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Prevalence, incidence and clearance of human papillomavirus infection among young primiparous pregnant women in Kampala, Uganda

Cecily Banura[1,2], Silvia Franceschi[3], Leen-Jan van Doorn[4], Annie Arslan[3], Bernhard Kleter[4], Fred Wabwire-Mangen[5], Edward K. Mbidde[6], Wim Quint[4] and Elisabete Weiderpass[2,7,8]*

[1]*Faculty of Medicine, Makerere University, Kampala, Uganda*
[2]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden*
[3]*International Agency for Research on Cancer, Lyon, France*
[4]*DDL Diagnostic Laboratory, Voorburg, The Netherlands*
[5]*School of Public Health, Makerere University, Kampala, Uganda*
[6]*Uganda Virus Research Institute, Entebbe, Uganda*
[7]*Cancer Registry of Norway, Oslo, and Department of Community Medicine, Tromso, Norway*
[8]*Samfundet Folkhalsan, Helsinki, Finland*

The proportion of women who have already been exposed to human papillomavirus (HPV) infection by the time they first become pregnant, and the influence of pregnancy and delivery on the course of HPV infection are unclear. In Kampala, Uganda, 987 young primiparous pregnant women aged <25 years had gynaecological examination and liquid-based cytology. In the follow-up, women acted as their own controls, *i.e.*, 1st/2nd *versus* 3rd trimesters (105 women), and during pregnancy *versus* after delivery (289 women). HPV was assessed using highly sensitive PCR assays. Prevalence of HPV and HIV infections at baseline were 60.0% and 7.3%, respectively. HPV16 and 18 were detected in 8.4% and 5.8%, respectively, *i.e.*, less frequently than HPV51 (8.7%) and 52 (12.1%). At follow-up new HPV infections were detected in 42.9% of women between the 1st/2nd and 3rd trimesters, and 38.1% between pregnancy and delivery, but 50.4% and 71.8% of HPV infections, respectively, cleared, leaving HPV prevalence unchanged in the different periods. Prevalence of cytological abnormalities diminished after delivery (from 21.2% to 12.4%). Presence of genital warts and sexually transmitted infections other than HPV were the strongest risk factors for prevalent or incident HPV infection. Clearance was lower among HIV-positive women. In conclusion, HPV prevalence was high in primiparous women in Uganda, but pregnancy did not seem to be a period of special vulnerability to the infection.
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Key words: human papillomavirus; HIV; Uganda; pregnant adolescents; epidemiology

Prophylactic vaccines against human papillomavirus (HPV) 16 and 18 are recommended for girls who are not yet sexually active.[1] In low-resource countries where the burden of cervical cancer is high, however, a catching up of less young women when they have contacts with medical facilities (*e.g.*, at first delivery) may be worth considering if the number of young women not yet infected by HPV vaccine types is low. Furthermore, although high parity and young age at first birth are risk factors for cervical cancer, even after adjustment for sexual behavior,[2] relatively few studies have provided information on the influence of pregnancy status on the incidence and clearance of HPV infection. Depending upon the study, HPV detection in pregnant women was shown to be higher than,[3,4] or similar to[5–8] HPV infection among nonpregnant women.

To further elucidate this issue, we evaluated prevalence, incidence and clearance of HPV infection between the 1st/2nd and 3rd trimesters of pregnancy, and between pregnancy and after delivery, among young primiparous pregnant women in Kampala, Uganda.

Material and methods

Study population and follow-up

Primiparous pregnant women aged below 25 years residing within a 20 km radius for the previous 6 months and presenting themselves for antenatal services at the Naguru Health Centre, located in a suburb of Kampala, Uganda, between May and November 2004, were invited to join the study. Refusals to participate were few (approximately 2%).

Trained midwives explained the study aims and procedures, which included answering a questionnaire, having a pelvic examination and returning for a follow-up visit during the 3rd trimester of pregnancy and/or after delivery. After obtaining informed consent, women were interviewed in English or Luganda (the most common local dialect). The questionnaires included information on socio-demographic characteristics, cigarette smoking, reproductive and menstrual factors, number of sexual partners in the last year and use of contraceptive methods. All women were offered pretest counseling for HIV, and testing for HIV and syphilis.

Follow-up visits were scheduled according to the trimester of pregnancy at recruitment. Women recruited during the 1st/2nd trimester (<26 weeks) were scheduled for visits both in the 3rd trimester and at least 6 weeks after delivery, whereas those recruited during the 3rd trimester were asked to return only after delivery.

Gynecological examination, collection of exfoliated cervical cells and cytological assessment

During all visits, trained midwives performed a pelvic examination and recorded visible abnormalities. After visual inspection of the vulva, a nonlubricated sterile speculum was inserted and exfoliated cervical cells were collected in a vial containing PreservCyt solution (Cytyc, Boxborough, MA) for liquid-based cytology and testing for HPV, *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). Briefly, a cytobrush (Cervex brush, Rovers Medical Devices B.V., Oss, The Netherlands) was inserted deep into the endocervical canal and rotated gently in a clockwise direction 5 times to collect cells from the endo and ectocervix. The cytobrush containing cervical cellular material was then placed in a vial containing PreservCyt solution (Cytyc) and rinsed by pushing

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; CI, confidence interval; CT, *Chlamydia trachomatis*; DEIA, DNA enzyme immunoassay; HPV, human papillomavirus; LiPA, line probe assay; LSIL, low-grade squamous intraepithelial lesion; NG, *Neisseria gonorrhoeae;* OR, odds ratio; SPF, short PCR fragment.

