Prevalence and correlates of HPV among women attending family-planning clinics in Thailand

Morgan A Marks¹,³*, Swati Gupta¹, Kai-Li Liaw¹, Amha Tadesse², Esther Kim³, Chailert Phongnarisorn⁴, Virach Wootipoom⁵, Pissamai Yuenyao⁵, Charoen Vipupinyo⁷, Sungwal Rugpao⁸*, Somchai Sriplienchan⁸, Patti E Gravitt³ and David D Celentano³

Abstract

Background: Cervical cancer is the most common cancer among women of reproductive age in Thailand. However, information on the prevalence and correlates of anogenital HPV infection in Thailand is sparse.

Methods: HPV genotype information, reproductive factors, sexual behavior, other STI and clinical information, and cervical cytology and histology were assessed at enrollment among one thousand two hundred and fifty-six (n = 1,256) HIV negative women aged 20–37 from Thailand enrolled in a prospective study of the natural history of HPV. The type-specific prevalence of HPV was estimated using cervical swab specimens from healthy women and women with a diagnosis of CIN 2/3 at baseline. Prevalence ratios (95% CI) were estimated using Poisson regression to quantify the association of demographic, behavioral, and clinical correlates with prevalent HPV infection.

Results: Overall, 307 (24.6%) and 175 (14.0%) of women were positive for any HPV type and any HR-HPV type, respectively; the most common types were 72, 52, 62, and 16. Among women diagnosed with CIN 2/3 at enrollment (n = 11), the most prevalent HPV types were 52 and 16. In multivariate analysis, HPV prevalence at enrollment was higher among women with: long-term combined oral contraceptive use, a higher number of lifetime sexual partners, a prior Chlamydia infection, and a current diagnosis of Bacterial Vaginosis.

Conclusion: The study findings provide important information that can be used in the evaluation of primary and secondary interventions designed to reduce the burden of cervical cancer in Thailand.

Keywords: HPV, Epidemiology, Thailand, CIN

Background

Cervical cancer affects nearly half a million women worldwide, making it the third most common cancer. Between the years 1983 and 2002, Thailand had a reported average annual cervical cancer incidence rate of 65.2 cases per 100,000 over the 20 year period making it the most common cancer among women between 30–74 years of age with a 2.6% per year increase in incidence [1]. Infection with anogenital types of the human papillomavirus (HPV) is the established cause of cervical cancer [2,3]. HPV types 16 and 18 account for nearly 70% of the total cervical cancer cases detected worldwide with HPV 16 alone accounting for approximately 50% [4].

In a prior population-based study in two districts in northern and southern Thailand, HPV types other than type 16 were as or more prevalent in women both with and without cervical disease [5]. However, this prior work provided only a limited view of the total HPV burden as it included women from only 2 regions in Thailand. Given the large burden of cervical cancer in Thailand, it is important to further understand the HPV type-specific distribution in cervical infections in other regions and populations. This information may help direct appropriate strategies for current and future interventions.

The current study assessed the prevalence and risk factors for anogenital HPV infection and CIN 2/3 in

* Correspondence: morgan.marks@merck.com
Deceased
¹Merck Research Laboratories, West Point, PA, USA
²Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA
Full list of author information is available at the end of the article

© 2015 Marks et al.; licensee BioMed Central. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
women aged 20–37 years recruited from family-planning clinics in 7 sites throughout the country.

Methods
Study population and enrollment
Women 20–37 years old attending family planning clinics in the northern (Chiang Mai), northeastern (Khon Kaen), central (Bangkok) and southern (Songkla/HatYai) regions in Thailand between 2002–2003 were recruited into a prospective study investigating the natural history of HPV and CIN 2/3. These women were previously enrolled in a two-year study addressing the effects of hormonal contraceptive use on HIV acquisition (HC-HIV). Selection criteria are described in detail elsewhere [6]. Briefly, inclusion criteria for enrollment in the HC-HIV study were: 1) HIV negative; 2) not pregnant; 3) intact uterus; 4) used some form of contraception in the 3 months prior to enrollment; and 5) willing to adhere to the self-selected contraceptive method for at least 1 year of follow-up. Among women who participated in the HC-HIV study, 79% were reconsented for inclusion in the current study (n = 1256). The study protocols were reviewed and approved by the committees on human subject research at Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, Merck Research Laboratories, West Point, PA, each study recruitment site, and the Institutional Review Board of the Thailand Ministry of Health (MOH), Thailand.

At enrollment, information on sociodemographic characteristics, sexual risk behavior, reproductive and contraceptive history, current contraceptive usage status, self-reported medical history, and woman’s report of the sexual behavior of her partner was collected at each study site by trained interviewers using a standardized questionnaire.

Sexual behavior variables included age of sexual debut, lifetime number of sexual partners, number of sexual partners in the last six months (L6M) and/or sexual partners acquired in the last year (L12M), commercial sex work L6M, condom use with primary partner L6M or most recent sexual partner if no primary partners are reported by the participant, and primary partner risk behaviors. Reproductive information included total number of pregnancies and live births. Contraceptive use was broadly classified as 1) combined low-dose oral contraceptives (COC); 2) depomedroxyprogesterone acetate (DMPA); 3) other injectable methods; and 4) non-hormonal contraceptive use and non-use (NHC). Current use and duration of use prior to enrollment for each category of hormonal contraception were assessed.

Questionnaire and physical exam data from the original HC-HIV study were extracted and linked to the participants. Laboratory-confirmed STI status was collected from the two year period prior to study enrollment and included gonorrhea (GC), chlamydia (CT), syphilis (SYP), and Bacterial Vaginosis (BV).

Physical examination and specimen collection
At enrollment, each participant underwent a pelvic examination. Exfoliated cervical cells were collected using a cytobrush and placed in PreservCyt™ for Thinprep™ liquid-based cytology. CT and GC detection from an ectocervical mucus specimen was performed using the Roche Amplicor assay per manufacturer’s instructions (Roche Molecular Systems, Alameda, CA). An endo/ecto cervical swab specimen was collected by study clinicians for HPV DNA genotyping using a Dacron swab stored in Specimen Transport Medium (STM) (Digene) at -20°C until testing. Bacterial Vaginosis was diagnosed by the Ames test.

