TITLE: Prevalence, Incidence, Clearance and Associated Factors of Genital Human papillomavirus Infection among Men: a population-based cohort study in rural China

Authors and affiliations

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Running title Natural history of genital Human papillomavirus infection among Chinese men

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Conflict of interests

All authors declare that they have no potential conflict of interests.

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ABSTRACT

Background The natural history of Human papillomavirus (HPV) infection in men on a population base has rarely been studied in general, particularly among Chinese men.

Methods 1286 men aged 25-65 years from rural China were enrolled during 2009-2010 and their genital HPV infection status were assessed bi-annually for up to 7 visits using PCR and sequencing method. Prevalence analysis was performed among men with at least one valid HPV result (N=1279) and men with at least two consecutive HPV results (N=1059) were included in incidence and clearance analyses (median follow-up time: 31.8 months; interquartile range: 15.4-37.9 months).

Results The prevalence and incidence of any HPV type, oncogenic and non-oncogenic HPV were 17.8%, 6.4%, 12.4% and 4.9, 10.8/1000 person months, respectively. The median duration of infection with any HPV type, oncogenic and non-oncogenic HPV was 11.5, 6.8 and 11.5 months, respectively. The number of lifetime sexual partner was consistently associated with increased risk of prevalent and incident infection of HPV. Men aged 25-50 years had a higher incidence and longer duration of HPV infection than older men (51-65 years).

Conclusions and impact This epidemiological investigation provide basic information of genital HPV infection among Chinese male population, these data are crucial for the consideration of primary strategies against HPV-related carcinoma in Chinese male and female population.
INTRODUCTION

Human papillomavirus (HPV) infection has been demonstrated to be the necessary cause of cervical cancer in women, and this virus also plays an important role in anogenital and oropharyngeal cancers (1). In the past decade, genital HPV infection in men has also brought about growing concern, not only because it may lead to benign or malignant pathologic changes in the external male genitalia, but also because the fact that men may act as a viral reservoir and significantly impact HPV infection and HPV disease risk in women (2-5).

In order to rationally implement screening and immunologic intervention for HPV related diseases, the natural history of HPV infection must be carefully investigated. Natural history studies in women have played a crucial role in developing vaccine-based HPV intervention programs for women (6-12). However, our knowledge regarding HPV infection in men is relatively inadequate at this time. To date, most of published data is based on volunteers, university students or soldiers, sexual transmitted disease clinic patients, men who have sex with men, HIV infected men, or men from a relatively narrow age range (13-19). Thus such studies have limited representation of the general population. Moreover, most available data is from North America and Europe and little data is available for Asian male populations, particularly for China.

In the current study, we established a cohort of rural Chinese men on the basis of a large scale esophageal cancer cohort study (20), and followed up their genital HPV status for 7 bi-annual visits. The aim of this study is to investigate the type-specific prevalence, incidence and clearance of HPV infection in men from rural China, and to evaluate potential risk factors.

MATERIAL AND METHODS:

Study subjects

In 2007–2009, a population-based esophageal cancer cohort study was initiated in rural Anyang, China
The current study utilizes a sub-cohort of the original study consisting of 3 target villages and the baseline investigation was started concurrently with the 2nd section of the AECCS. The eligibility criteria for this study were as follows: permanent residence in the target villages; age 25-65 years; no history of cancer, cardiovascular disease or mental disorder; no history of infection with Hepatitis B Virus, Hepatitis C Virus, or Human Immunodeficiency Virus; and willingness to cooperate with follow up and ability to provide informed consent. All eligible male cohort members were enrolled during 2009-2010 and were assessed bi-annually for up to 7 visits.

Research protocols and materials were approved by the Institutional Review Board of the Peking University School of Oncology, China. All participants in this study provided written informed consent.

Specimen and data collection

At each evaluation, exfoliated cells from the penile shaft, glans penis, coronal sulcus, and scrotum of all subjects were collected by an experienced doctor using saline-soaked swabs as described previously (21). Cells adherent to the swab were then rinsed into 0.9% saline solution, and centrifuged at 5000 rpm for 5 min. Supernatants were then discarded. All specimens were stored at -20°C and subsequently transported to our laboratory in Beijing and stored in ultralow temperature freezers (-70°C) pending DNA extraction and HPV detection. A computer aided questionnaire was completed by all cohort members during the baseline investigation to obtain demographic data, personal information and potential risk factors for HPV infection.

