Route of Sexual Exposure Is Independently Associated With Seropositivity to HPV-16 and HPV-18 Among Clients of an STI Clinic in the Netherlands

Maries Heiligenberg,1,2,* Catharina J. Alberts,1,2,* Tim Waterboer,3 Arjan G. C. L. Speksnijder,1 Henry J. C. De Vries,1,4,5 Michael Pawlita,3 and Maarten F. Schim van der Loeff1,2

1Department of Infectious Diseases, Public Health Service of Amsterdam, 2Department of Internal Medicine, Division of Infectious Diseases, Tropical Medicine and AIDS, Center for Infection and Immunity Amsterdam, Academic Medical Center, Amsterdam, The Netherlands; 3Infection and Cancer Program, German Cancer Research Center, Heidelberg, Germany; 4Centre for Infectious Diseases Control, National Institute for Public Health and the Environment, Bilthoven, and 5Department of Dermatology, Academic Medical Center, University of Amsterdam, The Netherlands

We investigated the route of sexual exposure as a determinant for human papillomavirus (HPV)-16 and HPV-18 seropositivity. At the Amsterdam sexually transmitted infections clinic we recruited 4 risk groups: (1) men who have sex with women only (MSW; n = 751); (2) women who have sex with men (WSM; n = 749); (3) men who have sex with men (MSM) reporting insertive anal sex only (insMSM; n = 156); and (4) MSM reporting receptive anal sex (recMSM; n = 415). In multivariable analyses, HPV-16 seropositivity was significantly more common in WSM vs MSW, recMSM vs MSW, and recMSM vs insMSM. HPV-18 results were similar. Route of sexual exposure is independently associated with HPV seropositivity.

**Keywords.** HPV; seroprevalence; HPV antibodies; route of sexual exposure; STI; men who have sex with men (MSM); the Netherlands.

Human papillomavirus (HPV) is a highly infectious sexually transmissible virus that can cause cancer at various anatomical sites (eg, cervix, vagina, anus, penis, or oral cavity), with HPV-16 and HPV-18 being the most prominent types.

Only a portion of people with an HPV infection will develop antibodies against HPV. About 20% of men develop antibodies against a prevalent HPV-16 infection within 24 months [1], and >90% of women within 13 months [2], resulting in higher HPV seroprevalences among women than men [3–5]. The limited data available show higher seroprevalence among men who have sex with men (MSM) than among heterosexual men [6,7]. It is not clear which factors influence seroconversion after HPV infection. It is likely that the route of transmission and therefore the site of infection (oral, anal, vaginal/cervical, or penile) as well as the duration of infection affect the probability of seroconversion. HPV infection of mucosal epithelium (eg, of the cervix or anus) may be more likely to induce a humoral immune response than HPV infection at keratinized epithelium (eg, penis) [3,6,8]. A possible explanation for the higher seroprevalence among MSM is the higher number of sexual partners. Various studies have shown that HPV seroprevalence is higher in human immunodeficiency deficiency (HIV)-infected individuals [8].

In this study we investigated whether the route of exposure (penile insertive intercourse, receptive vaginal intercourse, or receptive anal intercourse) is a determinant of seropositivity of HPV-16 and HPV-18. We did this by comparing seroprevalence in 4 risk groups (all HIV-negative) recruited from the sexually transmitted infections (STI) clinic in Amsterdam, the Netherlands: (1) MSW, (2) women who have sex with men (WSM), (3) MSM reporting to have had only insertive (but not receptive) anal intercourse (insMSM), and (4) MSM reporting receptive anal sex (recMSM).

**MATERIALS AND METHODS**

The STI clinic of the Public Health Service of Amsterdam offers free and anonymous STI testing and treatment. Since 1991 an anonymous survey among STI clinic clients has been conducted biannually [9]. During each survey period, all clinic attendees were invited to participate, totaling approximately 1000 attendees per period. The inclusion criteria were being age ≥16 years and being conversant in Dutch or English. During the routine consultation, a nurse explained the study and invited the client to participate. Written informed consent was obtained. Sociodemographic and sexual behavior characteristics were obtained via questions asked during the routine consultation by a nurse (education, history of sex work, being client of sex worker, and being transgender) and via questions asked by a
trained interviewer using a standardized questionnaire (country of birth of client and parents, number of recent and lifetime sexual partners, age of sexual debut, condom use, and type of sex [eg, oral, penile, vaginal, anal]). Ethnicity was defined according to Statistics Netherlands (Centraal Bureau voor de Statistiek, CBS) [10]. The medical ethics committee of the Academic Medical Center (Amsterdam) approved the study. Clients were tested for gonorrhea, chlamydia, and syphilis according to the clinic’s protocol. All clients (unless they actively refused) were tested for HIV [11].