All Pap smear and biopsy specimens were read by trained cytopathologists (Covance, Indianapolis, IN). Cytological smears were classified according to the Bethesda system [7]. Participants with an abnormal Pap smear diagnosis of atypical squamous cell of undetermined significance (ASC-US) or more severe (≥ ASC-US) were referred for colposcopic examination with biopsy and treatment as indicated. Colposcopy directed biopsies were reported as a diagnosis of Normal, Cervical Intraepithelial Neoplasia 1 (CIN 1), Cervical Intraepithelial Neoplasia II (CIN 2), and Cervical Intraepithelial Neoplasia 3 (CIN 3). Biopsies were read and interpreted by one study pathologist.

HPV DNA testing
All HPV DNA testing was performed on cervical cell samples stored in STM (Digene) at Johns Hopkins University, Baltimore, MD. DNA was extracted using the QIAamp DNA Blood Kit (Qiagen, Courtaboeuf, France) according to manufacturer’s instructions with modification. After extraction, DNA was tested using the Roche HPV Linear array© PCR assay (Roche Diagnostics, Indianapolis, IN). The HPV Linear Array® is based on the PGMY09/11 primer system that allows for high efficiency amplification of 37 types of HPV [8,9]. The quality and validity of the extracted DNA specimen was assessed by inclusion of β-globin gene-specific primers in the PCR reaction; only specimens with detectable β-globin were used in this analysis.

HPV types considered to be high risk (HR-HPV) for this analysis included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82 [3,10]. Low risk (LR) HPV types included all other HPV types detected by the HPV Linear Array®. Multiple infections that include a HR-HPV type were classified as high risk regardless of the presence of other low risk co-infections.

Statistical analysis
The prevalence of HPV any, high-risk HPV (HR-HPV), low-risk HPV (LR-HPV), and type-specific infection was
computed among the total study sample and among those with a diagnosis of CIN 2/3 at enrollment.

Contingency tables were created to assess the distribution of demographic, sexual, clinical, and reproductive factors by detection of HPV DNA. Due to the high prevalence of HPV in the study sample, univariate prevalence ratios (PR) were estimated using a generalized linear model with a Poisson function and robust variance to assess the strength of the association between a given risk factor and detection of any HPV or any HR-HPV type [11].

Covariates with a p-value of <0.1 in univariate analyses were considered for inclusion in the multivariate regression model. Following this initial selection, variables were removed from the model in a stepwise fashion and a likelihood ratio test was performed after removal of each variable to confirm the variables contribution to the model’s goodness-of-fit and to identify the most parsimonious model. Age and study site were retained in the multivariate model. After identifying the final multivariate model using an outcome of any HPV infection, separate multivariate models were then generated that assessed the relationship of the identified demographic, behavioral and clinical factors on other outcomes such as (1) any HR-HPV infections; (2) HPV 52 infections; and (3) HPV 52 infections without HPV 16 infections. The statistical significance of trends for PRs was assessed by including a categorical variable as a continuous variable in the regression model. The final model for any HPV infection was stratified by cytological diagnosis at baseline (Normal vs. Abnormal [including inflammation, ASCUS, LSIL, and HSIL]). A p-value of <0.05 was considered statistically significant in the final multivariate model for the association of a given variable and detection of HPV DNA. All analyses were conducted using STATA 11.0 (STATACORP, College Station, TX).

Results

At enrollment, 6 (0.4%) of the 1256 samples were β-globin negative leaving a total of 1250 samples for analysis. Overall, 307 (24.6%) and 175 (14.0%) of women were positive for any HPV or any HR-HPV type (57% of HPV DNA positive women), respectively (Table 1). There were 94 (7.6%) of women with multiple HPV infections (30.9% of HPV-positive women). The three most common types found in either a single or multiple infection were the low risk types 72 (4.1%) and 62 (2.9%) as well as the high-risk type 52 (3.6%). A total of 11 women had a histological diagnosis of CIN2/3 at enrollment. Among women diagnosed with CIN 2/3, the prevalence of any HPV type and any HR-HPV type was 90.9% for both categories. The two most prevalent HPV types detected among women with CIN 2/3 was HPV 52 (63.6%) and HPV 16 (27.3%).

Correlates of prevalent HPV infection

At enrollment, 33 (2.6%) women had missing parity data, 1 (0.1%) woman had a missing STI diagnosis data, and 5 women who reported use of other injectable contraceptives were excluded from the analysis leaving 1,201 women to examine the association of risk factors for prevalent HPV infection.

The prevalence of an HPV infection was significantly lower among women reporting one or more live births as compared to those with none (24.5% vs. 22.1%; p < 0.001) (Table 2). The prevalence of any HPV was significantly higher among women reporting greater than 6 years of COC use as compared to never users (31.5% vs. 21.1%; p < 0.001). The prevalence of any HPV was significantly higher among current smokers as compared to non-smokers (56.4% vs. 22.5%; p < 0.001). Lastly, a higher HPV prevalence was observed among women with risker sexual behavior such as younger age of sexual debut, increased number of recent and lifetime partners, and women reporting commercial sex work. Similar associations with demographic and reproductive factors were observed for HR-HPV infections.

A higher prevalence of any HPV was observed in women with a cytological diagnosis of inflammation (43.8%), AS-CUS (36.5%), LSIL (80.9%), or HSIL (71.4%) as compared to normal (20.3%) (p < 0.001) (Table 3). A higher prevalence of HPV was observed among women with a prior diagnosis of genital ulcers (36.2% vs. 22.0%; p < 0.001) and genital warts (42.2% vs. 23.5%; p = 0.01). No women had genital warts or ulcers upon physical examination at study enrollment. Women with a prior and current diagnosis of gonorrhea, chlamydia, and bacterial vaginosis had a significantly higher prevalence of any HPV infection. Similar associations were observed with HR-HPV infections.

In multivariate analysis (Table 4), prevalent infection with any HPV and any HR-HPV was associated with primary partner sexual behavior, >6 years cumulative use of COCs, an increased number of lifetime partners, prior detection of Chlamydia, and current diagnosis of bacterial vaginosis. The magnitude of the prevalence ratios were similar when analyses were restricted to individuals with HPV 52 infections with or without concurrent HPV 16 infections but the associations did not reach statistical significance due to reduced sample size. There was little to no difference in prevalence ratios among women with and without cytological abnormalities (Additional file 1: Table S1).