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***Correspondence to:** Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, PO Box 281, 171 77 Stockholm, Sweden. Fax: +468314975; E-mail: eliwei@ki.se

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TABLE I – PREVALENCE OF HUMAN PAPILLOMAVIRUS (HPV) TYPES BY HIV STATUS, 987 PRIMIPAROUS PREGNANT WOMEN, KAMPALA, UGANDA, 2004–2006

HPV type	HIV status								
	Negative (N = 913)			Positive (N = 72)			Total (N = 987)[1]		
	Single	Multiple	Total (%)[2]	Single	Multiple	Total (%)[2]	Single	Multiple	Total (%)[2]
HPV−	–	–	375 (41.1)	–	–	20 (27.8)	–	–	395 (40.0)
HPV+	314	224	538 (58.9)	19	33	52 (72.2)	334	258	592 (60.0)
High-risk infections									
16	21	49	70 (7.7)	3	10	13 (18.1)	24	59	83 (8.4)
18	5	47	52 (5.7)	0	5	5 (6.9)	5	52	57 (5.8)
31	9	35	44 (4.8)	0	6	6 (8.3)	9	41	50 (5.1)
33	6	35	41 (4.5)	1	6	7 (9.7)	7	42	49 (5.0)
35	11	35	46 (5.0)	0	5	5 (6.9)	12	40	52 (5.3)
39	7	21	28 (3.1)	1	8	9 (12.5)	8	30	38 (3.9)
45	7	23	30 (3.3)	0	3	3 (4.2)	7	26	33 (3.3)
51	30	49	79 (8.7)	1	5	6 (8.3)	31	55	86 (8.7)
52	34	71	105 (11.5)	3	10	13 (18.1)	37	82	119 (12.1)
56	10	34	44 (4.8)	0	9	9 (12.5)	10	44	54 (5.5)
58	12	25	37 (4.1)	0	2	2 (2.8)	12	27	39 (4.0)
59	2	12	14 (1.5)	0	3	3 (4.2)	2	15	17 (1.7)
68/73	12	33	45 (4.9)	1	11	12 (16.7)	13	44	57 (5.8)
Subtotal	166	214	380 (41.6)	10	32	42 (58.3)	177	247	424 (43.0)
Low-risk infections									
6	7	41	48 (5.3)	1	5	6 (8.3)	8	46	54 (5.5)
11	7	24	31 (3.4)	0	1	1 (1.4)	7	25	32 (3.2)
34	0	5	5 (0.6)	0	0	0	0	5	5 (0.5)
40	1	13	14 (1.5)	0	2	2 (2.8)	1	15	16 (1.6)
42	0	2	2 (0.2)	0	0	0	0	2	2 (0.2)
43	3	8	11 (1.2)	0	3	3 (4.2)	3	11	14 (1.4)
44	1	14	15 (1.6)	0	1	1 (1.4)	1	15	16 (1.6)
53	3	20	23 (2.5)	0	4	4 (5.6)	3	24	27 (2.7)
54	11	14	25 (2.7)	0	0	0	11	14	25 (2.5)
66	16	42	58 (6.4)	0	5	5 (6.9)	16	48	64 (6.5)
70	5	18	23 (2.5)	0	2	2 (2.8)	5	20	25 (2.5)
74	0	9	9 (1.0)	1	2	3 (4.2)	1	11	12 (1.2)
Subtotal	54	153	207 (22.7)	2	17	19 (26.4)	56	171	227 (23.0)
X[3]	94		94 (10.3)	7		7 (9.7)	101		101 (10.2)

[1]Includes 2 women whose HIV status was unknown.–[2]Percent of women.–[3]Percent of women with HPV X fell to 4.9%, 1.4% and 4.7% among HIV-negative, HIV-positive and all women, respectively, after additional testing for HPV26, 30, 55, 61, 62, 64, 67, 69, 71, 82, 83, 84, 85, 87, 89, 90, 91.

it into the bottom of the vial 10 times. The cytobrush was then discarded. The vial was closed and kept at room temperature until shipment in dry ice to DDL Diagnostic Laboratory, Voorburg, The Netherlands, for HPV testing. Cervical abnormalities in liquid-based cytology samples were read at the Cytology Department of the Slotervaart Hospital, Amsterdam, The Netherlands, and classified according to the 2001 Bethesda Classification.[9]

Before removal of the speculum, visual inspection with acetic acid and with Lugol's iodine was performed.[10] Two women were suspected to have cervical cancer but resulted to be cancer-free at liquid-based cytology (1 normal and 1 low-grade squamous intraepithelial lesion [LSIL]). The treatment of external genital warts was deferred until after delivery. Women with vaginal discharge and cervicitis received a 1-week syndromic treatment, and were further requested to talk to their sexual partners and to come back after treatment.

Isolation of HPV DNA

Total DNA was isolated from 200 µl of the suspension containing the cervical cells by the MagNA Pure LC instrument (Roche Diagnostics, Almere, The Netherlands), using the Total DNA isolation kit (Roche Diagnostics). DNA was eluted in 100 µl of elution buffer, and 10 µl was used for each PCR reaction. Each run contained positive and negative controls to monitor the DNA isolation, PCR, HPV detection and genotyping procedures.

PCR testing

The short PCR fragment $(SPF)_{10}$ primer set was used to amplify a broad spectrum of HPV genotypes, as described earlier.[11,12] Briefly, this primer set amplifies a fragment of 65 bp from the L1 region of HPV. Reverse primers contain a biotin label at the 5′ end, enabling capture of the reverse strand onto streptavidin coated microtiter plates. Captured amplimers are denatured by alkaline treatment, and the captured strand is detected by a defined cocktail of digoxigenin-labeled probes, detecting a broad spectrum of HPV genotypes. This method is designated HPV DNA enzyme immunoassay (DEIA), and provides an optical density value. If the SPF_{10}-DEIA yielded a borderline value (75–100% of the cut-off value), the SFP_{10} PCR was repeated and retested by DEIA.

The same SPF_{10} amplimers can be used to identify the HPV genotype by reverse hybridization on a reverse hybridization line probe assay (LiPA), containing probes for 25 different HPV genotypes (SPF_{10} HPV $LiPA_{25}$ version 1, Labo Bio-medical Products, Rijswijk, The Netherlands) (Table I). HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 and 73 were considered high-risk types.[13] Baseline samples that were positive at SPF_{10} PCR primer, but did not reveal any of the 25 types listed in Table I, were provisionally classified as positive for HPV X and subjected to a second reverse hybridization assay for 17 additional types (Table I).