Laboratory procedure

As described elsewhere (21), DNA was extracted using the Biomek 3000 automated workstation (Beckman Coulter, Brea, CA, USA) and then tested with Polymerase Chain Reaction (PCR) for the β-globin gene for quality evaluation. β-globin positive specimens were subsequently tested for HPV using PCR-based direct sequencing with a pair of SPF1/GP6+ primers which amplified a ~184bp fragment of L1 gene (22). The ABI
3730XL with bigdye 3.1 reagent (Applied Biosystems, USA) was used for the Sanger sequencing based genotyping procedure to evaluate the HPV types in this population, which was also used in previous studies (21, 23, 24). Samples with ambiguous HPV typing signals were subjected to further cloning and sequencing for multiple infections. The HPV types that were classified as oncogenic in this study were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (25, 26). The non-oncogenic types were 2, 3, 6, 7, 10, 11, 26, 27, 29, 30, 32, 37, 40, 42, 43, 44, 53, 54, 55, 57, 61, 62, 67, 69, 70, 72, 74, 75, 77, 81, 82, 84, 85, 87, 90, 91, and 94.

**Statistical analysis**

HPV prevalence was defined as the percentage of HPV positive visits out of all counted visits during the study period in participants with adequate HPV test results. Positive for any HPV type, oncogenic HPV or non-oncogenic HPV was defined as being positive for at least one of any type HPV, 13 oncogenic HPV and 38 non-oncogenic HPV, respectively. Prevalence estimates along with its 95% confidence intervals (CIs) were estimated using a null linear regression model implemented with the Generalized Estimating Equation (GEE) with a robust sandwich estimator of covariance to adjust for repeat measurements (27).

Participants were treated as the calculating unit in the incidence analysis and only participants who were negative for a specific type of HPV at enrollment were included. Incident infections of any HPV type, oncogenic and non-oncogenic HPV were defined as the first incident event of any type of HPV, 13 oncogenic HPV and 38 non-oncogenic HPV, respectively. Incident infection was assumed to occur at the midpoint of the interval prior to the first HPV positive visit. Person-time was calculated from enrollment to the first incident event and participants without HPV infection were censored at their last visits. Incidence rates were then calculated through dividing the number of transmission events by the total number of person months and the 95% CIs were estimated based on Poisson distribution (28). Kaplan-Meier method was used to estimate cumulative risk of incident infection of any HPV type, oncogenic HPV or non-oncogenic HPV and log-rank
test was used to compare incidence between groups.

Type specific infections were treated as the calculation unit in the clearance analysis. HPV clearance was defined as two sequential negative results and the clearance event was assumed to occur at the mid-interval following the last HPV positive visit. Clearance analysis was restricted in incident cases while newly acquired infections identified at a participant’s last visit were not included. Median time to clearance and 95% CIs were estimated for groups of HPV using the clustered Kaplan-Meier method (29) and the Wei Lin Weissfeld (WLW) marginal model approach was used to assess the statistical significance between HPV groups (30).

For the risk factor assessment, GEE logistic regression models, Cox regression models and WLW Cox regression models were used for HPV prevalence, incidence and clearance analysis, respectively. Backward-selection method with a significance threshold of 0.1 was used to identify variables included in final multivariate models. Candidate variables included age at enrollment, marital status, type of job, education level, current smoking status, lifetime number of female sexual partners, bathing frequency in the winter and washing external genitalia before sex. Age, education level and type of job were included in all multivariate models as design factors.

Single missing visits (12% of total visits) were imputed using “prior observation carried backward” approach. Statistical analyses were performed using STATA version 11.2 (STATA Corporation, College Station, TX, USA) and SAS version 9.2 (SAS Institute Inc., Cary, North Carolina, USA). Tests were two sided, and had a significance level of 0.05.

RESULTS

A total of 1286 participants participated in at least one visit of the study (Supplementary table 1). Human β-globin gene was positive in 94.4% (5439/5761 visits) of specimens form 1279 (99.5%) participants which
were therefore considered as adequate for HPV DNA evaluation.