Discussion

We identified a high prevalence of any HPV and any HR-HPV infection in a large population-based study of women recruited from seven family planning clinics across different geographic regions of Thailand. HPV 52
| Type                      | Prevalence (N = 1250) | Prevalence among those with prevalent CIN 2/3 (n = 11) |
|--------------------------|-----------------------|------------------------------------------------------|
|                          | n(%)                  | n (%)                                           |
|                          |                       | (95% CI)                                         |
| HPV DNA negative         | 943 (75.0)            | 1 (9.1)                                           |
|                          | (0.2, 41.3)           |                                                      |
| Any HPV positive         | 307 (24.6)            | 10 (90.9)                                         |
|                          | (58.7, 99.8)          |                                                      |
| Any HR-HPV positive      | 175 (14)              | 10 (90.9)                                         |
|                          | (58.7, 99.8)          |                                                      |
| Any LR-HPV positive      | 170 (13.6)            | 2 (18.2)                                          |
|                          | (2.3, 51.8)           |                                                      |
| High-Risk Infections:    |                       |                                                    |
| HPV 16                   | 26 (2.1)              | 3 (27.3)                                          |
|                          | (0.7, 20.2)           |                                                    |
| HPV 18                   | 9 (0.7)               | 2 (18.2)                                          |
|                          | (2.3, 51.8)           |                                                    |
| HPV 31                   | 7 (0.6)               | 0                                                  |
|                          |                       |                                                    |
| HPV 33                   | 7 (0.6)               | 1 (9.1)                                           |
|                          | (0.7, 20.2)           |                                                    |
| HPV 35                   | 3 (0.2)               | 0                                                  |
|                          |                       |                                                    |
| HPV 39                   | 20 (1.6)              | 1 (9.1)                                           |
|                          | (0.2, 41.3)           |                                                    |
| HPV 45                   | 3 (0.2)               | 1 (9.1)                                           |
|                          | (0.2, 41.3)           |                                                    |
| HPV 51                   | 23 (1.8)              | 0                                                  |
|                          |                       |                                                    |
| HPV 52                   | 45 (3.6)              | 7 (63.6)                                          |
|                          | (30.8, 89.1)          |                                                    |
| HPV 56                   | 6 (0.5)               | 0                                                  |
|                          |                       |                                                    |
| HPV 58                   | 11 (0.9)              | 0                                                  |
|                          |                       |                                                    |
| HPV 59                   | 12 (0.9)              | 0                                                  |
|                          |                       |                                                    |
| HPV 68                   | 21 (1.7)              | 1 (9.1)                                           |
|                          | (0.2, 41.3)           |                                                    |
| HPV 73                   | 6 (0.5)               | 0                                                  |
|                          |                       |                                                    |
| HPV 82                   | 6 (0.5)               | 0                                                  |
|                          |                       |                                                    |
| Low-Risk Infections:     |                       |                                                    |
| HPV 6                    | 1 (0.1)               | 0                                                  |
|                          |                       |                                                    |
| HPV 11                   | 1 (0.1)               | 0                                                  |
|                          |                       |                                                    |
| HPV 26                   | 0                     | 0                                                  |
|                          |                       |                                                    |
| HPV 39                   | 2 (0.2)               | 0                                                  |
|                          |                       |                                                    |
| HPV 40                   | 4 (0.3)               | 0                                                  |
|                          |                       |                                                    |
| HPV 42                   | 6 (0.5)               | 0                                                  |
|                          |                       |                                                    |
| HPV 53                   | 27 (2.2)              | 0                                                  |
|                          |                       |                                                    |
| HPV 54                   | 13 (1.0)              | 0                                                  |
|                          |                       |                                                    |
| HPV 55                   | 8 (0.6)               | 0                                                  |
|                          |                       |                                                    |
| HPV 61                   | 3 (0.2)               | 0                                                  |
|                          |                       |                                                    |
| HPV 62                   | 36 (2.9)              | 0                                                  |
|                          |                       |                                                    |
| HPV 64                   | 3 (0.2)               | 0                                                  |
|                          |                       |                                                    |
| HPV 66                   | 7 (0.6)               | 0                                                  |
|                          |                       |                                                    |
| HPV 67                   | 2 (0.2)               | 1 (9.1)                                           |
|                          | (0.2, 41.3)           |                                                    |
| HPV 69                   | 1 (0.1)               | 0                                                  |
|                          |                       |                                                    |
| HPV 70                   | 27 (2.2)              | 0                                                  |
|                          |                       |                                                    |
| HPV 71                   | 22 (1.8)              | 0                                                  |
|                          |                       |                                                    |
| HPV 72                   | 51 (4.1)              | 1 (9.1)                                           |
|                          | (0.2, 41.3)           |                                                    |
| HPV 81                   | 9 (0.7)               | 0                                                  |
|                          |                       |                                                    |
| HPV 83                   | 1 (0.1)               | 1 (9.1)                                           |
|                          | (0.2, 41.3)           |                                                    |
| HPV 84                   | 14 (1.1)              | 0                                                  |
|                          |                       |                                                    |
| HPV 89                   | 4 (0.3)               | 0                                                  |
|                          |                       |                                                    |
was identified as the most common HPV type. A higher prevalence of HPV was found to be associated with long-term use of combined oral contraceptives and prior and current diagnosis of sexually transmitted diseases, which appeared to remain significant after adjustment for sexual behavior. This study represents one of the largest and most comprehensive evaluations of HPV prevalence and correlates of infection in Thailand, a country where cervical cancer is one of most common cancers of women of reproductive age and a significant source of cancer-related mortality.