Sera for HPV antibody testing were centrifuged within 4 hours, stored at −20°C within 48 hours, and analyzed with the multiplex HPV assay as described previously [12]. Specific reactivity of L1 antibodies (median fluorescence intensity [MFI]) for a given HPV type was calculated as the difference between the MFI of antigen-loaded beads and the background MFI of beads without antigen. Serology cutoffs were estimated from the reactivities of 125 virgins’ sera (a cohort of young Korean women who were HPV DNA negative and self-reported to never have had sex) as the mean plus 3 standard deviations (excluding outliers) [13] and set to 400 MFI for both HPV types.

For this analysis, participants of the 2008 and 2009 study rounds were eligible. All MSM participating in those rounds (n = 927) were selected, and 800 MSW and 800 women were selected at random. HIV-infected individuals were excluded from this analysis, as were women who had sex with women only, those reporting never having had sex or not having had sex during the past 6 months, those reporting inconsistent sexual behavior, transgender people, and MSM not reporting the role taken during sex (insertive/receptive). See Supplementary Figure 1 for a flow chart that shows exclusion of participants.

Based on gender and sexual behavior (ie, gender of sexual contact and, for MSM, the role taken in sex) in the preceding 6 months, individuals were classified into 4 sexual risk groups: (1) MSW (n = 751), (2) WSM (n = 749), (3) insMSM (n = 156), and (4) recMSM (n = 415). The Pearson $\chi^2$ test was used to compare differences in demographic and sexual behavior characteristics between these risk groups.

HPV seropositivity was the main outcome. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated using bivariate and multivariable logistic regression analyses. HPV seroprevalences were compared between risk groups in 2 ways. The first approach compared all risk groups in 1 model, with MSW as the reference category. In the second approach, risk groups were compared head-to-head. In order to control for the confounding effects of age and sexual behavior, we adjusted all models for the following variables: (1) age in years, (2) age of sexual debut, and (3) number of lifetime sexual partners (LSPs). These variables were categorized into 4 categories based on quartiles. All multivariable models included this same set of a priori selected variables.

Statistical tests were 2-sided and performed using Stata version 11.2 (Stata Intercooled, College Station, TX).

RESULTS

Table 1 summarizes the demographic characteristics, reported sexual behavior, and STIs diagnosed at the time of clinic visit, by risk group. InsMSM and recMSM were older and WSM younger (P < .001). MSW and WSM were more often from Surinamese, Dutch Antillean, or Aruban origin than the 2 MSM groups. The majority of the study population was highly educated: 73% had at least higher general secondary education.

There were significant differences in sexual behavior between the 4 risk groups. Although the groups had similar median ages at first sexual intercourse, the distribution of these ages differed significantly (P < .001). Men who have sex with women only, WSM, insMSM, and recMSM reported a median number of LSPs of 15, 8, 80, and 80, respectively. Reported condom use during vaginal or anal sex in the preceding 6 months differed significantly among the 4 groups; insMSM reported most often to always use a condom (53%). Very few participants (1%) reported to be a sex worker; 11% of the MSW reported to have been clients of sex workers in the preceding 6 months. *Chlamydia trachomatis* (14%: 58/415) and gonorrhea (12%: 51/415) were most common in recMSM; newly diagnosed syphilis was absent in MSW and WSM and present in insMSM (3%: 4/156) and recMSM (1%: 6/415).

**HPV Seroprevalence**

The seroprevalence for HPV-16 and HPV-18 was 8% (60/751) and 5% (39/751) in MSW, 21% (158/749) and 13% (101/749) in WSM, 19% (30/156) and 16% (25/156) in insMSM, and 31% (130/415) and 28% (115/415) in recMSM, respectively. Supplementary Figure 2 shows the HPV antibody level vs the proportion of HPV-positive participants.

**HPV-16 Seropositivity by Risk Group**

The associations between risk group and HPV-16 seropositivity are shown in Table 2. Model 1 presents the analysis with all risk groups, and models 2–7 compare the risk groups head-to-head. Head-to-head comparisons allow for better adjustment because of differences in sexual behavior between groups. Bivariate ORs depict associations without adjusting for confounders; adjusted ORs (aORs) show associations after adjusting for age, age at sexual debut, and number of LSPs.

Model 1 shows that HPV-16 seropositivity was strongly associated with risk group, with the highest aOR for WSM compared with the reference group of MSW. Model 2 shows that the odds for HPV-16 seropositivity were significantly higher for WSM (aOR 4.50; 95% CI, 3.12–6.49) compared with MSW. Model 3 shows that insMSM had a nonsignificantly increased risk for being HPV-16 seropositive compared with MSW (aOR 1.59; 95% CI, 0.90–2.82), whereas model 4 shows that recMSM had a higher risk compared with MSW (aOR 4.00; 95% CI,
Model 5 shows that insMSM had a lower risk for being HPV-16 seropositive compared with WSM (aOR 0.41; 95% CI, .23–.74), whereas model 6 shows that recMSM had a similar risk as WSM (aOR 1.03; 95% CI, .69–1.54). Finally, model 7 shows that recMSM were significantly more likely to be HPV-16 seropositive than insMSM (aOR 1.89; 95% CI, 1.20–2.97).
DISCUSSION

To our knowledge, this is the first study to compare HPV-16 and HPV-18 seroprevalence among MSW, WSM, and MSM from the same source population. In a model adjusted for relevant sexual behavior factors, we observed that the route of sexual exposure was associated with HPV-16 and HPV-18 seropositivity. MSM who practiced receptive anal sex and WSM had similar risks of being HPV-16 and HPV-18 seropositive, while MSM who practiced insertive anal sex only and MSW had similar risks of HPV-16 seropositivity.