As shown in Table 1, the median age of the 1279 β-globin positive participants was 43 years (interquartile range: 36-54 years). Most of these individuals were married and had an education level of junior middle school or below. Participant job categories were distributed so that one-third of the participants held each of 3 types of jobs. Regarding to behavior factors, 1120 of 1279 (87.6%) participants reported having 0-1 lifetime female sexual partner, 718 (56.1%) were current smokers, 630 (49.3%) reported having bath every 8-30 days in winter, and 990 (77.4%) reported not washing genitalia before sex. Of these 1279 men, 1059 (82.8%) completed at least two consecutive visits in the study and were included in incidence analysis with a median follow-up time of 31.8 months (interquartile range: 15.4- 37.9). The 220 men excluded from the incidence analysis were younger and more likely to work outside the local area, had received a higher level of education, and had better personal hygienic habits than the subjects included in incidence analysis (Table 1).

The overall prevalence of any HPV infection was 17.8% (1099/6182 visits), and 114 out of 1099 positive visits (10.4%) had multiple infections (Supplementary table 1). Prevalence of oncogenic and non-oncogenic HPV infection was 6.4% (393/6182 visits) and 12.4% (765/6182 visits). Oncogenic HPV types with the highest prevalence were HPV-16, 18, 58. Non-oncogenic HPV types with the highest prevalence were HPV-3, 57, 54 (Table 2). As compared with older men (51-65 years), younger men (25-50 years) had a significantly higher prevalence of any type, oncogenic and non-oncogenic HPV infection (data not shown).

The incidence of any, oncogenic and non-oncogenic HPV infection was 14.6, 4.9 and 10.8 per 1000 person months respectively (Table 2). Oncogenic HPV types with the highest incidence were HPV-16, 18, 58. Non-oncogenic HPV types with the highest incidence were HPV-3, 57, 54. Incidence of non-oncogenic HPV was significantly higher than oncogenic type HPV (Figure 1). Younger men were at significantly higher risk for incident HPV infection both for any and non-oncogenic HPV types (Figure 1).
Median time to clearance of any, oncogenic or non-oncogenic HPV infection was 11.5, 6.8 and 11.5 months (Table 2). 55.5%, 57.3% and 54.8% of any, oncogenic and non-oncogenic HPV infection were cleared 12 months after new acquisition of infection, and 78.5%, 84.0% and 74.8% were cleared at 24 months respectively. Half of HPV-16 and HPV-18 infections were cleared in ~6 months. Non-oncogenic HPV types appeared to persist longer than oncogenic HPV types, but this difference did not reach a significant level ($P=0.106$) (Figure 1). Younger men cleared infections significantly slower than older men in any and oncogenic HPV types (Figure 1).

In the multivariate analyses, number of lifetime female sexual partners was consistently associated with an increased risk of HPV infection. The risk of acquisition of both any and non-oncogenic HPV infection was significantly increased in current smokers. Men who bathed infrequently in winter had a reduced risk of HPV infections in prevalent infection analysis, and also had a reduced risk of incident oncogenic HPV infection. Older age (51-65 years) was associated with a significantly lower risk of incident non-oncogenic HPV infection and shorter duration of oncogenic HPV infection (Table 3).

**DISCUSSION**

In this population-based study, we investigated the natural history of genital HPV infection in over 1000 men from rural China. Results of the study expanded our knowledge of the natural history of HPV infection in men. To our knowledge, this is the first report from China on this topic. As compared to previous studies, the population-based sampling frame and high response proportion in AECCS would maximally decrease the selection bias (20), and the conclusions of this study could thus be generalized to a broader rural population in China.

HPV prevalence and incidence for the male genitalia have been reported in some prospective cohort studies.
adopted relatively long (>12 months) and fixed follow-up design (4-6 months) in western countries (17-19).