HPV 52 was the most common HPV type detected among women both with and without cervical abnormalities with a prevalence of 63% among women diagnosed with prevalent CIN2/3 as compared to only 27% for HPV 16. This elevated prevalence of HPV 52 decreased to only 45% after exclusion of women with HPV 16 co-infection. The relatively high prevalence of HPV 52 relative to HPV 16 among those with prevalent CIN 2/3 stands in contrast to a recent hospital-based study of 100 cervical tissue specimens collected from women with HSIL that reported 44.2% and 11.8% attributed to HPV 16 and HPV 52, respectively [12]. Part of this difference could be explained by the fact that our study used cervical swab to detect HPV as compared to biopsied cervical tissue. This may have led to detection of HPV 52 infections that are truly not associated with CIN 2/3. However, prior population-based studies conducted in Thailand that measured HPV in cervical and vaginal samples identified HPV 52 as the dominant type among women with a diagnosis of CIN 2/3 [5,13]. Other population-based studies assessing HPV prevalence in low- and middle-income countries show a high level of variability in the dominance of specific HPV types, particularly among cytologically normal women, as compared to high-income countries in Europe and the US where HPV16 is the most common type detected [14,15]. A meta-analysis utilizing HPV genotype data from 115,789 HPV positive women from different geographic regions with normal cytology, low and high grade pre-cancer, and invasive cancer observed a higher prevalence and potential greater contribution of HPV52 among women with normal cytology and women with low and high grade neoplasia as compared to other non-HPV16 types, particularly in East Asian populations [16]. However, the relative contribution of HPV52 to CIN3 and invasive cancer was less robust than other oncogenic HPV types. These results suggest that, in East Asian populations, HPV52 may be playing a role in early stage neoplastic transformation but its role in the progression to CIN3 and invasive cancer could be potentially less important than other oncogenic HPV types. This observation provides impetus for future work exploring the epidemiology and impact of other, non-HPV 16 oncogenic HPV types on cervical disease in middle and low income settings.

Long-term use of combined oral contraceptives is associated with an increased risk of cervical cancer diagnosis [17]. In Thailand, data from the IARC has estimated that combined oral contraceptive use is attributed to 23.1% of all cervical cancer cases [18]. Current and long-term combined oral contraceptive use has also been shown to increase risk of prevalent HPV infection among women <30 years of age [19-21]. However, longitudinal studies conducted to assess the association of COC use with HPV acquisition, persistence, and progression to pre-cancerous lesions have been inconsistent [22-29]. A detailed cross-sectional study conducted in this population confirmed a higher prevalence of any HPV and any HR-HPV infection among long-term COC users [21]. This finding agrees with prior longitudinal analyses which revealed an increased risk of HPV persistence as compared to an increased risk of HPV acquisition among current COC users [22]. Similar analyses conducted among DMPA users in this study population did not show any association with HPV prevalence, incidence or persistence in this population, lending to the specificity of the association. A variety of mechanisms have been proposed to help explain the potential role of sex steroid hormones on the natural history of HPV.

### Table 1 Prevalence of cervical HPV infections overall and among those with prevalent CIN 2/3 cases (Continued)

| HPV Type                      | Overall Prevalence | Prevalent CIN 2/3 Prevalence | 95% CI | 95% CI |
|-------------------------------|--------------------|------------------------------|--------|--------|
| HPV16 (w/o HPV 52)            | 22 (1.8)           | 1 (9.1)                      | (0.2, 41.3) |
| HPV52 (w/o HPV 16)            | 41 (3.5)           | 5 (45.5)                    | (16.7, 76.6) |
| Other HR-HPV types (w/o HPV 16/52) | 105 (10.6)       | 2 (18.2)                    | (2.3, 51.8) |
| LR-HPV (w/o HR-HPV)           | 115 (8.4)          | 2 (18.2)                    | (2.3, 51.8) |

| Number of unique HPV types detected: | |
|--------------------------------------|---|
| 1                                    | 212 (16.9) |
| 2                                    | 62 (4.9)   |
| 3                                    | 22 (1.8)   |
| 4                                    | 7 (0.6)    |
| 5                                    | 4 (0.3)    |

Marks et al. BMC Infectious Diseases (2015) 15:159
### Table 2 Univariate association of demographic information and reproductive history with prevalent infection of any HPV and any HR-HPV type

