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Risk of seropositivity to multiple oncogenic human papillomavirus types among human immunodeficiency virus-positive and -negative Ugandan women

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To understand the prospects for human papillomavirus (HPV) mass vaccination in the setting of a developing country, we studied the co-occurrence of seropositivity to multiple high-risk (hr) HPV types among HIV-positive and HIV-negative Ugandan women. Our seroepidemiological study was conducted among 2053 women attending antenatal clinics. Sera were analysed for antibodies to eight hrHPV types of the α-7 (18/45) and α-9 (16/31/33/35/52/58) species of HPV by using a multiplex serology assay. Our results show that seropositivity for greater than one hrHPV type was as common (18 %) as for a single type (18 %). HIV-positive women had higher HPV16, HPV18 and HPV45 seroprevalences than HIV-negative women. In multivariate logistic regression analysis, age (>30 years) and level of education (secondary school and above) reduced the risk, whereas parity (>5) and HIV-positivity increased the risk for multiple hrHPV seropositivity. However, in stepwise logistic regression analyses, HIV-status remained the only independent, stand-alone risk factor [odds ratio (OR) 1.7, 95 % confidence interval (CI) 1.0–2.8). On the other hand, the risk of HPV16 or HPV18 seropositive women, as compared to HPV16 or HPV18 seronegative women, for being seropositive to other hrHPV types was not significantly different when they were grouped by HIV-status ($OR_{HPV16/HIV+}$ 12, 95 % CI 4.5–32 versus $OR_{HPV16/HIV-}$ 22, 95 % CI 15–31 and $OR_{HPV18/HIV+}$ 58, 95 % CI 14–242 versus $OR_{HPV18/HIV-}$ 45, 95 % CI 31–65). In conclusion, seropositivity to HPV16, HPV18 and to non-vaccine hrHPV types is common in Ugandan women, suggesting that there is little natural cross-protective immunity between the types. HIV-positivity was an independent, stand-alone, albeit moderate risk factor for multiple hrHPV seropositivity. HPV mass vaccination may be the most appropriate method in the fight against cervical cancer in the Ugandan population.

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INTRODUCTION

Owing to promising results of the HPV6/11/16/18 and HPV16/18 vaccine trials in developed countries (Ault & Future II Study Group, 2007; Paavonen *et al.*, 2009), several developing countries will consider implementing human papillomavirus (HPV) mass vaccination in the near future, for the control of cervical cancer (CxCa) and other HPV related cancers. However, in sub-Saharan African countries, such as Uganda, where human immunodeficiency virus (HIV) is highly prevalent, data on the occurrence and co-occurrence of oncogenic, high-risk (hr) HPV type DNA or hrHPV antibodies in HIV-positive and HIV-negative women, as well as HPV vaccine efficacy (VE) data are lacking.

So far, HPV prevalence data for Uganda has mainly been based on HPV DNA studies (Banura *et al.*, 2008a, b, 2010; Blossom *et al.*, 2007; Safaeian *et al.*, 2008; Serwadda *et al.*,

1999). Over time this underestimates the occurrence of multiple HPV types in the same individual, because the virus is effectively cleared by the immune system in 90 % of women within 18 months of infection (Banura *et al.*, 2010; Molano *et al.*, 2003; Trottier *et al.*, 2006). The sensitivity of conventional HPV serology in recent genital infections is between 50 and 75 % depending on the HPV type (Carter *et al.*, 2000; Kjellberg *et al.*, 1999). Although not all infected women seroconvert, HPV antibodies, once developed, remain detectable for at least 5–10 years (af Geijersstam *et al.*, 1998). Thus, antibody positivity to hrHPV types, i.e. hrHPV seroprevalence, is a plausible marker for epidemiological studies on the cumulative incidence and co-occurrence of hrHPV infections (Kibur *et al.*, 2000).

