		-	
≥17	0.66 (0.33-1.34)	0.25		
<b>Age of marriage (N=276)</b>				
<17	-		-	
≥17	0.97 (0.27-3.44)	0.96		
<b>age at first pregnancy (N=292)</b>				
<17	-		-	
≥17	0.60 (0.23-1.57)	0.30		
<b>Gesity</b>				
<6	-		-	
≥6	1.78 (0.89-3.57)	0.10		
<b>Parity</b>				
<6	-		-	
≥6	0.80 (0.30-2.18)	0.67	0.66 (0.16-2.84)	0.58
<b>Alcohol consumption</b>				
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.

361

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365

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

366

367

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
<b>Age group (years)</b>										
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		
<b>HIV infection</b>										
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				
<b>Number of lifetime sexual partners</b>		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		
<b>Profession</b>										
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
<b>Marital status</b>										
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		
<b>age sexual debut</b>										
<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		
<b>Age of marriage (N=276)</b>										
<17										
≥17	1.01 (0.41-2.52)		0.98			1.40 (0.54-3.65)		0.49		
<b>age at first pregnancy (N=292)</b>										
<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		
<b>Gestivity</b>										
<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
<b>Parity</b>										
<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
<b>Alcohol consumption</b>										
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										
<b>Cytology result</b>										
Abnormal	193.96 (26.05-1444.13)		<0.0001	148.72 (18.99-1164.60)	<0.0001					
Normal	Ref									

## 370 **Discussion**

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

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

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

388 Our results show a high overall HPV prevalence ranging from of 13.4% to 45.7% in urban HIV-  
389 negative and positive women respectively ( $p < 0.0001$ ). Our data show significant differences  
390 in HR/pHR-HPV prevalence between HIV-positive and negative women in urban (45.7% and  
391 12.8% respectively,  $p < 0.0001$ ). This pattern has been described in other studies [4;16;33;36].  
392 Studies done in Africa among HIV-positive women have reported a HR/pHR-HPV prevalence  
393 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



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

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

446 In the meta-analysis by Guan et al. [27], African women with LSIL/ASCUS were HPV 58 positive  
447 in important proportions, and women with HSIL/ICC were predominantly infected by HPV 16  
448 & 18, which is in agreement with our findings.

449 Our data from rural area cannot be disaggregated by cytological results but results are roughly  
450 similar to results found in Rwanda and Kenya [27;35]. Stratified by HIV status, the most  
451 frequent HPV types in our results are overall the same as results from a study done in Tanzania  
452 [32]. Our results highlight a high prevalence of the much known carcinogenic HPV types (HPV  
453 16, 18, 52, 51, 56, 33 and 58) and very likely to persist in this unscreened population, which  
454 translates into high rates of cervical precursor lesions, making this population a high priority  
455 for public health interventions, regardless of their HIV-status. This is reinforced by the fact  
456 that this population has never been screened for cervical cancer lesions and clearly shows the  
457 need for implementation of cervical cancer screening intervention in Burundian women  
458 population.

459 In rural, the results in figure 2 imply that the Cervarix® or Gardasil® vaccines would target  
460 32.1% and 30.8% of the HR/pHR-HPV infections among HIV-positive and negative women  
461 respectively. The nonavalent vaccine, Gardasil-9®, would target 60.7% and 53.9% of the  
462 circulating HR/pHR-HPV infections among HIV-positive and negative women respectively.

463 In urban women, Gardasil<sup>®</sup> or Cervarix<sup>®</sup> vaccine would target 8.7% and 15.8% of all HR/pHR-  
464 HPV infections in HIV-positive and negative women respectively and Gardasil-9<sup>®</sup> would  
465 prevent 31.9% and 42.1% of all HR/pHR-HPV infections in HIV-positive and negative women  
466 respectively. Overall, our results showed that Gardasil-9 vaccine covers most HR/pHR-HPV  
467 infections. If we look at the urban results, cervarix<sup>®</sup> would target 2/3<sup>rd</sup> and all HSIL/ASC-H/ICC  
468 among HIV-positives and negatives respectively; whereas Gardasil-9<sup>®</sup> would target 7 on 8 and  
469 100% of all HSIL/ASC-H/ICC among HIV-positives and negatives respectively (Table 2).  
470 We are aware, however, of the described cross-protection [45] of HPV vaccines and studies  
471 on HPV strain distributions in invasive cervical cancer cases are also needed. The results of  
472 these studies plus the related cost-effectiveness studies are necessary elements to provide  
473 Burundian health authorities a sound evidence to choose the appropriate HPV screening  
474 and/or vaccination programmes.