| Variable                        | Sample N = 1,201 | HPV positive n = 289 (24.1%) | Unadjusted prevalence ratio (95% CI) | HR-HPV positive N = 163 (13.6%) | Unadjusted prevalence ratio (95% CI) |
|---------------------------------|------------------|------------------------------|-------------------------------------|-------------------------------|-------------------------------------|
| **Age category, years**         |                  |                              |                                     |                               |                                     |
| <26                             | 224              | 26.3%                        | 1.0                                 | 18.3%                         | 1.0                                 |
| 26-30                           | 422              | 22.8%                        | 0.86 (0.65, 1.14)                   | 12.6%                         | 0.69 (0.47, 0.99)                   |
| 31-33                           | 279              | 25.1%                        | 0.95 (0.71, 1.28)                   | 11.8%                         | 0.65 (0.42, 0.99)                   |
| 34-38                           | 276              | 23.2%                        | 0.89 (0.65, 1.19)                   | 13.0%                         | 0.71 (0.47, 1.08)                   |
| **Study Site in Thailand**      |                  |                              |                                     |                               |                                     |
| North                           | 429              | 20.5%                        | 1.0                                 | 10.5%                         | 1.0                                 |
| North-East                      | 274              | 21.2%                        | 1.03 (0.77, 1.38)                   | 11.7%                         | 1.11 (0.73, 1.71)                   |
| South                           | 311              | 27.9%                        | 1.36 (1.05, 1.77)                   | 16.4%                         | 1.56 (1.08, 2.27)                   |
| Central                         | 187              | 29.9%                        | 1.46 (1.09, 1.95)                   | 18.7%                         | 1.78 (1.19, 2.68)                   |
| **Years of education**          |                  |                              |                                     |                               |                                     |
| >12                             | 149              | 28.9%                        | 1.0                                 | 14.8%                         | 1.0                                 |
| 10-12                           | 235              | 20.4%                        | 0.71 (0.49, 1.01)                   | 10.2%                         | 0.69 (0.40, 1.19)                   |
| 7-9                             | 277              | 25.9%                        | 0.90 (0.65, 1.24)                   | 15.5%                         | 1.05 (0.65, 1.69)                   |
| ≤6                              | 540              | 23.3%                        | 0.81 (0.60, 1.09)                   | 13.7%                         | 0.93 (0.59, 1.44)                   |
| **# of pregnancies:**           |                  |                              |                                     |                               |                                     |
| 0-1                             | 444              | 23.2%                        | 1.0                                 | 14.4%                         | 1.0                                 |
| 2                               | 500              | 23.2%                        | 1.00 (0.79, 1.26)                   | 12.4%                         | 0.86 (0.62, 1.19)                   |
| ≥3                              | 257              | 27.2%                        | 1.17 (0.90, 1.53)                   | 14.4%                         | 0.99 (0.69, 1.45)                   |
| **# of livebirths**             |                  |                              |                                     |                               |                                     |
| 0                               | 27               | 55.6%                        | 1.0                                 | 22.2%                         | 1.0                                 |
| 1                               | 604              | 24.5%                        | 0.44 (0.31, 0.64)                   | 15.7%                         | 0.71 (0.34, 1.47)                   |
| >1                              | 570              | 22.1%                        | 0.39 (0.27, 0.58)                   | 10.9%                         | 0.49 (0.23, 1.03)                   |
| **Contraceptive use at enrollment**: |              |                              |                                     |                               |                                     |
| NHC                             | 448              | 24.8%                        | 1.0                                 | 12.5%                         | 1.0                                 |
| DMPA                            | 347              | 19.9%                        | 0.80 (0.62, 1.05)                   | 11.8%                         | 0.95 (0.65, 1.38)                   |
| COC                             | 406              | 26.9%                        | 1.08 (0.86, 1.36)                   | 16.3%                         | 1.30 (0.94, 1.81)                   |
| **Cumulative use of COCs:**     |                  |                              |                                     |                               |                                     |
| Never                           | 194              | 21.1%                        | 1.0                                 | 12.4%                         | 1.0                                 |
| <4 years                        | 603              | 22.6%                        | 1.07 (0.78, 1.45)                   | 11.8%                         | 0.95 (0.62, 1.47)                   |
| 4-6 years                       | 310              | 23.6%                        | 1.11 (0.79, 1.56)                   | 13.6%                         | 1.09 (0.69, 1.75)                   |
| >6 years                        | 94               | 31.5%                        | 1.96 (1.37, 2.82)                   | 27.7%                         | 2.24 (1.36, 3.68)                   |
| **Cumulative use of DMPA:**     |                  |                              |                                     |                               |                                     |
| Never                           | 272              | 27.6%                        | 1.0                                 | 15.1%                         | 1.0                                 |
| <4 years                        | 558              | 25.3%                        | 0.92 (0.72, 1.16)                   | 13.9%                         | 0.93 (0.65, 1.32)                   |
| 4-6 years                       | 315              | 18.7%                        | 0.68 (0.50, 0.92)                   | 11.8%                         | 0.78 (0.52, 1.18)                   |
| >6 years                        | 56               | 25.0%                        | 0.91 (0.55, 1.48)                   | 12.5%                         | 0.83 (0.39, 1.75)                   |
| **Current Smoker:**             |                  |                              |                                     |                               |                                     |
| No                              | 1146             | 22.5%                        | 1.0                                 | 12.5%                         | 1.0                                 |
| Yes                             | 55               | 56.4%                        | 2.50 (1.94, 3.23)                   | 36.4%                         | 2.91 (1.99, 4.27)                   |
| **Age of sexual debut, years**  |                  |                              |                                     |                               |                                     |
| >20                             | 459              | 20.3%                        | 1.0                                 | 10.2%                         | 1.0                                 |
including (a) enhancement of cervical ectopy leading to enhanced acquisition of HPV; (b) modulation of host immune response by sex steroid hormones facilitating HPV persistence and development of cervical pre-cancer; (c) facilitating progression of an already established pre-cancerous lesion to invasive disease [30]. Additional studies in populations from other geographic regions are needed to clarify the epidemiologic association between COC use and HPV to strengthen the mechanistic hypothesis.

We observed higher prevalence of HPV infection among women diagnosed with either a current or prior history of bacterial vaginosis or Chlamydia Trachomatis. These associations remain significant even after adjustment for sexual behavior. Bacterial vaginosis is characterized as an alteration of the vaginal microflora that can result in inflammation and significant morbidity. Bacterial vaginosis has been suggested to increase the risk of HPV acquisition, presumably through disruption of non-specific physical immune barriers by alteration of vaginal pH [31]. A recent prospective study of over 9000 women from Costa Rica has shown that increases in vaginal pH were positively associated with HPV infection [32]. Chlamydia infection is associated with complications such as pelvic inflammatory disease, cervicitis, ectopic pregnancy and infertility. Chlamydia infection is associated with increasing the risk of HPV persistence, presumably through disruption of the mucosal immune response [33]. Although we cannot determine the temporal relationship of these genital tract infections on detection of HPV DNA in this study, our findings agree with other large cohort studies in where there was a similar detailed collection

| Table 2 Univariate association of demographic information and reproductive history with prevalent infection of any HPV and any HR-HPV type (Continued) |
|---|
| 17-19 | 550 | 23.6% | 1.17 (0.92, 1.48) | 13.6% | 1.33 (0.94, 1.88) |
| <17 | 192 | 34.4% | 1.69 (1.29, 2.22) | 21.4% | 2.09 (1.42, 3.06) |
| Lifetime # of sex partners: | | | | | |
| 1 | 852 | 17.4% | 1.0 | 9.7% | 1.0 |
| 2 | 179 | 31.8% | 1.83 (1.41, 2.38) | 13.9% | 1.43 (0.94, 2.18) |
| 3 | 61 | 32.8% | 1.89 (1.28, 2.78) | 18.0% | 1.85 (1.04, 3.28) |
| ≥4 | 109 | 58.7% | 3.38 (2.73, 4.19) | 40.4% | 4.14 (3.05, 5.63) |
| # Partners L6M**: | | | | | |
| 0 | 29 | 10.3% | 1.0 | 3.5% | 1.0 |
| 1 | 1,133 | 22.8% | 2.20 (0.75, 6.46) | 12.4% | 3.58 (0.52, 24.8) |
| >1 | 39 | 71.8% | 6.94 (2.33, 20.6) | 56.4% | 16.4 (2.34, 114.6) |
| New partner L12M***: | | | | | |
| No | 1128 | 22.7% | 1.0 | 12.5% | 1.0 |
| Yes | 44 | 68.2% | 3.00 (2.39, 3.78) | 47.7% | 3.82 (2.70, 5.39) |
| Commercial Sex Work L6M: | | | | | |
| No | 1167 | 22.7% | 1.0 | 12.3% | 1.0 |
| Yes | 34 | 70.6% | 3.11 (2.44, 3.96) | 55.9% | 4.53 (3.24, 6.34) |
| Condom use L6M: | | | | | |
| No | 977 | 20.9% | 1.0 | 11.6% | 1.0 |
| Yes | 224 | 37.5% | 1.79 (1.45, 2.20) | 22.3% | 1.93 (1.43, 2.60) |
| Primary partner L6M: | | | | | |
| Yes | 1164 | 23.9% | 1.0 | 13.5% | 1.0 |
| No | 8 | 87.5% | 3.65 (2.76, 4.84) | 62.5% | 4.63 (2.66, 8.08) |
| Primary partner had sex with other† L6M****: | | | | | |
| No | 902 | 20.6% | 1.0 | 11.2% | 1.0 |
| Yes | 76 | 48.7% | 2.36 (1.81, 3.07) | 30.3% | 2.70 (1.83, 3.98) |
| Don’t Know | 186 | 30.1% | 1.46 (1.13, 1.88) | 17.1% | 1.58 (1.11, 2.27) |