Despite being one of the countries with the highest age-standardized incidence rates of CxCa (41.7/100 000 person-years), Uganda has no organized screening/treatment programmes (Parkin *et al.*, 2002). Also, awareness of CxCa and other hrHPV associated diseases is low (Katahoire *et al.*, 2008). Therefore, most CxCa cases are diagnosed at a late stage, with very low survival rates (Wabinga *et al.*, 2003). Furthermore, risk-taking sexual behaviour is increasing among young people (Katahoire *et al.*, 2008; Molano *et al.*, 2003; Slaymaker *et al.*, 2009). The prevalence of HIV, which has been associated with multiple hrHPV infections (Banura *et al.*, 2008b; Clifford *et al.*, 2006; Clifton, 2009; Molano *et al.*, 2003; Moscicki *et al.*, 2004; Slaymaker *et al.*, 2009), is 6 % in the Ugandan population. Our previous results indicated that infections with hrHPV types 16, 18, 31, 33, or 45 are very common among young Ugandan women <20 years of age (Namujju *et al.*, 2010). In the current HPV-seroprevalence study we measured antibodies to eight hrHPV genotypes, and evaluated the occurrence of antibodies against several hrHPV genotypes among HIV-positive and HIV-negative Ugandan women.

RESULTS

Seroprevalence for genital HPV types

Two thousand and fifty-three women with a mean and median age 23 years (SD of 5 years) were enrolled, but 110 women had to be excluded because of insufficient serum sample volumes. Overall, 702 (36 %) and 366 (19 %) women were seropositive for at least one of the eight genital HPV types (16, 18, 31, 33, 35, 45, 52 or 58) and at least two of these types, respectively (Fig. 1). Antibodies to four single