For the oncogenic types, the prevalence estimates ranged from 16.6% to 30.0% (17, 18), and incidence estimates ranged from 15.5 to 24.7 per 1000 person months (18, 19). In this study, we found a much lower prevalence (6.4%) and also a lower incidence (4.9 /1000 person months) for oncogenic HPV infection in Chinese men. Our previous cross-sectional investigation in the very same population found a similar but slightly lower prevalence estimate (6.1%) and the most common oncogenic types were HPV-16, HPV-18 and HPV-58 in both studies (21). An international study also reported a lower prevalence of oncogenic HPV infection among men from the Asia-Pacific region (31). Differences among races, sampling methods and the considerable more conservative sexual behavior in the population we studied may account for the low HPV prevalence and incidence in oncogenic types. For non-oncogenic types, exploratory study had shown a broad distribution of non-oncogenic HPV types at the male genital region could not be genotyped by linear array which was used in almost all prior studies (32). And this had largely limited the comparability between our results and the others’. On the basis of our result, we could infer that non-oncogenic HPVs existed extensively at the male external genital site in this population.

We found that the median time to clearance of oncogenic HPV was 6.8 months. The US men study reported that median time for clearance of oncogenic HPV infections was 5.8 months (18). In the HPV in Men (HIM) study, the corresponding median time was 7.2 months (17). These findings were in stand with our current recognition that most HPV infections in male are transient and would be cleared quickly. For type specific clearance, HPV-16 cleared more rapidly in our population as compared with the HIM study (6.6 months vs 12.2 months). However, the US men study reported a median duration for HPV-16 (6.0 months) which is much closer to our study. Difference in clearance duration may be the result of both demographic and behavioral factors cross populations and this requires further evaluation with large studies. However, this
discrepancy should be interpreted with caution since there would be a lack of variation in the estimates of median duration and they would be very close to the integer multiples of the follow-up interval due to the period sampling. This was particularly obvious in our study, since the biannual centralized follow-up was adopted and all study subjects were investigated within 2-3 weeks in each section.

Significantly higher prevalence, incidence and longer duration of HPV infection was observed in the younger group (25-50 years) as compared to the older group (51-65 years), and in our previous descriptive study, we also observed similar age pattern of HPV infection (p=0.039) (21). In other studies, however, this age trend was rarely detected, though faster clearance of HPV infections with increasing age was also found in the HIM study (17). We found younger age is positively associated with more sexual partners in this population (Supplementary table 2) which was not observed in the HIM cohort (17). As such, more sexual activity in younger men and higher exposure levels to HPV may have led to the age related pattern observed for HPV acquisition and clearance in this study. Despite all this, further longitudinal studies in the Chinese population were required to verify our results.

Similar to previous studies (17-19, 33-36), the number of lifetime sexual partner was consistently associated with an increased risk of HPV infection. We also found current smokers had a significantly elevated risk of non-oncogenic HPV infection, which was in keeping with the HIM study (17). A potential mechanism is that smoking may increase the HPV viral load by weakening the cellular immune response (37). To investigate the possibility that lifetime number of sexual partner acted as a confounder within the association between smoking and non-oncogenic HPV incidence, stratification analysis by lifetime sexual partner group was conducted. And similar effects (OR and HR) were observed in both groups, which suggesting the association between smoking and non-oncogenic HPV was independent of sexual behavior. In addition, we also unexpectedly found better personal hygiene is associated with a significantly elevated risk of HPV infection,
and this has not been evaluated in previous studies of men. In this population, levels of personal hygiene (i.e., frequency of bathing), may reflect overall socio-economic status (SES) due to the relatively poor economic and cultural conditions in rural China (38). Men with higher SES were more likely to work outside the local area for most of the year, which suggests these individuals may engage in more unprotected sexual behavior and higher risk for HPV exposure. Lastly, we found that age group was associated with incident infection of non-oncogenic HPV and clearance of oncogenic HPV. This age trend should be interpreted with caution due to the potential pitfall of multiple testing in these borderline significant findings.

Two limitations of this study should be noted. First, 220 (17.2%) men without follow-up data were excluded from the incidence and clearance analyses, which might lead to a certain degree of underestimation of overall incidence and duration of HPV. But it would not significantly affect our main conclusions concerning prevalence, age-stratified analyses of natural history and risk factor analysis. Second, although we have investigated over 1000 men for up to 7 evaluations, we were still limited by statistical power when analyzing minor types. Large studies with more evaluation cycles are needed to validate our conclusions.

Basic information regarding the natural history of HPV infection across a broad age range is a pre-requisite for the development of cost effective prophylactic strategy for HPV-related cancer. This study was the first prospective HPV natural history study among men from China. Results from this study will be both important in establishing primary prevention project of HPV-related carcinoma in Chinese women, and in evaluating male HPV vaccination in China.