HIV testing

HIV-1 testing was performed in Kampala using first the Determine rapid test (Abbot Diagnostics, Abbot Park, IL). If it was not reactive, the sample was considered HIV-negative. Otherwise, the Statpak rapid test (ChemoBio Diagnostics Systems, Medford, NY) was used to confirm HIV positivity. In case of disagreement between the two tests, a tie-breaker test, the Unigold rapid test (Orgenics, Waltham, MA), was used. All HIV-positive results were finally confirmed by enzyme-linked immunoabsorbent assay (ELISA) or PCR assays.[14] Women found to be HIV-positive were

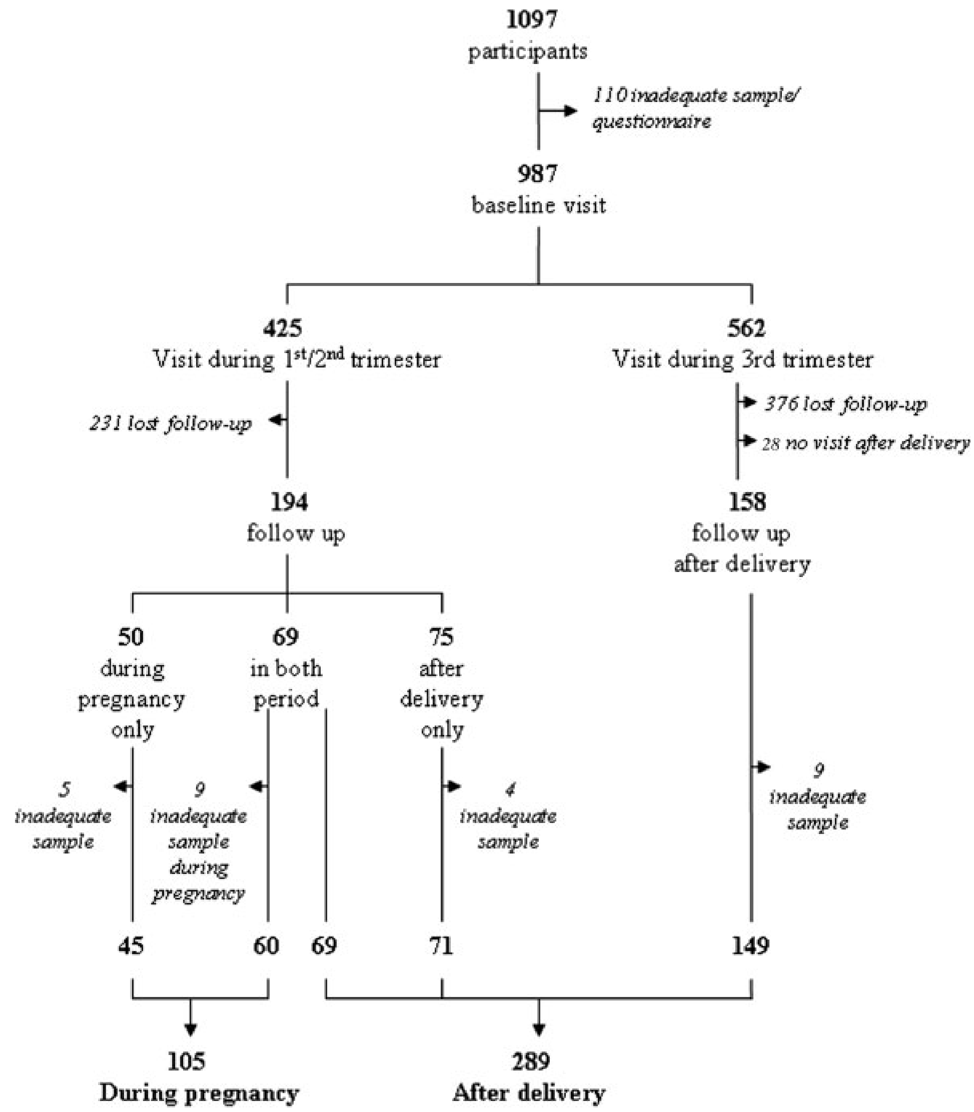

FIGURE 1 – Flow chart of the study of primiparous pregnant women in Kampala, Uganda, 2004–2006.

referred to health institutions for treatment, and for the prevention of mother-to-child transmission of HIV.

Syphilis, CT and NG testing

Syphilis testing was performed in Kampala using a commercially available standard Rapid Plasma Reagin 18 mm circle card test (Quorum Diagnostics, Sacramento, CA). Samples with reactive Rapid Plasma Reagin test results were referred to established laboratories for confirmation before treatment was offered to the women concerned and to their partners.

Isolated DNA for HPV analysis was also used for CT and NG testing as described earlier.[15,16] The CT detection and genotyping assay was performed in The Netherlands according to the manufacturer's instructions (Laboratory Biomedical Products BV, Rijswijk, The Netherlands). Briefly, the CT detection and genotyping assay is a multiplex broad-spectrum PCR for the cryptic plasmid and the VD2-region of the *omp1* gene. Like HPV testing, CT-amplimers generated by PCR from both the cryptic plasmid and the smp1 amplimers are simultaneously detected in a DEIA test. The target of amplification of NG is the cppB gene 16 and the NG amplimers were also detected in a DEIA test.

Ethical issues

All study participants signed an informed consent, or assent form, if minors, according to the recommendations of the ethical review committees of the Faculty of Medicine, Makerere University, the Uganda National Council of Science and Technology, and the International Agency for Research on Cancer, which approved the study.

Statistical analysis

We evaluated HPV prevalence at baseline and then computed incidence and clearance of HPV infection between the 1st/2nd and 3rd trimesters of pregnancy, and between the earliest visit during pregnancy and the visit after delivery (Fig. 1). In the few instances where more than 1 follow-up visit after delivery was available from the same woman, we chose the earliest one. Results were not materially different if the last visit after delivery was used (data not shown).

Incidence was calculated for any HPV type that had not been detected at baseline visit, using women as the unit of analysis. Conversely, we used infections as the denominator of clearance because of our interest in clearance of individual HPV types. Women positive for HPV X at recruitment who tested positive for a known HPV type at the follow-up visit were considered to have developed a new infection in the analyses of incidence, but were excluded from the analyses of clearance as the persistence of HPV X types could not be known. Second, the association between selected baseline characteristics and incidence and clearance of HPV was evaluated in all women who had at least 1 follow-up visit, choosing preferentially the visit after delivery (Fig. 1). Incidence and clearance of cytological abnormalities was evaluated only before and after delivery.