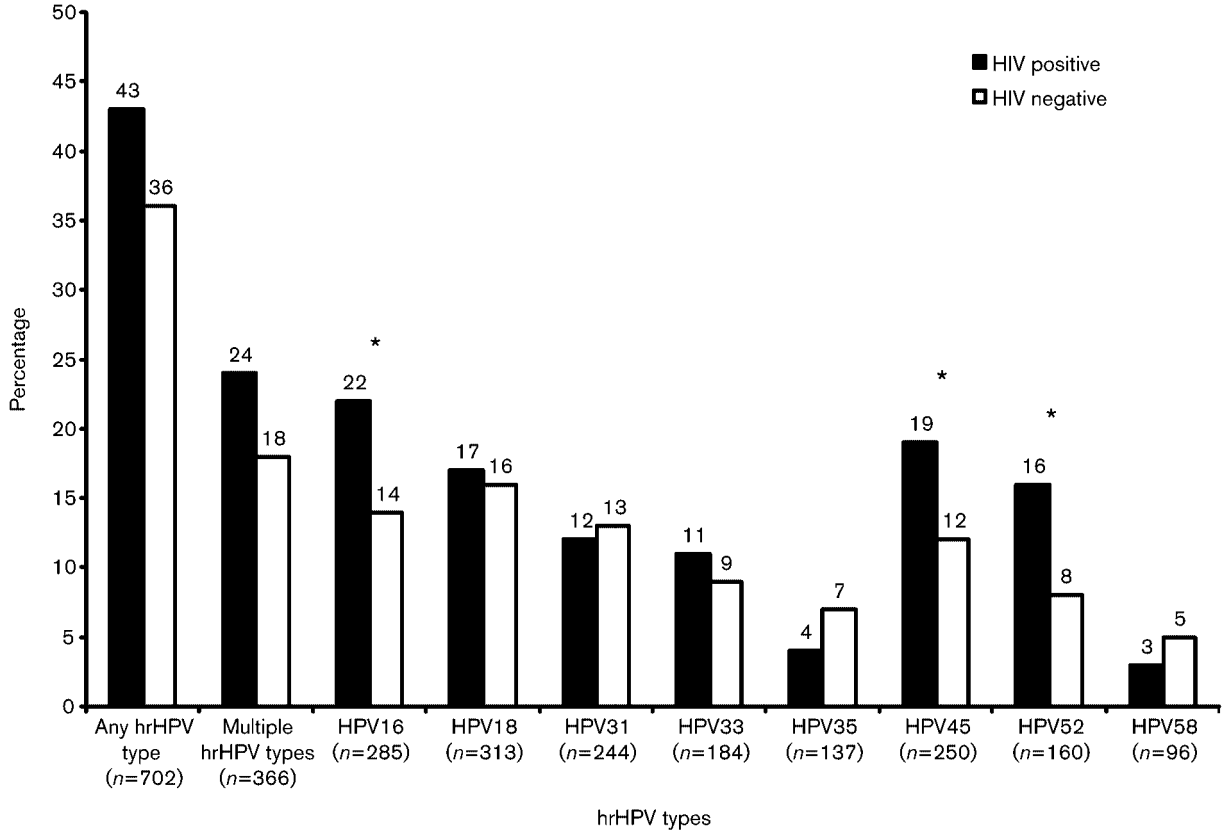

Fig. 1. HPV seropositivity among 1943 pregnant Ugandan women by HIV status. *, Statistically significant difference ($P<0.01$); *n*, number of seropositive women.

hrHPV types were common: HPV18 (16 %), HPV16 (15 %), HPV31 (13 %) and HPV45 (13 %). Antibodies to HPV16 or HPV18 were detected in 23 % of the women. Among the women seropositive for multiple hrHPV types, 39.6, 24.3, 10.4, 6.0, 5.7, 5.5 and 8.5 % were seropositive for 2, 3, 4, 5, 6, 7 and 8 hrHPV types, respectively.

The prevalences of HIV and *Chlamydia trachomatis* antibodies were 7 and 25 %, respectively. HIV-positive women, as compared with HIV-negative women, had, statistically, significantly higher HPV type 16, 45 and 52 seroprevalences of 22 versus 14 %, 19 versus 12 % and 16 versus 8 %, respectively (Fig. 1).

Risk factors for multiple hrHPV seropositivity

We assessed the risk factors for multiple hrHPV serotypes by using the questionnaire data collected from 1209 women in phase two of our study, and serology data for HIV and *C. trachomatis* (Table 1). In the univariate logistic regression analysis we observed that visiting the antenatal clinic at an older age (>30 years), and a higher level of education (secondary school and above) had a protective effect against multiple hrHPV seropositivity (Table 1). In contrast, seropositivity to HIV and seropositivity to *C. trachomatis* were associated with multiple hrHPV seropositivity (Table 1). In the multivariate model, older age, and

Table 1. Univariate and multivariate estimation of the relative risk (RR) of seropositivity for multiple high-risk HPV types among pregnant Ugandan women (n=1209)

Data for risk factors of multiple hrHPV seropositivity were analysed for only 1209 women enrolled in the second phase of our study. CI, Confidence interval.

Characteristic	*n*	Percentage	hrHPV antibody		Univariate multiple hrHPV types RR (95 % CI)	Multivariate multiple hrHPV types RR (95 % CI)
			Positive	%		
Age						
<20	150	12	38	25	1	
20–24	425	35	83	20	0.7 (0.5–1.1)	0.7 (0.4–1.1)
25–29	385	32	86	22	0.8 (0.5–1.3)	0.8 (0.5–1.3)
⩾30	249	21	42	17	0.6 (0.4–1.0)*	0.5 (0.3–0.8)*
Age at sexual debut						
<15	120	10	29	24	1	
15–20	903	75	185	20	0.8 (0.5–1.3)	1.0 (0.6–1.7)
>20	186	15	35	19	0.7 (0.4–1.3)	1.1 (0.5–2.2)
Lifetime partners						
1	314	26	64	20	1	
2	377	31	76	20	1.0 (0.7–1.4)	1.1 (0.7–1.6)
3	238	20	52	22	1.1 (0.7–1.6)	1.0 (0.6–1.6)
>3	280	23	57	20	1.0 (0.7–1.5)	1.0 (0.6–1.6)
Pregnancies						
<5	970	80	192	20	1	
⩾5	239	20	57	24	1.3 (0.9–1.8)	1.6 (1.0–2.5)*
Education†						
⩽Primary	352	33	92	26	1	
Secondary	445	42	91	20	0.7 (0.5–1.0)	0.8 (0.6–1.2)
Tertiary	266	25	43	16	0.5 (0.4–0.8)*	0.6 (0.4–1.0)
Smoking‡						
No	1103	96	228	21	1	
Yes	47	4	10	21	1.0(0.5–2.0)	0.9 (0.4–1.9)
HIV						
Negative	1114	92	297	27	1	
Positive	54	8	35	37	1.8 (1.1–2.8)*	1.7 (1.0–2.8)*§
***Chlamydia*‖**						
Negative	875	73	164	19	1	
Positive	322	27	81	25	1.5 (1.1–2.0)*	1.1 (0.8–1.6)