#### 475 Study limitations

476 Our study population choose 4 similar strata according to HIV status and urban/rural and  
477 participants were not randomly selected. Thus, our overall study results do not represent the  
478 general female population of Burundi. This was a deliberate choice as we wanted to avoid  
479 that urban women and HIV infected women would be underrepresented. According to the  
480 2010 Burundi Demographic Health Survey, 91% of Burundian women live in rural areas where  
481 HIV prevalence is estimated at 1.2% in women aged 15-49 years old, we assume that the  
482 results of the HIV negative women in Kirundo may be most representative result for the  
483 Burundian situation. This limitation turns indeed out to be a strength as we provide  
484 comprehensive baseline estimates of HPV prevalence and type-specific distribution in HIV-  
485 positive and negative women in rural and urban Burundi. And we observed the limited  
486 additional risk of rural HIV-infected women (who are relatively few) and the increased risk of

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

## 492 **Conclusions**

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

502

503

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653

654 **List of abbreviations**

- 655 ABUBEF: Association Burundaise pour le Bien-Etre Familial
- 656 AGC: Atypical Glandular Cells
- 657 ANSS: Association Nationale de Soutien aux Séropositifs et malades du SIDA
- 658 ASC-H: Atypical Squamous Cells cannot exclude High-grade lesion
- 659 ASCUS: Atypical Squamous Cells of Undetermined Significance
- 660 CI: Confidence Interval
- 661 CIN: Cervical Intraepithelial Neoplasia
- 662 DNA: DesoxyriboNucleic Acid
- 663 HIV: Human Immunodeficiency Virus
- 664 HPV: Human Papillomavirus
- 665 HR/pHR-HPV: High-risk/possible high-risk human papillomavirus
- 666 HSIL: High-grade Squamous Intraepithelial Lesions
- 667 ICC: Invasive Cervical cancer
- 668 LMICs: Low and Middle Income Countries
- 669 LR-HPV: Low-risk human papillomavirus
- 670 LSIL: Low-grade Squamous Intraepithelial Lesions
- 671 OR: Odds ratio
- 672 PCR: Polymerase Chain Reaction
- 673 S.D: Standard Deviation
- 674 SSA: Sub-Saharan Africa
- 675 STI: Sexually Transmitted Infection
- 676 VIA: Visual Inspection with 5% Acetic acid
- 677 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, JPGV, DVB