We computed odds ratios (ORs) for HPV positivity at baseline visit and for HPV incidence or clearance by different women's characteristics using unconditional, multiple logistic regression models and considering HPV infection as the dependent variable. ORs and corresponding 95% confidence intervals (CIs) presented are adjusted for age group (14–18, 19–20, 21–24 years). We assessed the statistical significance of OR trends considering the categorical variable as a continuous variable in the logistic model.

Results

Baseline

One thousand ninety-seven primiparous pregnant women accepted to participate, but essential information (*e.g.*, age) was missing for 15, and exfoliated cervical cell samples were missing or inadequate in 34 and 61 women, respectively. Hence 987 women were included (Fig. 1). Of these, 985 women accepted to be tested for HIV, and 72 (7.3%) were HIV-positive. The median age was 19 (range 14–24 years).

Overall, 60.0% of our study participants were positive for one HPV type or more (Table I). High-risk types were found more often (43.0%) than low-risk types (23.0%). Among high-risk types, HPV52 (12.1%), 51 (8.7%) and 16 (8.4%) were the most frequently detected, whereas, among low-risk types, HPV66 (6.5%) and 6 (5.5%) predominated. One hundred one women (10.2%) had samples that were HPV X-positive at the first reverse hybridization assay, which then went through a second round of testing for 17 additional types. After this second round, 55 women could be assigned to a specific type or more, most frequently HPV61 (12 women), and 55 (9 women), whereas 4.7% of all women (1.4% of HIV-positive women) could not be assigned any HPV type.

Study participants who were HIV-positive showed higher HPV prevalence (72.2%) than HIV-negative ones (58.9%) ($p = 0.027$) (Table I), and the largest difference was found in respect to multiple-type infections, which were detected in 24.5% of HIV-negative and 45.8% of HIV-positive women ($p < 0.001$). Mean number of HPV types detected among HPV-positive women was higher among HIV-positive women (2.4, range: 1–10) than among HIV-negative women (1.8, range 1–12) (Student's t-test = 2.79, $p = 0.005$).

Table II shows the association of HPV positivity at baseline with different women's characteristics after adjustment for age group. There was a tendency of HPV positivity to decline with age, but the trend was of borderline statistical significance. HPV positivity was significantly associated with HIV positivity (OR = 1.8; 95% CI: 1.1–3.1), cytological abnormalities (OR = 3.8 and 3.5 for presence of atypical squamous cells of undetermined significance [ASCUS] and LSIL, respectively) and young age at first sexual intercourse (OR for ≤15 *vs.* 18–24 = 1.6; 95% CI: 1.1–2.4). In addition, higher HPV prevalence was detected among women who presented with genital warts (OR = 3.1; 95% CI: 1.6–6.1), and tested positive for syphilis (OR = 2.4; 95% CI: 1.0–5.5), CT (OR = 3.1; 95% CI: 1.5–6.2) or NG (OR = 2.7; 95% CI: 1.0–7.2). Trimester of pregnancy when the baseline visit took place, education level, marital status and multiple sexual partners in the last year were unrelated to HPV positivity (Table II). When risk factors for positivity for high-risk and low-risk types were assessed separately, no significant heterogeneity was found (data not shown).

TABLE II – ODDS RATIOS (OR) FOR HUMAN PAPILLOMAVIRUS (HPV) POSITIVITY AT BASELINE AND CORRESPONDING 95% CONFIDENCE INTERVALS (CI) ACCORDING TO SELECTED CHARACTERISTICS, 987[1] PRIMIPAROUS PREGNANT WOMEN, KAMPALA, UGANDA, 2004–2006

Characteristic	HPV prevalence		
	N	%	OR (95% CI)[2]
Total	987	60.0	–
Trimester of pregnancy			
First	81	64.2	1
Second	344	61.3	0.9 (0.5–1.5)
Third	562	58.5	0.8 (0.5–1.3)
χ^2 for trend			1.33 $p = 0.25$
Age (years)			
14–18	363	63.1	1
19–20	373	59.8	0.9 (0.6–1.2)
21–24	251	55.8	0.7 (0.5–1.0)
χ^2 for trend			3.30 $p = 0.07$
HIV status			
Negative	913	58.9	1
Positive	72	72.2	1.8 (1.1–3.1)
Cytology result			
Normal	756	54.0	1
ASCUS	147	81.6	3.8 (2.4–5.9)
LSIL	42	81.0	3.5 (1.6–7.7)
Education			
Illiterate or primary	367	62.4	1
Secondary	576	58.7	0.9 (0.7–1.2)
University	44	56.8	0.9 (0.5–1.9)
χ^2 for trend			0.38 $p = 0.54$
Marital status			
Ever married	842	60.0	1
Single	145	60.0	1.0 (0.7–1.4)
Age at 1st sexual intercourse			
18–24	314	53.5	1
16–17	401	61.1	1.3 (1.0–1.8)
≤15	272	65.8	1.6 (1.1–2.4)
χ^2 for trend			6.25 $p = 0.02$
Sexual partners in last year			
1	742	59.8	1
≥2	245	60.4	1.0 (0.7–1.4)
Genital warts			
Absent	921	58.6	1
Present	61	82.0	3.1 (1.6–6.1)
Syphilis test			
Negative	953	59.4	1
Positive	32	78.1	2.4 (1.0–5.5)
CT test			
Negative	903	58.1	1
Positive	54	81.5	3.1 (1.5–6.2)
NG test			
Negative	932	58.9	1
Positive	25	80.0	2.7 (1.0–7.2)

[1]Some figures do not add up to the total because of missing values.–[2]Adjusted for age. ASCUS, atypical squamous cells of undetermined significance; CT, *Chlamydia trachomatis*; LSIL, low-grade squamous intraepithelial lesions; NG, *Neisseria gonorrhoeae*.