*Statistically significant risk factor for multiple hrHPV seropositivity.
†n=1063.
‡n=1150.
§Statistically significant, independent risk factor for multiple hrHPV seropositivity.
‖n=1197.

higher education (tertiary) continued to show the protective effect (RR 0.5, 95 % CI 0.3–0.8 and RR 0.6, 95 % CI 0.4–1.0), whereas HIV positivity was associated with an increased risk for multiple hrHPV seropositivity (RR 1.7, 95 % CI 1.0–2.8) (Table 1). Furthermore, an increased number of pregnancies ($\geqslant 5$) [odds ratio (OR) 1.6, 95 % CI 1.0–2.5] also appeared, and *C. trachomatis* disappeared, amongst the risk factors for multiple hrHPV seropositivity (Table 1). Finally, by eliminating variables from the model one by one (step wise multivariate model), HIV positivity remained as the only independent, stand-alone risk factor for multiple hrHPV seropositivity.

Risk of seropositivity for hrHPV types by specific HPV type

Next we assessed, stratifying by HIV status, the differences in the occurrence of seropositivity to multiple hrHPV types among women who were antibody positive to either one of the hrHPV types included in the licensed HPV vaccines: HPV16 or HPV18. Irrespective of HIV-status, HPV16 seropositive women had at least a tenfold increased risk of being seropositive to multiple hrHPV types [OR 11.8, 95 % CI 4.5–31.5 (HIV positive); OR 21.8, 95 % CI 15.4–30.8 (HIV-negative)], compared with HPV16 seronegative women (Table 2). Women who were HPV18 seropositive had an even higher occurrence of multiple hrHPV seropositivity, as compared with HPV18 seronegative women, albeit again irrespective of HIV status [OR 58.1, 95 % CI 13.9–242 (HIV-positive); OR 44.8, 95 % CI 31.1–64.7 (HIV-negative); Table 2].

We also investigated the occurrence of joint seropositivity to a given hrHPV type (16, 18, 31, 33, 35, 45, 52 or 58) among women who were antibody positive to at least one of the HPV types, HPV16 or HPV18, again by HIV status (Tables 3 and 4). There was no material (statistically significant) difference in the risk of double seropositivity of vaccine types (HPV16 or HPV18) and non-vaccine types among HIV-negative and HIV-positive women (Tables 3 and 4).

DISCUSSION

To the best of our knowledge, this is the first large study to evaluate the occurrence of multiple hrHPV seroprevalence among HIV-positive and HIV-negative women in sub-Saharan Africa who would mostly benefit from prophylactic HPV vaccination. Among the pregnant Ugandan women tested, young age, low education, number of pregnancies (>5) and HIV positivity were the independent risk factors for being seropositive for multiple hrHPV types. Even if HIV positivity was the only independent, stand-alone risk factor for multiple hrHPV seropositivity, cumulative incidence of hrHPV infections, as determined by multiple HPV seropositivity, appeared to cluster similarly among HIV-negative and HIV-positive women.