685 Data curation: ZN, JPGV

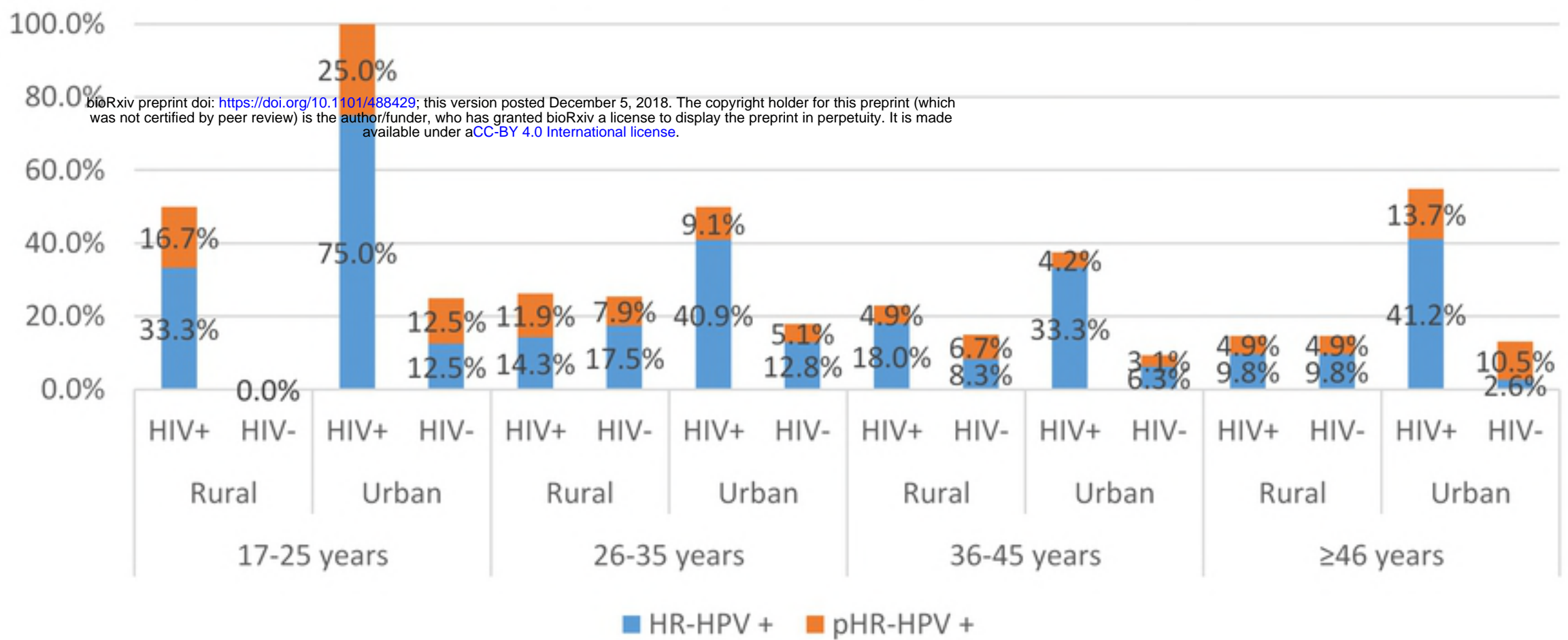
686 Analysis and interpretation of data: ZN, JPGV, DVB