*nhc = Non-Hormonal Contraception; DMPA = DepotMedroxyprogesterone Acetate; COC = Combined oral contraception **L6M = Last six months prior to enrollment; L12M = Last twelve months prior to enrollment ***Among those who report ≥1 sexual partner L6M ****Among those who report a primary partner L6M †Includes commercial and non-commercial sexual partners.
of sexual risk behavior and/or longitudinal analyses were conducted.

This study has several strengths. First, the use of highly sensitive and specific laboratory assays for HPV detection and genotyping allows for a higher degree of internal validity and better assessment of the outcome measures. The use of histological and cytological methods for diagnosis of CIN helps minimize potential overreporting of disease endpoints. Second, given the study cohort was derived from a study assessing the effects of hormonal contraceptive use on HIV acquisition, detailed information regarding reproductive factors such

### Table 3 Univariate association of cytological diagnosis, clinical and STI history with prevalent infection with any HPV and any HR-HPV type

| Variable                      | Sample N = 1201 | HPV + ve N = 289 (24.1%) | HR-HPV + ve N = 163 (13.6%) |
|-------------------------------|-----------------|---------------------------|-----------------------------|
| Pap smear diagnosis:          |                 |                           |                             |
| Normal                        | 1077            | 20.3%                     | 10.3%                       |
| Inflammation                  | 16              | 43.8%                     | 25.0%                       |
| AS-CUS                        | 52              | 36.5%                     | 28.9%                       |
| LSIL                          | 42              | 80.9%                     | 57.1%                       |
| HSIL                          | 14              | 71.4%                     | 64.3%                       |
| Genital ulcer ever:           |                 |                           |                             |
| No                            | 1027            | 22.0%                     | 12.5%                       |
| Yes                           | 174             | 36.2%                     | 20.1%                       |
| Genital warts ever:           |                 |                           |                             |
| No                            | 1168            | 23.5%                     | 13.4%                       |
| Yes                           | 33              | 42.2%                     | 18.2%                       |
| PID ever:                     |                 |                           |                             |
| No                            | 1151            | 23.6%                     | 13.5%                       |
| Yes                           | 50              | 34.0%                     | 16.0%                       |
| Ever Gonnorhea:               |                 |                           |                             |
| No                            | 1162            | 23.2%                     | 12.6%                       |
| Yes                           | 39              | 48.7%                     | 43.6%                       |
| Ever Chlamydia:               |                 |                           |                             |
| No                            | 1044            | 21.7%                     | 11.3%                       |
| Yes                           | 157             | 40.1%                     | 28.7%                       |
| Ever Syphilis:                |                 |                           |                             |
| No                            | 1183            | 24.0%                     | 13.5%                       |
| Yes                           | 18              | 27.8%                     | 16.7%                       |
| Current gonnorhea:            |                 |                           |                             |
| No                            | 1199            | 24.0%                     | 13.5%                       |
| Yes                           | 2               | 50.0%                     | 50.0%                       |
| Current Chlamydia:            |                 |                           |                             |
| No                            | 1186            | 23.6%                     | 13.1%                       |
| Yes                           | 15              | 60.0%                     | 53.3%                       |
| Ever Bacterial Vaginosis:     |                 |                           |                             |
| No                            | 980             | 21.5%                     | 11.9%                       |
| Yes                           | 221             | 35.3%                     | 20.8%                       |
| Current Bacterial Vaginoses:  |                 |                           |                             |
| No                            | 1142            | 22.8%                     | 12.7%                       |
| Yes                           | 59              | 49.2%                     | 30.5%                       |

Marks et al. BMC Infectious Diseases (2015) 15:159
as contraceptive usage history as well as sexual behavior and other risk factors were collected which allowed for a thorough investigation of these exposures on the risk of HPV prevalence.

Limitations of the study include the cross-sectional design which limits the ability to ascribe a temporal relationship between the factors examined and HPV outcomes. Prospective studies are therefore needed to address the association of these factors on endpoints of HPV such as acquisition and persistence. Additionally, it is important to note that sexual behavior information, in particular male partner behavior, was based on participant self-report and therefore may underestimate the prevalence of certain risky sexual behaviors that may increase the association with HPV infection. Finally, the generalizability of the study’s findings may be limited. The reported prevalence of any HPV and any HR-HPV in this study is about 4-times higher than previously reported population-based prevalence survey’s in Thailand using similar HPV detection and genotyping assay’s with similar levels of sensitivity [5,13]. The current study sampled women between the ages of 20–37 years from varied geographic settings in Thailand who were family-planning clinic attendees. These women may, therefore be at elevated risk of HPV exposure and infection relative to the general population. However, the similarity of HPV type distribution among both diseased and non-diseased women, particularly with respect to HPV 52, in this population as compared to previous studies lends strength to the relevance and generalizability of these findings in the broader context of the epidemiology of HPV in Thailand.