Pregnant women represent the sexually active female population. In Uganda, this group has been used previously to estimate HPV DNA and HIV prevalences (Banura *et al.*, 2008a, b, 2010). In our study, a comprehensive multiplexed serological approach was used to examine risk factors for multiple hrHPV types and differences in the risk of co-occurrence of eight genital hrHPV types (16, 18, 31, 33, 35, 45, 52 and 58) among HIV-positive and HIV-negative women. Very good type specificity and stability for a longer time (af Geijersstam *et al.*, 1998) make hrHPV antibodies, detected with improved sensitivity, a suitable marker to study the cumulative incidence of multiple hrHPV types (Michael *et al.*, 2010; Syrjänen *et al.*, 2009; Waterboer *et al.*, 2005, 2006). Our large study also yielded ample power (see Methods) to distinguish independent risk factors for the multiple hrHPV seropositivity detected, and to compare differences in the relative risk estimates between HIV-positive and HIV-negative women.

There were, however, also limitations. We did not have HPV DNA data. The enrolled women may have been infected with different HPV types at various times before they became pregnant or infected with HIV (Woodman *et al.*, 2001). We also lacked CD4 counts for those who

Table 2. Occurrence of seropositivity for multiple high-risk human papillomavirus (hrHPV) types by absence (reference) or presence of antibodies to HPV types 16 or 18 among HIV-positive and HIV-negative, pregnant Ugandan women ($n=1943$)

OR, Odds ratio; CI, confidence interval.

HPV type	HIV positive women ($n=140$)		HIV negative women ($n=1803$)	
	Multiple hrHPV types positive/negative	OR* (95 % CI)	Multiple hrHPV types positive/negative	OR* (95 % CI)
HPV16				
Negative	11/95	1	68/1403	1
Positive	20/14	11.8 (4.5–31.5)	286/246	21.8 (15.4–30.8)
HPV18				
Negative	3/103	1	58/1413	1
Positive	21/13	58.1 (13.9–242)	231/101	44.8 (31.1–64.7)

*Adjusted for age and antibodies to *C. trachomatis*.

Table 3. Occurrence of seroreactivity (positive/negative) for a given HPV type by absence or presence of antibodies to another HPV type among HIV positive (n=140; upper right corner) and HIV negative (n=1803; lower left corner) Ugandan women

NA, Not applicable.

HPV type	HPV16 (pos/neg)	HPV18 (pos/neg)	HPV31 (pos/neg)	HPV33 (pos/neg)	HPV35 (pos/neg)	HPV45 (pos/neg)	HPV52 (pos/neg)	HPV58 (pos/neg)
HPV16 (n=285)								
Negative	NA	9/100	4/105	10/99	2/107	14/95	9/100	2/107
Positive		15/16	13/18	6/25	4/27	13/18	13/18	2/29
HPV18 (n=313)								
Negative	161/1388	NA	7/109	10/106	2/114	12/104	10/106	2/114
Positive	128/126		10/14	6/18	4/20	15/9	12/12	2/22
HPV31 (n=244)								
Negative	112/1437	104/1410	NA	8/115	2/121	18/105	10/113	14/122
Positive	115/139	123/166		8/9	4/13	9/8	12/5	3/1
HPV33 (n=184)								
Negative	83/1466	70/1444	84/1492	NA	4/120	21/103	13/111	1/123
Positive	85/169	98/191	84/143		2/14	6/10	9/7	3/13
HPV35 (n=137)								
Negative	53/1496	54/1460	47/1529	68/1567	NA	24/110	18/116	3/131
Positive	78/176	77/212	84/143	63/105		3/3	4/2	1/5
HPV45 (n=250)								
Negative	107/1442	75/1439	122/1454	149/1486	157/1515	NA	10/103	3/110
Positive	116/138	148/141	101/126	74/94	66/65		12/15	1/26
HPV52 (n=160)								
Negative	61/1488	59/1455	54/1522	62/1573	74/1598	63/1517	NA	0/118
Positive	77/177	79/210	84/143	76/92	64/67	75/148		4/18
HPV58 (n=96)								
Negative	36/1513	34/1480	36/1540	31/1604	51/1621	42/1538	42/1623	NA
Positive	56/198	58/231	56/171	61/107	41/90	50/173	50/88	

Table 4. Occurrence of seropositivity for a given HPV type by absence (reference, OR=1) or presence of antibodies to another HPV type among HIV-positive (n=140; upper right corner) and HIV-negative (n=1803; lower left corner) Ugandan women. The data are presented as the odds ratios with the 95% CI in parentheses.