Follow-up

Of 425 women recruited in 1st/2nd trimester of pregnancy, 105 had a follow-up visit and adequate cervical cell samples during the 3rd trimester and, hence, contributed to the comparison of HPV prevalence between the 1st/2nd and 3rd trimesters (mean interval between visits: 16 weeks). Of 562 women recruited during the 3rd trimester of pregnancy, 149 had a follow-up visit and adequate cervical cell samples after delivery. After the addition of 140 women recruited during the 1st/2nd trimester of pregnancy, 289 women contributed to the comparison of HPV status between pregnancy and after delivery (mean interval between visits: 48 weeks). Overall 334 women had at least 1 follow-up visit and adequate cervical cell samples either during pregnancy (45) or after delivery (289) (Fig. 1).

Women who were not adequately followed up were younger (OR for 14–18 *vs.* 21–24 years = 1.9; 95% CI: 1.3–2.7) and had a lower educational level (OR for illiterate or primary *vs.* secondary or more = 1.4; 95% CI: 1.1–1.9) than women who were adequately followed up. Conversely, women with and without adequate follow-up did not differ significantly by any other characteristics, including positivity for HPV, HIV and other sexually transmitted infections (Table III).

TABLE III – ODDS RATIOS (ORS) FOR LACK OF ADEQUATE FOLLOW-UP AND CORRESPONDING 95% CONFIDENCE INTERVALS (CI) ACCORDING TO SELECTED CHARACTERISTICS, 987[1] PRIMIPAROUS PREGNANT WOMEN, KAMPALA UGANDA, 2004–2006

Characteristics	Adequate follow-up				OR (95% CI)[2]
	Yes (N = 334)		No (N = 653)		
	N	%	*N*	%	
HPV					
Negative	144	43.1	251	38.4	1
Positive	190	56.9	402	61.6	1.2 (0.9–1.5)
Age (years)					
21–24	105	31.4	146	22.4	1
19–20	129	38.6	244	37.4	1.4 (0.98–1.9)
14–18	100	29.9	263	40.3	1.9 (1.3–2.7)
HIV status					
Negative	309	92.5	604	92.5	1
Positive	25	7.5	47	7.2	1.0 (0.6–1.6)
Cytology result					
Normal	252	79.0	504	80.5	1
ASCUS	52	16.3	95	15.2	0.9 (0.6–1.3)
LSIL	15	4.7	27	4.3	0.8 (0.4–1.5)
Education					
Secondary or more	234	70.1	386	59.1	1
Illiterate or primary	100	29.9	267	40.9	1.4 (1.1–1.9)
Marital status					
Single	41	12.3	104	15.9	1
Ever married	293	87.7	549	84.1	0.8 (0.5–1.2)
Age at 1st sexual intercourse					
18–24	118	35.3	196	30.0	1
16–17	142	42.5	259	39.7	0.9 (0.6–1.2)
≤15	74	22.2	198	30.3	1.2 (0.8–1.8)
Sexual partners in the last year					
1	244	73.1	498	76.3	1
≥2	90	27.0	155	23.7	0.8 (0.6–1.1)
Genital warts					
Absent	306	91.6	615	94.2	1
Present	25	7.5	36	5.5	0.7 (0.4–1.1)
Syphilis test					
Negative	326	97.6	627	96.0	1
Positive	8	2.4	24	3.7	1.5 (0.6–3.3)
CT test					
Negative	309	95.1	594	94.0	1
Positive	17	4.9	38	6.0	1.2 (0.6–2.1)
NG test					
Negative	317	97.5	615	97.3	1
Positive	8	2.5	17	2.7	1.0 (0.4–2.3)

[1]Some figures do not add up to the total because of missing values.–[2]Adjusted for age. ASCUS, atypical squamous cells of undetermined significance; CT, *Chlamydia trachomatis*; LSIL, low-grade squamous intraepithelial lesions; NG, *Neisseria gonorrhoeae*.

Table IV shows number and percent of new HPV infections among women who were negative at baseline for the corresponding HPV type, and the number and percent of individual HPV infections that cleared between the 1st/2nd and 3rd trimesters, and between pregnancy and after delivery. Overall, 42.9% and 38.1% of women, respectively acquired new HPV infection(s). As for HPV prevalence at baseline, the incidence of high-risk HPV types was greater than the incidence of low-risk types. Fifty percent of individual HPV infections cleared between the 1st/2nd and 3rd trimesters, and 71.8% between pregnancy and after delivery.

Figure 2 shows the net effects of new infections and HPV clearance. The prevalence of HPV16/18, high- and low-risk types, and all HPV types between the (*i*) 1st/2nd and 3rd trimesters, and (*ii*) between pregnancy and after delivery, did not change significantly.

The comparison of liquid-based cytological findings during pregnancy and after delivery was possible among 217 women. Of 171 women cytologically normal at baseline, 9.4% (95% CI: 5.4%–14.7%) developed ASCUS or LSIL, whereas of 46 women who had ASCUS or LSIL, 76.1% (95% CI: 61.2%–87.4%) became cytologically normal. Thus the prevalence of ASCUS/LSIL decreased from 21.2% before to 12.4% after delivery (χ^2_1 = 5.95; $p = 0.015$, data not shown).

Association between selected baseline characteristics, and incidence and clearance of HPV among 334 women followed-up either during pregnancy (45) or after delivery (289) is shown in Table V. Incidence of HPV infection did not vary with the time interval between the visits and the number of sexual partners in the interval between visits, but was related inversely and significantly with age (OR for 21–24 *vs.* 14–18 years = 0.3; 95% CI: 0.1–0.5). Cytological abnormalities (OR = 1.9; 95% CI: 1.1–3.4) and the presence of genital warts, syphilis, CT or NG (OR = 2.1; 95% CI: 1.1–4.1), but not HIV infection, were associated with significantly increased HPV incidence. Long interval between the two visits (OR ≥12 *vs.* ≤5 months = 7.8; 95% CI: 3.8–16.2), presence of HIV infection (OR = 0.5; 95% CI: 0.3–0.9) and 2 or more sexual partners in the interval between visits (OR = 0.1; 95% CI: 0.0–0.7) were significantly associated with HPV clearance. Clearance was marginally, but not significantly, less frequent for HPV16/18 (OR = 0.6; 95% CI: 0.3–1.3) and other high-risk HPV types (OR = 0.6; 95% CI: 0.4–1.1) than for low-risk types (Table V). One woman seroconverted to HIV between the 1st/2nd and 3rd trimesters, and 5 seroconverted between pregnancy and after delivery (data not shown).