Acknowledgements

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References


38. Zou L, Bao YP, Li N, Dai M, Ma CP, Zhang YZ, et al. Life-style and genital human papillomavirus in a
Table 1. Selected demographic and behavior variables for genital HPV infection among men from rural China included in the prevalence analysis, included in the incidence analysis and not included in the incidence analysis, 2009-2013.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Participants included in the prevalence analysis</th>
<th>Participants included in the incidence analysis</th>
<th>Participants NOT included in the incidence analysis</th>
<th>( P \text{ value} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Follow-up time(months)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(IQR)</td>
<td>25.3 (7.6-37.2)</td>
<td>31.8 (15.4-37.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean(SD)</td>
<td>22.4 (14.9)</td>
<td>27.0 (11.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at enrollment(years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median(IQR)</td>
<td>43 (36-54)</td>
<td>44 (37-55)</td>
<td>36 (29.5-43)</td>
<td></td>
</tr>
<tr>
<td>25-50</td>
<td>888 (69.4)</td>
<td>697 (65.8)</td>
<td>191 (86.8)</td>
<td></td>
</tr>
<tr>
<td>51-65</td>
<td>391 (30.6)</td>
<td>362 (34.2)</td>
<td>29 (13.2)</td>
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</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not married</td>
<td>66 (5.2)</td>
<td>56 (5.3)</td>
<td>10 (4.6)</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>1213 (94.8)</td>
<td>1003 (94.7)</td>
<td>210 (95.4)</td>
<td>0.651</td>
</tr>
<tr>
<td>Type of job</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farming</td>
<td>486 (38.0)</td>
<td>441 (41.6)</td>
<td>45 (20.5)</td>
<td></td>
</tr>
<tr>
<td>Work in local area</td>
<td>374 (29.2)</td>
<td>301 (28.4)</td>
<td>73 (33.2)</td>
<td></td>
</tr>
<tr>
<td>Category</td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
<td>P-value</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Work outside</td>
<td>419 (32.8)</td>
<td>317 (30.0)</td>
<td>138 (46.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Primary school or below</td>
<td>384 (30.0)</td>
<td>335 (31.6)</td>
<td>49 (22.3)</td>
<td></td>
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<tr>
<td>Junior middle School</td>
<td>690 (53.9)</td>
<td>570 (53.8)</td>
<td>120 (54.5)</td>
<td></td>
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<tr>
<td>Senior middle school or above</td>
<td>157 (12.3)</td>
<td>121 (11.4)</td>
<td>36 (16.3)</td>
<td></td>
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<tr>
<td>Unknown&lt;sup&gt;d&lt;/sup&gt;</td>
<td>48 (3.8)</td>
<td>33 (3.1)</td>
<td>15 (6.8)</td>
<td>0.001</td>
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<tr>
<td>No. of lifetime sexual partner</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>1120 (87.6)</td>
<td>934 (88.2)</td>
<td>186 (84.6)</td>
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<tr>
<td>≥2</td>
<td>159 (12.4)</td>
<td>125 (11.8)</td>
<td>34 (15.4)</td>
<td>0.135</td>
</tr>
<tr>
<td>Current smoker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>561 (43.9)</td>
<td>463 (43.7)</td>
<td>98 (44.6)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>718 (56.1)</td>
<td>596 (56.3)</td>
<td>122 (55.4)</td>
<td>0.823</td>
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<tr>
<td>Bath frequency in winter (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-7</td>
<td>459 (35.9)</td>
<td>354 (33.4)</td>
<td>105 (47.7)</td>
<td></td>
</tr>
<tr>
<td>8-30</td>
<td>630 (49.3)</td>
<td>531 (50.1)</td>
<td>99 (45.0)</td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>190 (14.8)</td>
<td>174 (16.5)</td>
<td>16 (7.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Wash genitalia before sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>990 (77.4)</td>
<td>839 (79.2)</td>
<td>151 (68.6)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>289 (22.6)</td>
<td>220 (20.8)</td>
<td>69 (31.4)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

<sup>a</sup> Participants had valid HPV results for at least one visit were included in the prevalence analysis.