OR are adjusted for age and antibodies to *C. trachomatis*. NA, not applicable.

HPV type	HPV16	HPV18	HPV31	HPV33	HPV35	HPV45	HPV52	HPV58
HPV16 positive	NA	9.1 (3.2–25.9)	20.1 (5.4–78.9)	2.1 (0.7–6.8)	6.1 (1.0–37.0)	3.8 (1.4–10.1)	6.7 (2.4–18.7)	5.6 (0.6–50.0)
HPV18 positive	6.6 (4.8–9.0)	NA	10.3 (3.1–34.2)	3.3 (1.0–11.4)	11.9 (1.6–88.7)	11.3 (3.8–33.2)	9.0 (3.0–27.0)	9.4 (0.8–109.3)
HPV31 positive	8.5 (6.1–11.7)	7.8 (5.6–10.7)	NA	14.7 (4.0–53.4)	16.4 (2.5–108.4)	5.5 (1.7–17.8)	26.6 (7.3–97.6)	36.4 (3.3–397.9)
HPV33 positive	6.7 (4.7–9.5)	7.8 (5.5–11.1)	7.9 (5.5–11.4)	NA	3.2 (0.5–22.0)	3.2 (0.9–10.8)	11.2 (3.3–38.3)	32.4 (2.8–371–7)
HPV35 positive	10.7 (7.2–16.1)	7.8 (5.2–11.6)	17.1 (11.2–26.0)	10.6 (7.0–16.0)	NA	3.2 (0.5–20.1)	9.2 (1.5–58.1)	26.0 (1.0–657.0)
HPV45 positive	8.4 (6.0–11.8)	14.5 (10.3–20.4)	7.2 (5.2–10.2)	5.2 (3.6–7.5)	7.2 (4.8–10.9)	NA	6.8 (2.3–19.8)	2.1 (0.2–24.9)
HPV52 positive	8.0 (5.4–11.8)	6.6 (4.5–9.6)	12.8 (8.6–19.1)	15.0 (10.0–22.6)	15.8 (10.2–24.3)	8.2 (5.5–12.2)	NA	1.0 (0.9–1.1)
HPV58 positive	9.5 (5.9–15.1)	8.0 (5.0–12.8)	11.1 (6.9–17.7)	22.3 (13.6–36.5)	10.4 (6.5–16.9)	7.2 (4.5–11.5)	15.6 (9.6–25.2)	NA

were HIV positive to establish their immune competence at enrolment. Two-thirds of the enrolled women had provided questionnaire data on the risk factors of multiple hrHPV seropositivity. While follow-up studies have shown a clearly increased risk of seroconversion to another hrHPV type among baseline seropositive women (Kaasila *et al.*, 2009; Merikukka *et al.*, 2011; Palmroth *et al.*, 2010), our cross-sectional design did not allow inferences about the order in which the infections were acquired. Furthermore, not all women exposed to HPV infections seroconvert (Carter *et al.*, 2000; Ho *et al.*, 2004). Even if this were true, multiplex serology is not 100 % sensitive (Syrjänen *et al.*, 2009; Waterboer *et al.*, 2005), and, despite its robustness, the method is not 100 % type specific (Michael *et al.*, 2010).