The current study demonstrates a uniquely high prevalence of HR-HPV types such as HPV 52 among women with cervical pre-cancer in Thailand. These data provide descriptive information for development and

Table 4 Multivariate association of factors with prevalent infection of any HPV and any HR-HPV

| Variable                        | Adjusted PR (95% CI)** |
|---------------------------------|------------------------|
|                                 | Any HPV | Any HR-HPV | HPV 52 | HPV 52 (w/o HPV 16) |
| Age at enrollment:              |          |           |        |                    |
| <26                             | 1.0      | 1.0       | 1.0    | 1.0                |
| 26-30                           | 0.89 (0.68, 1.16) | 0.73 (0.49, 1.06) | 0.99 (0.46, 2.11) | 0.99 (0.46, 2.11) |
| 31-33                           | 1.02 (0.77, 1.37) | 0.72 (0.47, 1.09) | 0.51 (0.19, 1.35) | 0.51 (0.19, 1.35) |
| 34-38                           | 0.95 (0.70, 1.29) | 0.73 (0.48, 1.13) | 0.35 (0.12, 1.03) | 0.35 (0.12, 1.03) |
| Cumulative use of COCs*:        |          |           |        |                    |
| Never                           | 1.0      | 1.0       | 1.0    | 1.0                |
| <4 years                        | 1.17 (0.87, 1.58) | 1.06 (0.71, 1.59) | 0.50 (0.22, 1.10) | 0.49 (0.22, 1.10) |
| 4-6 years                       | 1.21 (0.87, 1.69) | 1.24 (0.79, 1.94) | 1.03 (0.46, 2.32) | 1.03 (0.46, 2.33) |
| >6 years                        | 2.03 (1.39, 2.96) | 2.47 (1.45, 4.19) | 1.16 (0.39, 3.45) | 1.16 (0.39, 3.45) |
| # lifetime partners             |          |           |        |                    |
| 1                               | 1.0      | 1.0       | 1.0    | 1.0                |
| 2                               | 1.66 (1.29, 2.14) | 1.21 (0.80, 1.82) | 1.05 (0.43, 2.57) | 1.05 (0.43, 2.57) |
| 3                               | 1.54 (1.04, 2.26) | 1.25 (0.69, 2.28) | 1.99 (0.67, 5.89) | 1.99 (0.67, 5.89) |
| ≥4                              | 2.02 (1.54, 2.66) | 1.98 (1.35, 2.91) | 1.97 (0.87, 4.45) | 1.97 (0.87, 4.45) |
| Primary partner had sex w/others:|          |           |        |                    |
| No                              | 1.0      | 1.0       | 1.0    | 1.0                |
| Yes                             | 1.42 (1.05, 1.91) | 1.35 (0.89, 2.04) | 2.32 (0.98, 5.50) | 2.32 (0.98, 5.50) |
| Don’t Know                       | 1.29 (1.02, 1.64) | 1.34 (0.94, 1.91) | 1.48 (0.69, 3.15) | 1.48 (0.69, 3.15) |
| Chlamydia infection ever:       |          |           |        |                    |
| No                              | 1.0      | 1.0       | 1.0    | 1.0                |
| Yes                             | 1.32 (1.04, 1.66) | 1.74 (1.27, 2.38) | 1.25 (0.54, 2.92) | 1.25 (0.54, 2.92) |
| Bacterial Vaginosis at enrollment:|      |           |        |                    |
| No                              | 1.0      | 1.0       | 1.0    | 1.0                |
| Yes                             | 2.11 (1.54, 2.91) | 1.98 (1.28, 3.06) | 2.28 (0.96, 5.39) | 2.28 (0.96, 5.39) |

*COC = Combined oral contraception.

**All variables mutually adjusted for in final model.
Additional file 1: Table S1. Multivariate association of factors with prevalent infection of any HPV and any HR-HPV by Pap Smear Status.

Abbreviations
HPV: Human Papillomavirus; HR-HPV: High Risk Human Papillomavirus; COC: Combined oral contraception; DMPA/DepoPrevea: Depomedroxyprogesterone Acetate; GEE: generalized estimating equation.

Competing interests
KLL, MM, SG – Employee of Merck, Sharp and Dohme, which manufactures the quadrivalent HPV vaccine. Owns Merck stocks & options.

Authors’ contributions
MM participated in the acquisition of the HPV genotype data and analyzed data and authored manuscript. SG and KLL participated in the conception, design and conduct of the study as well as reviewed and commented on the manuscript. AT participated in the design and conduct of the study and acquisition of the data. EK participated in the acquisition of the HPV genotype data as well as reviewed and commented on the manuscript. CP, WW, PY, CV, SS participated in the conduct of the study as well as reviewed and commented on the manuscript. SR participated in the conduct of the study. PEG participated in the acquisition of the HPV genotype data as well as assisted with the analysis and reviewed/commented on the manuscript. DDC participated in the conception, design and conduct of the study as well as reviewed and commented on the manuscript. All authors read and approved the final manuscript.

Acknowledgements
We thank Dr. Christine Velicer who provided critical review of this manuscript. Dr. Morgan A. Marks, Dr. Swati Gupta, and Dr. Kai-Li Liaw are all current employees of Merck and Co. Inc which provided funding to conduct the design and collection of the data presented in this manuscript.

Financial support
This work was funded in part by the NIAID pre-doctoral training fellowship in sexually transmitted infections (ST32-20050056-09). This study was funded by Merck & Co. Inc. This data was previously presented as an oral talk entitled "Human papillomavirus genotype and proportion of CIN 2/3 attributable to high-risk HPV type in a large cohort of Thai women" at the 24th International Papillomavirus Conference and Clinical Workshop, Beijing, China (2007).

Author details
1Merck Research Laboratories, West Point, PA, USA. 2PRA Health Sciences, Fort Washington, PA, USA. 3Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA. 4Chiang Mai University, Chiang Mai, Thailand. 5Prince of Songkla University, Songkla, Thailand. 6Khon Kaen University, Khon Kaen, Thailand. 7Research Institute for Health Sciences, Chiang Mai, Thailand.