<sup>b</sup> Participants had valid HPV results for at least two consecutive visits were included in the incidence analysis.
Chi-square test was used to compare between participants included in the incidence analysis and not included.

The unknown category was not included in the Chi-square test.

Abbreviations: IQR, interquartile range; SD, standard deviation.
Table 2. Prevalence, incidence and clearance of HPV infection among men from rural China, 2009–2013.

<table>
<thead>
<tr>
<th>HPV type</th>
<th>Prevalent cases</th>
<th>Prevalence &amp; 95%CI (N=1279)</th>
<th>Incident cases &amp; 95%CI (N=6182)</th>
<th>Incidence rate &amp; 95%CI (/1000 person months)</th>
<th>Newly acquired infections (N=1059)</th>
<th>Cleared infections &amp; 95%CI (N=207)</th>
<th>Median time to clearance (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any</td>
<td>526</td>
<td>17.8 (16.2-19.3)</td>
<td>291</td>
<td>14.6 (13.1-16.4)</td>
<td>359</td>
<td>207</td>
<td>11.5 (6.5-12.4)</td>
</tr>
<tr>
<td>Oncogenic</td>
<td>218</td>
<td>6.4 (5.4-7.3)</td>
<td>123</td>
<td>4.9 (4.1-5.8)</td>
<td>115</td>
<td>76</td>
<td>6.8 (6.4-12.6)</td>
</tr>
<tr>
<td>HPV-16</td>
<td>105</td>
<td>2.7 (2.1-3.3)</td>
<td>59</td>
<td>2.2 (1.7-2.9)</td>
<td>43</td>
<td>31</td>
<td>6.6 (6.2-12.4)</td>
</tr>
<tr>
<td>HPV-18</td>
<td>43</td>
<td>1.0 (0.7-1.3)</td>
<td>26</td>
<td>0.9 (0.6-1.4)</td>
<td>22</td>
<td>20</td>
<td>6.6 (6.0-6.8)</td>
</tr>
<tr>
<td>HPV-58</td>
<td>34</td>
<td>1.0 (0.6-1.3)</td>
<td>23</td>
<td>0.8 (0.6-1.2)</td>
<td>16</td>
<td>14</td>
<td>6.4 (5.9-12.6)</td>
</tr>
<tr>
<td>HPV-45</td>
<td>19</td>
<td>0.5 (0.2-0.6)</td>
<td>11</td>
<td>0.4 (0.2-0.7)</td>
<td>9</td>
<td>3</td>
<td>NE</td>
</tr>
<tr>
<td>HPV-35</td>
<td>13</td>
<td>0.5 (0.2-0.7)</td>
<td>7</td>
<td>0.3 (0.1-0.5)</td>
<td>6</td>
<td>2</td>
<td>NE</td>
</tr>
<tr>
<td>HPV-33</td>
<td>7</td>
<td>0.2 (0.0-0.4)</td>
<td>6</td>
<td>0.2 (0.1-0.5)</td>
<td>6</td>
<td>1</td>
<td>NE</td>
</tr>
<tr>
<td>HPV-52</td>
<td>8</td>
<td>0.2 (0.0-0.3)</td>
<td>6</td>
<td>0.2 (0.1-0.5)</td>
<td>4</td>
<td>2</td>
<td>18.9 (7.3-NE)</td>
</tr>
<tr>
<td>HPV-68</td>
<td>9</td>
<td>0.4 (0.1-0.7)</td>
<td>5</td>
<td>0.2 (0.1-0.4)</td>
<td>4</td>
<td>1</td>
<td>NE</td>
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<tr>
<td>HPV-56</td>
<td>6</td>
<td>0.1 (0.0-0.2)</td>
<td>3</td>
<td>0.1 (0.0-0.3)</td>
<td>2</td>
<td>1</td>
<td>11.7 (NE-NE)</td>
</tr>
<tr>
<td>Non-oncogenic</td>
<td>404</td>
<td>12.4 (11.1-13.6)</td>
<td>239</td>
<td>10.8 (9.6-12.3)</td>
<td>244</td>
<td>131</td>
<td>11.5 (6.5-12.6)</td>
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<tr>
<td>HPV-3</td>
<td>136</td>
<td>3.3 (2.7-4.0)</td>
<td>88</td>
<td>3.3 (2.7-4.1)</td>
<td>61</td>
<td>33</td>
<td>11.6 (6.6-12.8)</td>
</tr>
<tr>
<td>HPV-57</td>
<td>76</td>
<td>1.9 (1.4-2.3)</td>
<td>58</td>
<td>2.1 (1.6-2.7)</td>
<td>41</td>
<td>23</td>
<td>11.9 (6.6-18.5)</td>
</tr>
<tr>
<td>HPV-54</td>
<td>46</td>
<td>1.3 (0.8-1.7)</td>
<td>29</td>
<td>1.0 (0.7-1.5)</td>
<td>20</td>
<td>11</td>
<td>6.6 (6.5-NE)</td>
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<tr>