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

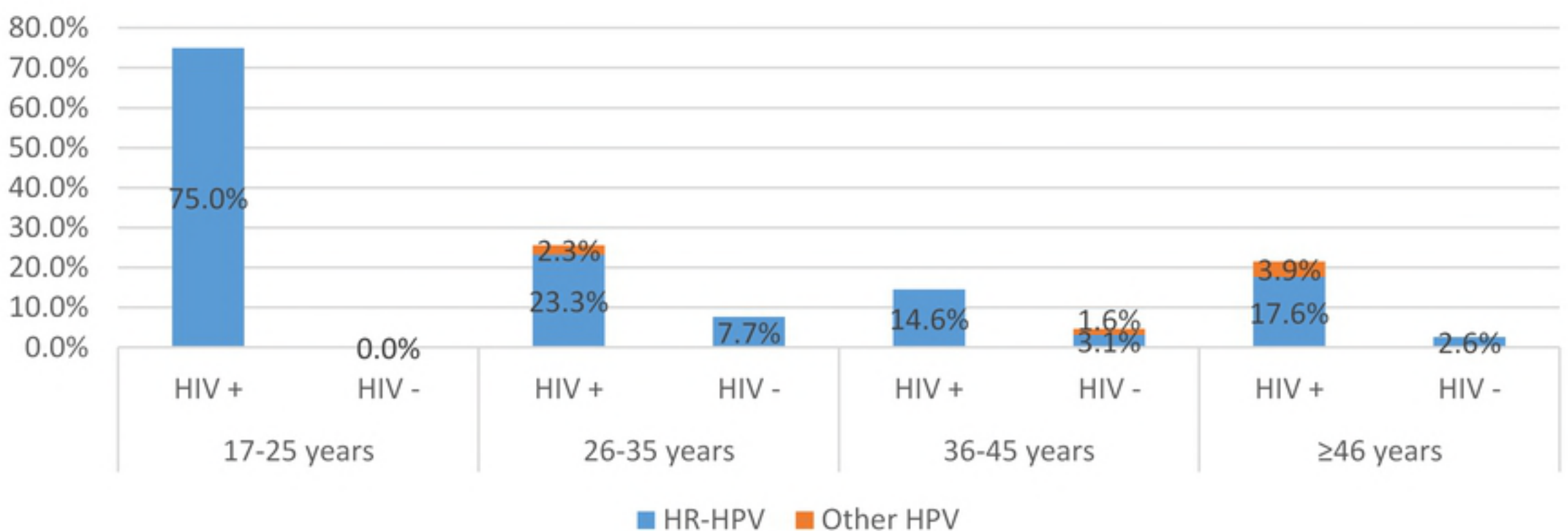
688 Methodology: ZN, JPGV, DVB

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

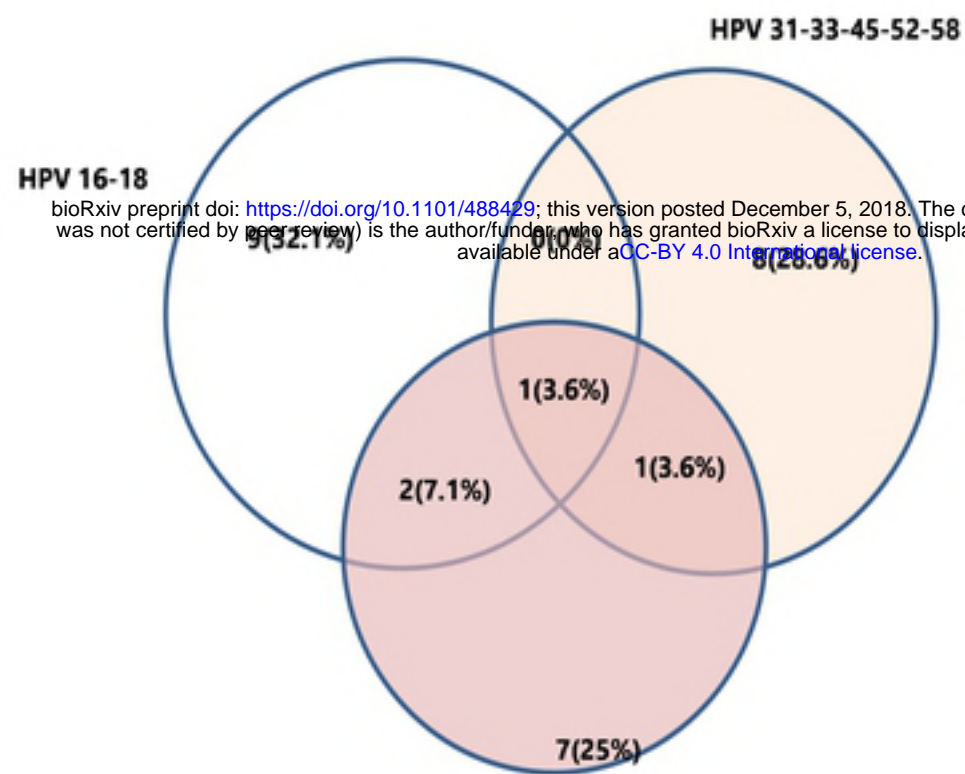
### A. Age-specific prevalence of HR and pHR-HPV by HIV status and by study area



### B. Age-specific prevalence of abnormal cytology by HIV status in Urban women and stratification by HPV oncogenic type infection.