The large sample size enabled stratified multivariable analyses and comparisons between various sexual risk groups. This study provides estimates of natural exposure, since the study was conducted before the introduction of HPV vaccination in the Netherlands in 2009. MSM were divided in risk groups (receptive or insertive-only) on the basis of their sexual behavior in the preceding 6 months; this may have lead to a dilution of the effect when comparing these 2 groups because of discrepancies between lifetime and recent behavior. Because the study was conducted among clients of an STI clinic, the results may not apply to the general population. Care should be taken when comparing our results with previous studies as different serological assays may yield different results.

Several studies [3, 6, 8] suggest that HPV infection in mucosal epithelium (eg, of the cervix or anus) is more likely to induce a humoral immune response than HPV infection of keratinized epithelium, resulting in higher HPV seroconversion rates in groups with sexual exposure to mucosal epithelium. Our findings of similar risk of HPV-16 and HPV-18 seropositivity in recMSM and WSM (model 6) and of a higher risk of HPV-16 and HPV-18 seropositivity in WSM compared with MSW (model 2) are compatible with this hypothesis. Also the observation that the seroconversion of HPV-16 was similar between insMSM and MSW (model 3) supports this hypothesis. In contrast, we found that the risk of HPV-18 seropositivity was higher in insMSM than in MSW (model 3). However, since information about anal sex practices was limited to the preceding 6 months, several MSM who practiced receptive anal sex longer ago may have been classified as insMSM.

In conclusion, seroprevalence of HPV-16 and HPV-18 was high among visitors to the STI clinic but varied strongly by route of sexual exposure: lower seroprevalence among those practicing insertive intercourse only, and higher seroprevalence in those who practiced receptive (vaginal or anal) intercourse. This appears to confirm the hypothesis that exposure of HPV-16 or HPV-18 to keratinized epithelium leads less easily to seroconversion than exposure to mucosal epithelium.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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Table 2. Association Between Risk Group and Human Papillomavirus-16 Seropositivity in 2071 Clients of the Sexually Transmitted Infection Clinic in Amsterdam (2008–2009)

<table>
<thead>
<tr>
<th>Model</th>
<th>Risk group</th>
<th>ORs 95% CI</th>
<th>aORs 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>MSW</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>WSM</td>
<td>3.08 (2.24–4.23)</td>
<td>4.20 (2.94–5.99)</td>
</tr>
<tr>
<td></td>
<td>insMSM</td>
<td>2.74 (1.70–4.42)</td>
<td>1.98 (1.19–3.29)</td>
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<tr>
<td></td>
<td>recMSM</td>
<td>5.25 (3.76–7.35)</td>
<td>3.80 (2.60–5.55)</td>
</tr>
<tr>
<td>Model 2</td>
<td>MSW</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>WSM</td>
<td>3.08 (2.24–4.23)</td>
<td>4.50 (3.12–6.49)</td>
</tr>
<tr>
<td>Model 3</td>
<td>MSW</td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>insMSM</td>
<td>2.74 (1.70–4.42)</td>
<td>1.59 (.90–2.82)</td>
</tr>
<tr>
<td>Model 4</td>
<td>MSW</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>recMSM</td>
<td>5.25 (3.76–7.35)</td>
<td>4.00 (2.68–5.97)</td>
</tr>
<tr>
<td>Model 5</td>
<td>WSM</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>insMSM</td>
<td>0.89 (.58–1.38)</td>
<td>0.41 (.23–.74)</td>
</tr>
<tr>
<td>Model 6</td>
<td>WSM</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>recMSM</td>
<td>1.71 (1.30–2.24)</td>
<td>1.03 (.69–1.54)</td>
</tr>
<tr>
<td>Model 7</td>
<td>insMSM</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>recMSM</td>
<td>1.92 (1.22–3.00)</td>
<td>1.89 (1.20–2.97)</td>
</tr>
</tbody>
</table>

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; insMSM, MSM reporting to have had only insertive (but not receptive) anal intercourse; MSM, men who have sex with men; MSW, men who have sex with women only; OR, odds ratio; recMSM, MSM reporting receptive anal sex; WSM, women who have sex with men.

* Multivariable model are adjusted for: age in years (categorical), lifetime number of sexual partners (categorical), and age of sexual debut in years (categorical).
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