<td>HPV-90</td>
<td>33</td>
<td>0.9 (0.5-1.3)</td>
<td>21</td>
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<td>17</td>
<td>10</td>
<td>12.0 (6.6-12.4)</td>
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<tr>
<td>HPV-87</td>
<td>30</td>
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<td>20</td>
<td>0.7 (0.5-1.1)</td>
<td>16</td>
<td>13</td>
<td>6.5 (5.8-11.7)</td>
</tr>
<tr>
<td>HPV Type</td>
<td>Prevalence Count</td>
<td>Prevalence</td>
<td>Incidence Count</td>
<td>Incidence</td>
<td>Clearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-----------------</td>
<td>------------</td>
<td>-----------------</td>
<td>-----------</td>
<td>-----------</td>
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<tr>
<td>HPV-67</td>
<td>24</td>
<td>0.6 (0.3-0.8)</td>
<td>16</td>
<td>0.6 (0.4-0.9)</td>
<td>14 11 6.6 (5.9-11.5)</td>
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<td></td>
</tr>
<tr>
<td>HPV-81</td>
<td>26</td>
<td>0.7 (0.4-1.0)</td>
<td>13</td>
<td>0.5 (0.3-0.8)</td>
<td>7 4 6.6 (5.8-NE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-91</td>
<td>14</td>
<td>0.4 (0.2-0.6)</td>
<td>8</td>
<td>0.3 (0.1-0.6)</td>
<td>7 3 12.8 (6.5-NE)</td>
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<td></td>
</tr>
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<td>HPV-94</td>
<td>11</td>
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<td>8</td>
<td>0.3 (0.1-0.6)</td>
<td>7 2 NE</td>
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<tr>
<td>HPV-43</td>
<td>15</td>
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<td>8</td>
<td>0.3 (0.1-0.6)</td>
<td>7 3 NE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-11</td>
<td>11</td>
<td>0.2 (0.1-0.4)</td>
<td>8</td>
<td>0.3 (0.1-0.6)</td>
<td>5 2 11.7 (6.6-NE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-30</td>
<td>10</td>
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<td>6</td>
<td>0.2 (0.1-0.5)</td>
<td>5 3 13.2 (6.6-NE)</td>
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<td></td>
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<tr>
<td>HPV-10</td>
<td>13</td>
<td>0.4 (0.2-0.6)</td>
<td>6</td>
<td>0.2 (0.1-0.5)</td>
<td>4 0 NE</td>
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<td></td>
</tr>
<tr>
<td>HPV-6</td>
<td>7</td>
<td>0.2 (0.0-0.3)</td>
<td>5</td>
<td>0.2 (0.1-0.4)</td>
<td>4 2 6.6 (5.8-NE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-27</td>
<td>11</td>
<td>0.2 (0.1-0.4)</td>
<td>5</td>
<td>0.2 (0.1-0.4)</td>
<td>0 0 NE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-84</td>
<td>6</td>
<td>0.1 (0.0-0.2)</td>
<td>4</td>
<td>0.1 (0.1-0.4)</td>
<td>4 1 6.7 (6.7-NE)</td>
<td></td>
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<tr>
<td>HPV-75</td>
<td>7</td>
<td>0.2 (0.0-0.3)</td>
<td>4</td>
<td>0.1 (0.1-0.4)</td>
<td>4 1 NE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV-70</td>
<td>7</td>
<td>0.3 (0.0-0.5)</td>
<td>4</td>
<td>0.1 (0.1-0.4)</td>
<td>3 1 6.6 (6.6-NE)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Only HPV types with >5 prevalent cases were listed.