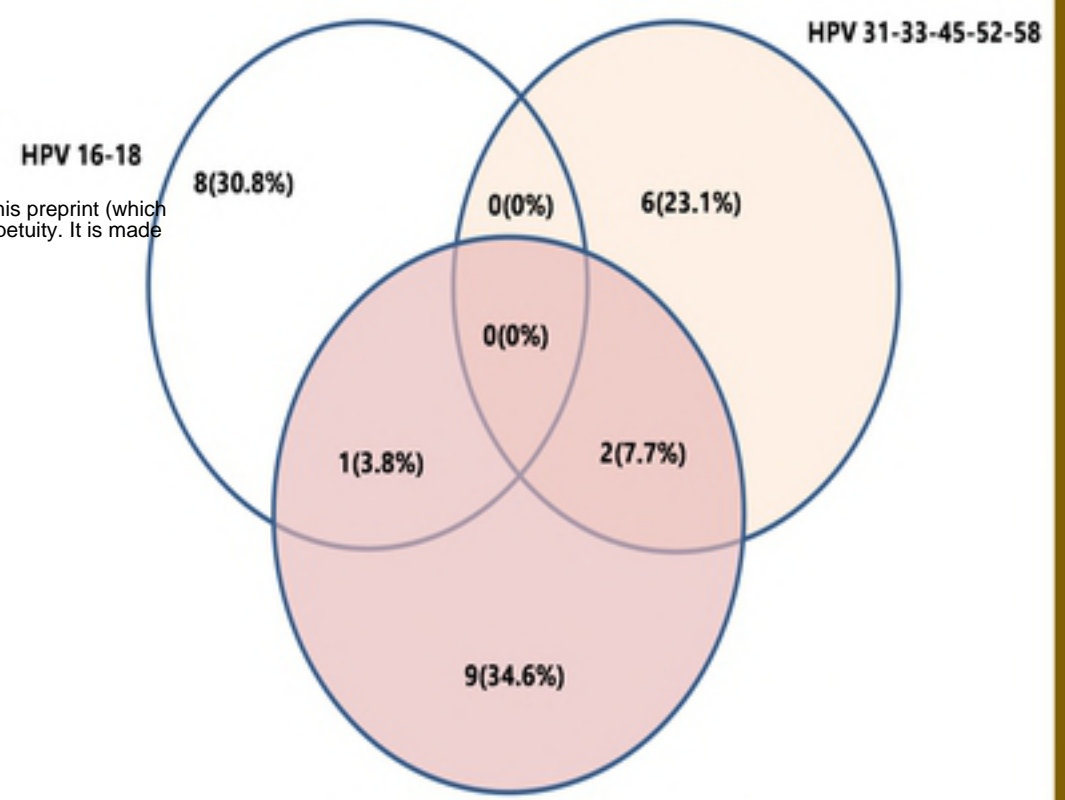


### HIV-positive rural women



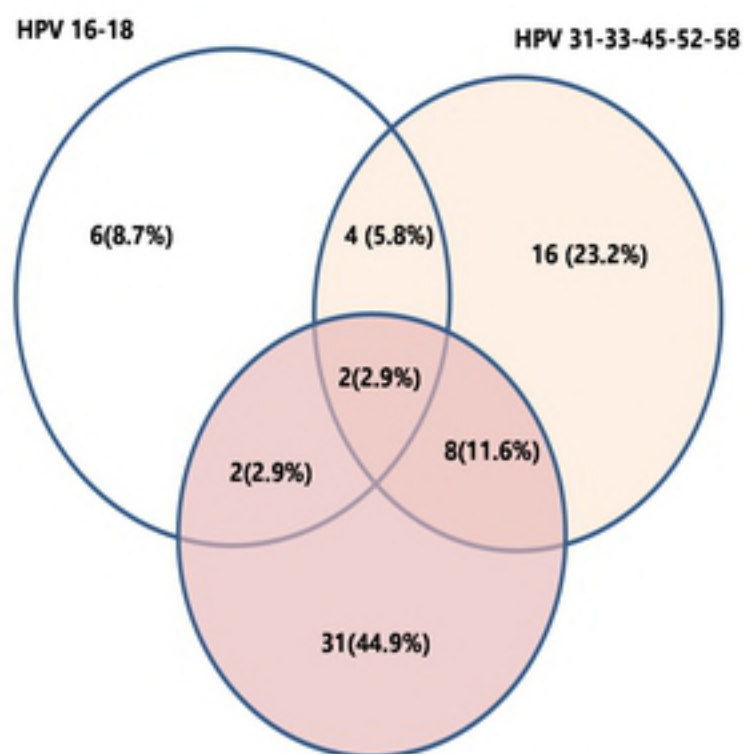
Other HR/pHR-HPV not included in currently available vaccines

### HIV-negative rural women



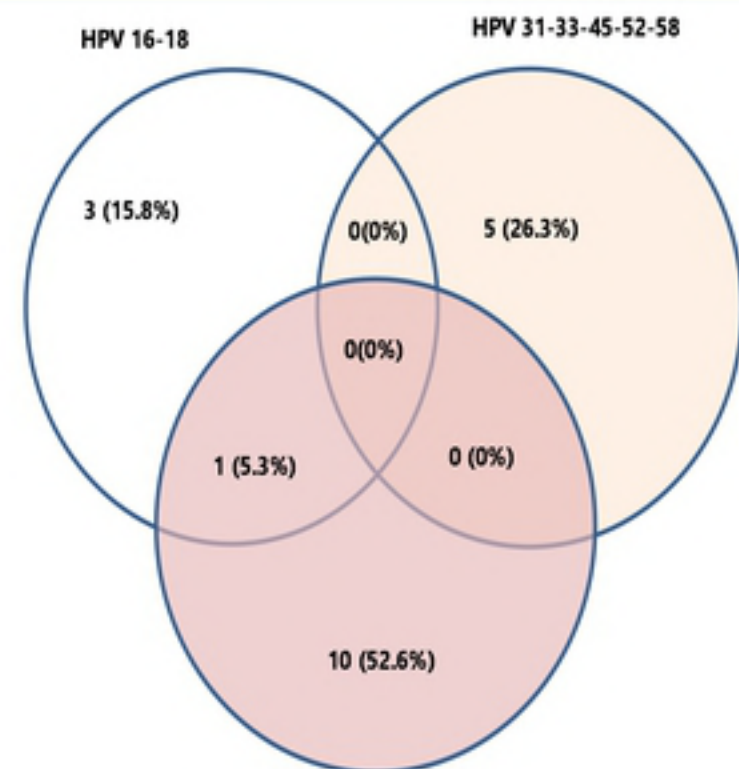
Other HR/pHR-HPV not included in currently available vaccines