Population-based PCR studies report HPV types 16, 52 and 58 as being the most common HPV types in African populations (Banura *et al.*, 2008b; Blossom *et al.*, 2007; De Vuyst *et al.*, 2008). Our results on the seroprevalences of HPV16 and HPV52 were consistent with these studies, and also with others reporting HPV types 16 and 52 to be more prevalent among HIV-positive women than among HIV-negative women (Blossom *et al.*, 2007; Serwadda *et al.*, 1999). However, HPV58 antibodies were relatively rare in our study, which could be because of poor seroconversion associated with HPV58, or previously unknown geographical differences in the occurrence of hrHPV types.

Age, age at sexual debut, parity, education, number of lifetime partners, smoking, infection with *C. trachomatis* and HIV have been reported previously as being risk factors for HPV infections (Banura *et al.*, 2008b; Soto-De Leon *et al.*, 2011). In our multivariate analysis, age, education, parity and HIV positivity were, however, the only independent risk factors for multiple hrHPV seropositivity. Eventually, HIV positivity was identified as the only independent, stand-alone risk factor. Hence, further analyses were done by stratifying for HIV status.

Seropositivity to multiple hrHPV types was very common, probably indicating a truly increased cumulative incidence of hrHPV infections both in HIV-negative and HIV-positive women. This is in line with previous follow-up studies, which have looked at seroconversion for related HPV types among baseline HPV16- or HPV18-antibody positives (Kaasila *et al.*, 2009; Palmroth *et al.*, 2010; Woodman *et al.*, 2001), but in contrast to a study by Ho *et al.* (2002) that suggested a protective effect among HPV16 antibody positives against the other clade α9 HPV types. HPV vaccine trials in developed countries have reported 100-fold antibody levels, as compared with those occurring after natural HPV infection, and cross-protection against a number of phylogenetically related HPV types (HPV31, -33 and -45) (Brown *et al.*, 2009; Paavonen *et al.*, 2009). Why antibodies induced in natural HPV infections do not offer cross-protection but vaccine induced antibodies do is unclear, but this may have to do with antibody levels and/or the avidity of the antibodies (Namujju *et al.*, 2011). Studies into whether current HPV vaccines will offer a sufficiently broad protection against different HPV types in the Ugandan and other sub-Saharan African populations are warranted.

A study by Odida *et al.* (2008) recently showed that, apart from HPV16 and HPV18, HPV31 and HPV45 are also common among Ugandan women with invasive CxCa. We observed that multiple HPV antibody positivity to several hrHPV types (16, 18, 31, 33, 35, 45 and 52) was common in HIV-negative pregnant women, who had antibodies to at least one hrHPV type. Clustering of the multiple hrHPV antibody positivity was, however, not significantly different between HIV-positive and HIV-negative women, which suggests that the acquisition of multiple, oncogenic hrHPVs, as indicated by type-specific hrHPV antibodies, is largely determined by other factors that do not undermine HPV vaccination. Hence, HPV mass vaccination may play a pivotal role in both the HIV-positive and HIV-negative populations, even though data on HPV VE and the effectiveness of vaccination strategies among HIV-infected populations are still lacking.

In conclusion, antibodies to vaccine and non-vaccine hrHPV types are very common in sub-Saharan (Ugandan) women, and they are somewhat more common in HIV-positive than HIV-negative women. HIV was the only independent, stand-alone risk factor for multiple hrHPV seropositivity, but the acquisition of multiple oncogenic hrHPV infections was comparable in HIV-positive and HIV-negative women. HPV vaccine trials in populations where HIV is endemic are needed to determine the efficacy and effectiveness of vaccination with the currently licensed HPV vaccines.

METHODS

Study design and population. A seroepidemiological study was carried out among 2053 antenatal clinic attendees at Entebbe Hospital, Naguru Health Center and Nsambya Hospital in Uganda as previously described (Namujju *et al.*, 2010). In conjunction with the prevention of mother-to-child HIV transmission (PMTCT) programme, a comprehensive questionnaire on living conditions (age at the antenatal clinic visit, education and number of pregnancies), life-style habits (smoking) and sexual risk-taking behaviour characteristics (age at sexual debut and number of lifetime partners), a serological study on sexually transmitted infections was introduced amongst the health promotion services offered to pregnant women (Banura *et al.*, 2008a, Mpairwe *et al.*, 2005). All syphilis-positive women were treated, and HIV-positive women were referred to HIV programmes for management.