Discussion

Our large study shows a high prevalence of cervical HPV infection (60%) among young primiparous pregnant women (7.3% of whom were HIV-positive) who attended antenatal care in Kampala, Uganda. In over two-thirds of HPV-positive women high-risk types were involved, most notably HPV16, 51 and 52,

TABLE IV – INCIDENCE AND CLEARANCE OF PREVALENT HUMAN PAPILLOMAVIRUS (HPV) INFECTIONS IN PRIMIPAROUS PREGNANT WOMEN, KAMPALA, UGANDA, 2004–2006

HPV type(s)	Incidence[1]		Clearance[2]	
	Women (*N*)	% (95% CI)	Infections (*N*)	% (95% CI)
Between 1st/2nd and 3rd trimester of pregnancy				
HPV16/18	8/103	7.8 (3.4–14.7)	11/19	57.9 (33.5–79.7)
High-risk[3]	32/105	30.5 (21.9–40.2)	45/92	48.9 (38.3–59.6)
Low-risk	16/105	15.2 (9.0–23.6)	19/35	54.3 (36.6–71.2)
All	45/105	42.9 (33.2–52.9)	64/127	50.4 (41.4–59.4)
Between pregnancy and after delivery				
HPV16/18	7/287	2.4 (1.0–5.0)	19/31	61.3 (42.2–78.2)
High-risk[3]	73/289	25.3 (20.4–30.7)	129/189	68.3 (61.1–74.8)
Low-risk	49/289	17.0 (12.8–21.8)	82/105	78.1 (69.0–85.6)
All	110/289	38.1 (32.4–43.9)	211/294	71.8 (66.3–76.8)

[1]Calculated for any HPV type not detected at baseline, women used as the unit of analysis.–[2]Calculated for any HPV type present at baseline, except HPV X, infections used as unit of analysis.–[3]Includes HPV16/18. CI, confidence interval.

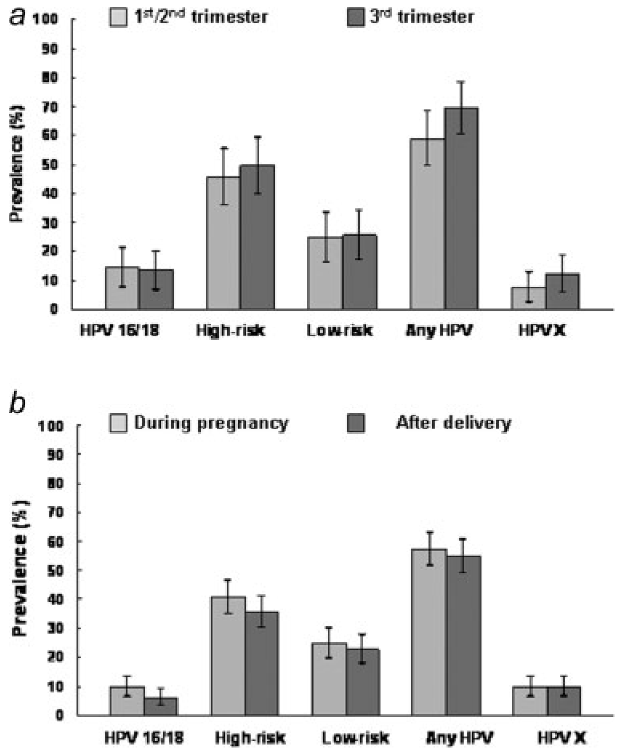

FIGURE 2 – Comparison of human papillomavirus (HPV) prevalence between the 1st/2nd and 3rd trimesters of pregnancy (105 women), and between pregnancy and after delivery (289 women) among primiparous pregnant women, Kampala, Uganda, 2004–2006.

and multiple-type infections were common (43.6% of HPV infections). HPV prevalence at baseline was similar in women who were seen in different periods of pregnancy. In a similar study conducted at the same time among equally young nonpregnant women attending a teenage clinic in the same Health Centre in Kampala,[17] the prevalence of HPV (74.6%) and HIV (8.6%) were slightly higher than among women included in our study.

The aim of the follow-up in this study was to assess changes in HPV status between early and late pregnancy and after delivery. High parity, young age at first birth[2] and long-term use of hormonal contraceptives[18] are associated with an increased risk for cervical cancer that is not totally eliminated by careful adjustment for sexual behavior. Although multiparous women and hormonal contraceptive users did not show higher HPV prevalence than women with fewer or no children or non-users in a large cross-sectional study of women aged 15-59,[19] endocrinological or lifestyle changes during pregnancy and after delivery might affect, at least temporarily, the acquisition or clearance of HPV infections.

Maternal and then placental production of progesterone continues to rise until about the 32nd week of pregnancy, when it reaches a plateau, to subsequently fall abruptly after the expulsion of the placenta.[20] High progesterone levels, in conjunction with high estrogen levels, downregulate cell-mediated immunity that could be harmful to the fetus,[20] but are essential for clearance of HPV infection.[21] Reduced humoral response to HPV16 has also been reported in pregnant women.[22]

Several studies, mainly cross-sectional ones, have compared HPV prevalence in pregnant and nonpregnant women[3–5,8,23] and showed inconclusive results. Inconsistencies may derive from differences in the selection of nonpregnant women and the use of a variety of HPV detection methods. Two prospective studies used sensitive PCR-based assays able to detect a broad range of HPV types. Nobbenhuis *et al.*[7] compared pregnant and nonpregnant women with cytological abnormalities in The Netherlands. Preg-

TABLE V – ODDS RATIOS (OR) AND 95% CONFIDENCE INTERVALS (CI) OF DEVELOPING A NEW HUMAN PAPILLOMAVIRUS (HPV) INFECTION AND CLEARING A PREVALENT HPV INFECTION IN PRIMIPAROUS PREGNANT WOMEN BY SELECTED VARIABLES, KAMPALA, UGANDA, 2004–2006