### HIV-positive urban women



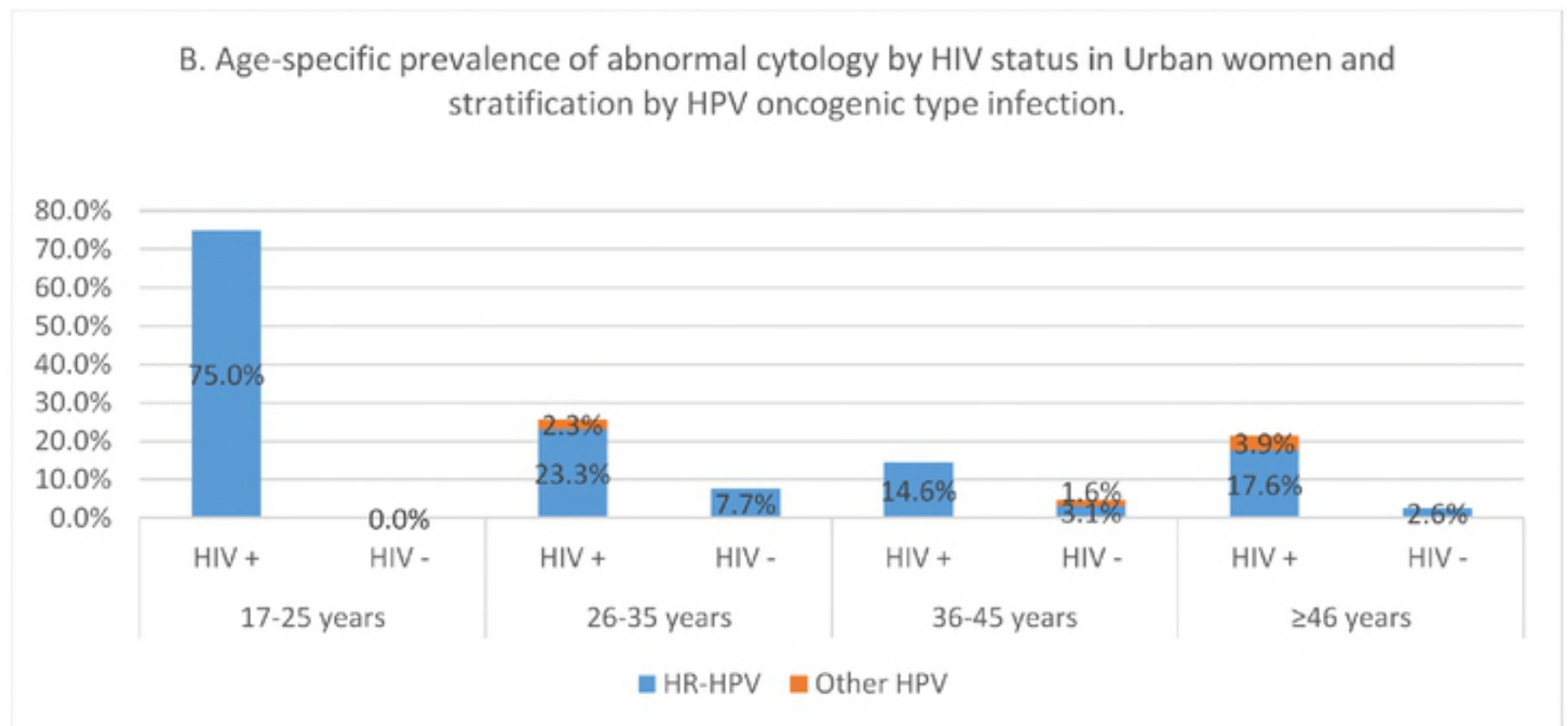
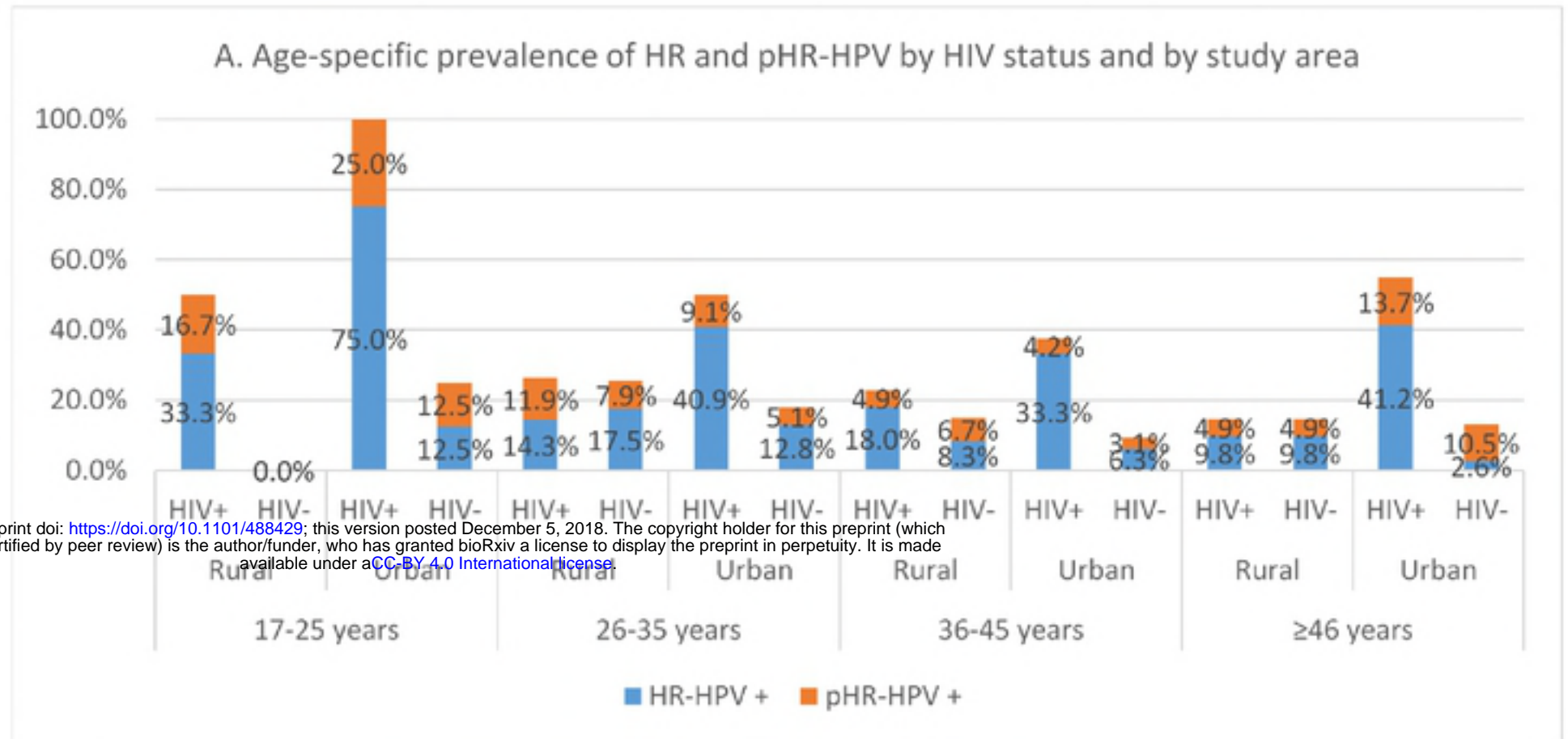
Other HR/pHR-HPV not included in currently available vaccines

### HIV-negative urban women



Other HR/pHR-HPV not included in currently available vaccines

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