Received: 11 December 2014 Accepted: 12 March 2015
Published online: 27 March 2015

References
1. Vaccarella S, Lortet-Tieulent J, Plummer M, Franceschi S, Bray F. Worldwide trends in cervical cancer incidence: impact of screening against changes in disease risk factors. Eur J Cancer. 2013;49(15):3262–73.
2. Walboomers JM, Jacobs MV, Monos MM, Bosch FX, Kummer JA, Shah KV, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189(1):12–9.
3. Munoz N, Castellsague X, de Gonzalez AB, Gissmann L. Chapter 1: HPV in the etiology of human cancer. Vaccine. 2006;24:531-510.
4. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer. 2003;88(1):163–73.
5. Sukvirach S, Smith JS, Tursakul S, Munoz N, Kesaranar V, Opaosotan O, et al. Population-based human papillomavirus prevalence in Lampang and Songkla, Thailand. J Infect Dis. 2003;187(8):1246–56.
6. Morrison CS, Richardson BA, Mmiro F, Chipato T, Celentano DD, Luoto J, et al. Hormonal contraception and the risk of HIV acquisition. AIDS. 2007;21(1):85–95.
7. Solomon D, Davey D, Kurman R, Moriaty A, O’Connor D, Prey M, et al. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA. 2002;287(16):2114–9.
8. Gaviit P, Peyton CL, Alessi TQ, Wheeler CM, Couteel F, Hildesheim A, et al. Improved amplification of genital human papillomaviruses. J Clin Microbiol. 2000;38(1):357–61.
9. Couteel F, Gaviit P, Komagay J, Hankins C, Richardson H, Lapointe N, et al. Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. J Clin Microbiol. 2002;40(3):9102–7.
10. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med. 2003;348(3):518–27.
11. Thompson ML, Myers JE, Kriebel D. Prevalence odds ratio or prevalence ratio in the analysis of cross sectional data: what is to be done? Occup Environ Med. 1998;55(4):272–7.
12. Chanaamong J, Jinyangdikul P, Chinchai T, Swangvaree S, Karalak A, Gemma N, et al. Large scale study of HPV genotypes in cervical cancer and different cytological cervical specimens in Thailand. J Med Virol. 2014;86(4):601–7.
13. Wongwimonrat K, Keawwicheat W, Sanruen B, Dogru S, Ruangsuttikarn C, Sripisophon S, et al. Detection of human papillomavirus from self-collected vaginal samples of women in Chiang Mai, Thailand. Sex Transm Dis. 2008;35(2):172–3.
14. Clifford GM, Gallus S, Herrero R, Munoz N, Snijders Pj, Vaccarella S, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. Lancet. 2005;366(9490):991–8.
15. Dunne EF, Unger ER, Sternberg M, McLiann G, Swan DC, Patel SS, et al. Prevalence of HPV infection among females in the United States. JAMA. 2007;297(8):813–9.
16. Guan P, Howell-Jones R, Li N, Bruni L, de Sanjose S, Franceschi S, et al. Human papillomavirus types in 115,789 HPV-positive women: a meta-analysis from cervical infection to cancer. Int J Cancer. 2012;131(10):2349–59.

17. Moreno V, Bosch FX, Munoz N, Meijer CJ, Shah KV, Walboomers JM, et al. Effect of oral contraceptives on risk of cervical cancer in women with human papillomavirus infection: the IARC multicentric case-control study. Lancet. 2002;359(9211):1085–92.

18. Castellsague X, de Sanjose S, Aguado T, Louie KS, Bruni L, Munoz J, et al. HPV and Cervical Cancer in the World 2007 report. Vaccine. 2007;25(Supplement 3):C198.

19. Kjaer SK, van den Brule AJ, Bock JE, Poll PA, Engholm G, Sherman ME, et al. Determinants for genital human papillomavirus (HPV) infection in 1000 randomly chosen young Danish women with normal Pap smear: are there different risk profiles for oncogenic and nononcogenic HPV types? Cancer Epidemiol Biomarkers Prev. 1997;6(10):799–805.

20. Ley C, Bauer HM, Reingold A, Schiffman MH, Chambers JC, Tashiro CJ, et al. Determinants of genital human papillomavirus infection in young women. J Natl Cancer Inst. 1991;83(14):1085–92.

21. Marks M, Gavett PE, Gupta SB, Liaw KL, Kim E, Tadesse A, et al. The association of hormonal contraceptive use and HPV prevalence. Int J Cancer. 2011;128(12):2962–70.

22. Marks M, Gavett PE, Gupta SB, Liaw KL, Tadesse A, Kim E, et al. Combined oral contraceptive use increases HPV persistence but not new HPV detection in a cohort of women from Thailand. J Infect Dis. 2011;204(10):1505–13.

23. Winer RL, Lee SK, Hughes JP, Adam DE, Kiviat NB, Koutsy LA. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. Am J Epidemiol. 2003;157(3):218–26.

24. Moscicki AB, Hills N, Shiboski S, Powell K, Hanson E, et al. Risks for incident human papillomavirus infection and low-grade squamous intraepithelial lesion development in young females. JAMA. 2001;285(25):2995–3002.

25. Sycuro U, Xi LF, Hughes JP, FengQ, Winer RL, Lee SK, et al. Persistence of genital human papillomavirus infection in a long-term follow-up study of female university students. J Infect Dis. 2008;198(7):971–8.

26. Munoz N, Hernandez-Suarez G, Mendez F, Molano M, Posso H, Moreno V, et al. Persistence of HPV infection and risk of high-grade cervical intraepithelial neoplasia in a cohort of Colombian women. Br J Cancer. 2009;100(7):1184–90.

27. Castle PE, Wacholder S, Lorincz AT, Scott DR, Sherman ME, Glass AG, et al. A prospective study of high-grade cervical neoplasia risk among human papillomavirus-infected women. J Natl Cancer Inst. 2002;94(18):1406–14.

28. Deacon JM, Evans CD, Yule R, Desai M, Binns W, Taylor C, et al. Sexual behaviour and smoking as determinants of cervical HPV infection and of CIN3 among those infected: a case–control study nested within the Manchester cohort. Br J Cancer. 2000;83(11):1565–72.

29. Marks M, Klein S, Gavett P. Hormonal contraception and HPV: a tale of differing and overlapping mechanisms. Contraception. 2011;84(4):265–71.

30. Watts DH, Fazzari M, Minkoff H, Hillier SL, Sha B, Glesby M, et al. Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. J Infect Dis. 2005;191(7):1129–39.

31. Clarke MA, Rodriguez AC, Gage JC, Herrero R, Hildesheim A, Wacholder S, et al. A large, population-based study of age-related associations between vaginal pH and human papillomavirus infection. BMC Infect Dis. 2012;12:53.

32. Samoff E, Koumans EH, Markowitz LE, Sterneberg M, Sawyer MK, et al. Association of Chlamydia trachomatis with persistence of high-risk types of human papillomavirus in a cohort of female adolescents. Am J Epidemiol. 2005;162(7):668–75.