Baseline characteristic	Incidence (334 women)[1]			Clearance (339 HPV infections)[1]		
	N	%	OR (95% CI)[2]	N	%	OR (95% CI)[2]
Months between 2 visits[3]						
≤5	95	46.3	1	94	52.1	1
6–11	130	30.0	0.5 (0.3–0.9)	130	66.2	1.8 (1.0–3.2)
≥12	109	45.9	1.0 (0.6–1.9)	115	89.6	7.8 (3.8–16.2)
χ^2 for trend			0.08 $p = 0.77$			34.41 $p < 0.001$
Age (years)						
14–18	100	54.0	1	118	67.8	1
19–20	129	42.6	0.6 (0.4–1.1)	128	75.8	1.5 (0.8–2.6)
21–24	105	22.9	0.3 (0.1–0.5)	93	65.6	0.9 (0.5–1.6)
χ^2 for trend			20.78 $p < 0.001$			0.05 $p = 0.82$
HIV status						
Negative	309	39.2	1	294	72.5	1
Positive	25	48.0	1.5 (0.6–3.4)	45	55.6	0.5 (0.3–0.9)
Cytology result						
Normal	252	36.9	1	190	65.3	1
ASCUS/LSIL	67	52.2	1.9 (1.1–3.4)	121	71.9	1.4 (0.8–2.3)
Sexual partners in the interval						
1	323	40.6	1	329	71.1	1
≥2	8	25.0	0.5 (0.1–2.9)	6	16.7	0.1 (0.0–0.7)
Genital warts/syphilis /CT/NG						
Absent	278	36.3	1	233	65.2	1
Present	47	59.6	2.1 (1.1–4.1)	83	75.9	1.6 (0.9–2.9)
HPV types						
Low-risk	–	–	–	111	76.6	1
HPV16/18	–	–	–	41	65.9	0.6 (0.3–1.3)
Other high-risk	–	–	–	187	67.4	0.6 (0.4–1.1)
Multiplicity of infection						
Single	–	–	–	89	69.7	1
Multiple	–	–	–	250	70.4	1.0 (0.6–1.7)

[1]Some numbers do not add up to the total because of missing values.–[2]Adjusted for age.–[3]If follow-up visits during pregnancy and after delivery were both available, the latter was used. ASCUS, atypical squamous cells of undetermined significance; CT, *Chlamydia trachomatis*; LSIL, low-grade squamous intraepithelial lesions; NG, *Neisseria gonorrhoeae*.

nant women showed nonsignificantly reduced clearance rates of high-risk HPV types compared to nonpregnant women during the 1st trimester of pregnancy, but compensatory higher clearance rates after delivery. Minkoff *et al*.[6] evaluated the impact of pregnancy on 628 HIV-positive women in the United States among whom baseline HPV prevalence (32.8% for high-risk and 33.3% for low-risk HPV types) was high, as in our study women in Uganda. In addition, as in this study, women acted as their own controls, *i.e*., they were assessed before, during and shortly after pregnancy. The prevalence and copy number of high-risk and low-risk types did not significantly differ between pregnancy and either the pre or postpregnancy period. HPV incidence, however, was significantly lower during pregnancy than after delivery.[6]

In this study, we found high HPV incidence during pregnancy and after delivery, but clearance was also frequent, especially when the interval between the 2 visits was relatively long. Among study women seen at a 12-month interval or more, 90% of HPV infections had cleared, *i.e*., a finding consistent with what has been reported among nonpregnant women.[24]

Young age, cytological abnormalities and presence of sexually transmitted infections other than cervical HPV were associated with acquisition of a new HPV infection. Clearance was lower among HIV-positive women. HPV16/18 and other high-risk types also showed lower clearance than low-risk types, but this difference was not statistically significant. Very few women reported more than 1 sexual partner in the interval between the 2 examinations, but a strong association of multiple partners with reduced clearance suggested that reinfections with the same types might have masked infection clearance in some women.

It is worth noting, however, that despite a substantial level of HPV incidence and clearance, HPV prevalence during early and late pregnancy, and during pregnancy and after delivery, was similar. These findings suggest a balance between acquisition and clearance of HPV infection during and after pregnancy among our young study women.

This study included the largest number of liquid-based cytology tests ever reported among pregnant women. Despite the very high burden of high-risk HPV infections, and the reading of the cytology test by experienced cytologists in The Netherlands, no high-grade lesions were detected. Cytology results became available months after baseline visit and, therefore, women with ASCUS or LSIL were neither biopsied nor treated. Nevertheless, clearance of HPV was not significantly different in women with and without cytological abnormalities. Our findings agree with the notion that low-grade cervical lesions are merely signs of HPV infection and do not predict a worse prognosis than cytologically normal HPV infections.[24] Three-quarters of ASCUS and LSIL detected during pregnancy, and nearly the same percent of HPV infections, disappeared after delivery, thus confirming that screening very young women using either cytology or HPV testing should not be encouraged.[25]

The main weaknesses of this study are the high proportion (61%) of women who were lost to follow-up and the difficulty in having women return at well-defined intervals. It is common for pregnant women in Kampala to return to their villages of origin to deliver a baby and obtain family assistance. Some women may have also chosen to deliver in other hospitals or simply to drop out of the study. The evaluation of changes in HPV status during different trimesters of pregnancy was further hampered by the typically late referral to antenatal care in Kampala, Uganda. Women who were lost to follow-up were significantly younger and less educated than women who were followed up, but, rather reassuringly, no difference was found in respect to the presence of cytological abnormalities, HIV, genital warts, CT, NG, syphilis and, most notably, HPV prevalence at baseline. Other important limitations of our present study are the variation in the intervals between visits, and the use, in order to assess clearance, of prevalent infections, whose duration was unknown, rather than incident infections. Major strengths of this study include the location in a high-risk area for HPV infection and cervical cancer,[17] the large number of pregnant women included at baseline, and the use of liquid-based cytology and highly sensitive PCR-based assays to detect HPV, CT and NG.