The recruitment was done in two phases (Namujju *et al.*, 2010). Since CxCa and HPV are not commonly known among the general population, comprehensive HPV information was given to the women before they were asked to consent to donation of serum samples for the PMTCT programme to be used for HPV and other STI serology. The questionnaire data on risk factors for multiple HPV infections was collected by trained counsellors/nurses in the second phase. All women were advised to seek screening for CxCa post-partum. The serum samples were stored at −20 °C, and were shipped frozen to the National Institute for Health and Welfare (THL), Oulu, Finland. An aliquot was later shipped to the German Cancer Research Center (DKFZ) for multiplex HPV serology. The study was approved

by the ethical review boards of Uganda Virus Research Institute, St Raphael of St Francis Hospital Nsambya, The Uganda National Council of Science and Technology, and the National Institute of Health and Welfare (THL), Finland.

Laboratory analysis. HPV serology was conducted at the DKFZ. Serum IgG antibodies were analysed by using multiplex serology, simultaneously analysing antibodies to eight hrHPV types (Waterboer *et al.*, 2005). Briefly, fluorescence-coded bead sets (3000 beads per set per well) carrying different antigens were mixed. Fifty microlitres of diluted serum and 50 µl of mixed beads were mixed in 96-well plates. The plate was incubated on a shaker in the dark at room temperature for 1 h. The beads were washed three times with 100 µl casein buffer on a vacuum manifold. Secondary antibody and conjugate were added and incubated for 1 h and 30 min, respectively, with washing steps in between. The reporter fluorescence of the beads was determined with a Luminex analyser and expressed as the median fluorescence intensity (MFI) of at least 100 beads per set per well. The MFI cut-offs for specific types were: 422, 394, 712, 515, 552, 368, 547 and 592 for hrHPV types 16, 18, 31, 33, 35, 45, 52 and 58, respectively (Clifford *et al.*, 2007, Dondog *et al.*, 2008).

HIV serology was done in Uganda by using a rapid HIV test (Banura *et al.*, 2008b, Kizito *et al.*, 2008) and in Oulu by using the Abbott Combo test (Namujju *et al.*, 2010). IgG antibodies to *C. trachomatis* were analysed by using a commercial kit (Anilabsystems) (Dillner *et al.*, 1996).

Statistical analysis. The main outcome measurements of the RR and OR estimates being seropositive to at least two hrHPV types. Univariate logistic regression was used to estimate RR with 95 % CI of being seropositive to multiple hrHPV types according to different questionnaire variables, a surrogate of sexual risk-taking behaviour (antibodies to *C. trachomatis*) and HIV status. Logistic regression was used in the multivariate and stepwise multivariate models, where eventually the independent and independent stand-alone risk indicators were identified, respectively. The latter were estimated by removing variables one at a time, and by identifying those variables that retained statistical significance in all of the different alternatives.

This large study also yielded ample power (at 80 % and a significance level of 5 % to detect odds ratios of <0.44 and $\geqslant 1.82$) to distinguish independent risk factors for the multiple hrHPV seropositivity detected, and to compare differences in the relative risk estimates between HIV-positive and HIV-negative women.

In addition, analysis for the relative risk of clustering, i.e. co-occurrence of multiple HPV seropositivity with HPV16 and HPV18 seropositivity was done by calculating OR with 95 % CI using HPV16 or HPV18 seronegatives as the reference (Kibur *et al.*, 2000). The risk of being seropositive for another HPV type among those seropositive as compared to those seronegative (reference) for a specific HPV type was estimated. These analyses were adjusted for age and antibodies to *C. trachomatis*, and stratified by HIV status. All the outcome measurements were treated as binary variables.

All statistical analyses were done using STATA 8 (StataCorp).

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