In conclusion, pregnancy does not seem to be a period of special vulnerability to HPV infection, but the burden of the infection among young primiparous pregnant women in Uganda is very high, thus reducing substantially the cost-effectiveness of the administration of a prophylactic HPV vaccine after first delivery. Antenatal care clinics like the one in our study attended by large numbers of young primiparous pregnant women would, however, be ideal locations for Sentinel Surveys in low-resource countries to monitor the early effect of large-scale immunization of girls on the prevalence of HPV16/18 and other HPV types.[21]

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References

1. Cutts FT, Franceschi S, Goldie S, Castellsagué X, de Sanjosé S, Garnett G, Edmunds WJ, Claeys P, Goldenthal KL, Harper DM, Markowitz L. Human papillomavirus and HPV vaccines: a review. Bull World Health Organ 2007;85:719–26.
2. International Collaboration of Epidemiological Studies of Cervical Cancer. Cervical carcinoma and reproductive factors: collaborative reanalysis of individual data on 16,563 women with cervical carcinoma and 33,542 women without cervical carcinoma from 25 epidemiological studies. Int J Cancer 2006;119:1108–24.
3. Fife KH, Katz BP, Roush J, Handy VD, Brown DR, Hansell R. Cancer-associated human papillomavirus types are selectively increased in the cervix of women in the first trimester of pregnancy. Am J Obstet Gynecol 1996;174:1487–93.
4. Hernandez-Giron C, Smith JS, Lorincz A, Lazcano E, Hernandez-Avila M, Salmeron J. High-risk human papillomavirus detection and related risk factors among pregnant and nonpregnant women in Mexico. Sex Transm Dis 2005;32:613–18.
5. Chang-Claude J, Schneider A, Smith E, Blettner M, Wahrendorf J, Turek L. Longitudinal study of the effects of pregnancy and other factors on detection of HPV. Gynecol Oncol 1996;60:355–62.
6. Minkoff H, Shen X, Watts DH, Leighty R, Hershow R, Palefsky J, Tuomala R, Neu N, Zorrilla CD, Paul M, Strickler H. Relationship of pregnancy to human papillomavirus among human immunodeficiency virus-infected women. Obstet Gynecol 2006;108:953–60.
7. Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, Rozendaal L, Bezemer PD, Voorhorst FJ, Meijer CJ. High-risk human papillomavirus clearance in pregnant women: trends for lower clearance during pregnancy with a catch-up postpartum. Br J Cancer 2002;87:75–80.
8. Chan PK, Chang AR, Tam WH, Cheung JL, Cheng AF. Prevalence and genotype distribution of cervical human papillomavirus infection: Comparison between pregnant women and non-pregnant controls. J Med Virol 2002;67:583–8.
9. Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, Raab S, Sherman M, Wilbur D, Wright T, Jr, Young N. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287:2114–19.
10. Sellors JW, Sankaranarayanan R. Colposcopy and treatment of cervical intraepithelial neoplasia: a beginner's manual. Lyon: International Agency for Research on Cancer, 2003.
11. Kleter B, van Doorn LJ, ter Schegget J, Schrauwen L, van Krimpen K, Burger M, ter Harmsel B, Quint W. Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. Am J Pathol 1998;153:1731–9.
12. Kleter B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, Lindeman J, ter Harmsel B, Burger M, Quint W. Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. J Clin Microbiol 1999;37:2508–17.

13. Muñoz N, Bosch FX, Castellsagué X, Diaz M, de Sanjosé S, Hammouda D, Shah KV, Meijer CJ. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. Int J Cancer 2004;111:278–85.
14. Downing RG, Otten RA, Marum E, Biryahwaho B, Alwano-Edyegu MG, Sempala SD, Fridlund CA, Dondero TJ, Campbell C, Rayfield MA. Optimizing the delivery of HIV counseling and testing services: the Uganda experience using rapid HIV antibody test algorithms. J Acquired Immune Defic Syndr Hum Retrovirol 1998;18:384–8.
15. Quint KD, van Doorn LJ, Kleter B, de Koning MN, van den Munckhof HA, Morre SA, ter Harmsel B, Weiderpass E, Harbers G, Melchers WJ, Quint WG. A highly sensitive, multiplex broad-spectrum PCR-DNA-enzyme immunoassay and reverse hybridization assay for rapid detection and identification of *Chlamydia trachomatis serovars*. J Mol Diagn 2007;9:631–8.
16. Ho BS, Feng WG, Wong BK, Egglestone SI. Polymerase chain reaction for the detection of *Neisseria gonorrhoeae* in clinical samples. J Clin Pathol 1992;45:439–42.
17. Banura C, Franceschi S, van Doorn LJ, Arslan A, Wabwire-Mangen F, Mbidde EK, Quint W, Weiderpass E. Infection with human papillomavirus and HIV among young women in Kampala, Uganda. J Infect Dis 2008;197:555–62.
18. International Collaboration of Epidemiological Studies of Cervical Cancer. Cervical cancer and hormonal contraceptives: collaborative reanalysis of individual data on 16,573 women with cervical cancer and 35,509 women without cervical cancer from 24 epidemiological studies. Lancet 2007;370:1609–21.
19. Vaccarella S, Herrero R, Dai M, Snijders PJF, Meijer CJLM, Thomas JO, Anh PTH, Ferreccio C, Matos E, Posso H, de Sanjose S, Shin HR, et al. Reproductive factors, oral contraceptive use and HPV infection: pooled analysis of the IARC HPV Prevalence Surveys. Cancer Epidemiol Biomarkers Prev 2006;15:2148–53.
20. Druckmann R, Druckmann MA. Progesterone and the immunology of pregnancy. J Steroid Biochem Mol Biol 2005;97:389–96.
21. Dillner J, Arbyn M, Dillner L. Translational mini-review series on vaccines: Monitoring of human papillomavirus vaccination. Clin Exp Immunol 2007;148:199–207.
22. Sethi S, Muller M, Schneider A, Blettner M, Smith E, Turek L, Wahrendorf J, Gissmann L, Chang-Claude J. Serologic response to the E4, E6, and E7 proteins of human papillomavirus type 16 in pregnant women. Am J Obstet Gynecol 1998;178:360–4.
23. Peng TC, Searle CP, III, Shah KV, Repke JT, Johnson TR. Prevalence of human papillomavirus infections in term pregnancy. Am J Perinatol 1990;7:189–92.
24. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007;370:890–907.
25. Sasieni P, Adams J, Cuzick J. Benefit of cervical screening at different ages: evidence from the UK audit of screening histories. Br J Cancer 2003;89:88–93.