a Prevalence was defined as the percentage of HPV positive visits out of all counted visits during the study period in participants with adequate HPV test results (n=6182). 95% CIs were adjusted for correlations of repeat measurements.

b Incident cases were defined as the first HPV infection for a specific HPV type in one participant (n=1059).

c Incidence rate and 95% CIs were calculated based on the number of events modeled as a Poisson variable for the total person months.

d Only incident infections were included. Multi-type incident infections in one participant were judged as different newly acquired infections. Incident infections detected at a participant’s last visit were not included for clearance analysis.

e Clearance was defined as two consecutive negative results after testing HPV positive for a specific type.
Median time to clearance and 95% CIs were estimated using Kaplan-Meier method.

Oncogenic types in this study were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.

Non-oncogenic types in this study were 2, 3, 6, 7, 10, 11, 26, 27, 29, 30, 32, 37, 40, 42, 43, 44, 53, 54, 55, 57, 61, 62, 66, 67, 69, 70, 72, 74, 75, 77, 81, 82, 84, 85, 87, 90, 91, and 94.

95% CIs were adjusted for possible within subject correlations.

Abbreviations: HPV, human papillomavirus; CI, confidence interval; NE, not estimable because too few cases.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Prevalence of HPV infection</th>
<th>Incidence of HPV infection</th>
<th>Clearance of HPV infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any HPV</td>
<td>Oncogenic</td>
<td>Non-oncogenic</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt;50</td>
<td>0.91 (0.68-1.22)</td>
<td>0.96 (0.61-1.53)</td>
<td>0.90 (0.66-1.23)</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary school or below</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Junior middle School or above</td>
<td>0.90 (0.71-1.14)</td>
<td>0.81 (0.56-1.18)</td>
<td>0.95 (0.73-1.23)</td>
</tr>
<tr>
<td>Type of job</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Worker in local area</td>
<td>1.01 (0.77-1.34)</td>
<td>1.05 (0.70-1.58)</td>
<td>1.01 (0.74-1.37)</td>
</tr>
<tr>
<td>Worker outside</td>
<td>0.99 (0.74-1.31)</td>
<td>0.72 (0.47-1.12)</td>
<td>1.13 (0.82-1.55)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>No</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td>Yes</td>
<td>1.29 (1.04-1.60)</td>
<td>NA</td>
<td>1.26 (1.00-1.59)</td>
</tr>
<tr>
<td>No. of lifetime sexual partner</td>
<td>0-1</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>≥2</td>
<td>1.86 (1.40-2.46)</td>
<td>2.00 (1.35-2.96)</td>
<td>1.69 (1.24-2.30)</td>
</tr>
<tr>
<td>Bath frequency in winter (days)</td>
<td>1-7</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>8-30</td>
<td>0.70 (0.55-0.87)</td>
<td>0.53 (0.36-0.77)</td>
<td>0.84 (0.65-1.08)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>0.50 (0.35-0.71)</td>
<td>0.43 (0.24-0.76)</td>
<td>0.55 (0.37-0.83)</td>
</tr>
</tbody>
</table>

Backward selection with a threshold of 0.1 was used to generate the final multivariate model. Age, education level and type of job were included in all multivariate models as design factors.

Oncogenic types in this study were 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68.
Non-oncogenic types in this study were 2, 3, 6, 7, 10, 11, 26, 27, 29, 30, 32, 37, 40, 42, 43, 44, 53, 54, 55, 57, 61, 62, 66, 67, 69, 70, 72, 74, 75, 77, 81, 82, 84, 85, 87, 90, 91, and 94.

All the listed variables were remained in the final multivariate model.

All the listed variables and wash genitalia before sex were remained in the final multivariate model.

Only age, education level and type of job were remained in the model.

Abbreviations: HPV, human papillomavirus; OR, odds ratio; HR, hazard ratio; CI, confidence interval; NA, not applicable.
Figure Legend:

**Figure 1.** Kaplan Meier estimates of the incidence (per 1000 person months) and time to clearance (months)\(^a\) of HPV infection among 1059 men from rural China, 2009-2013, stratified by carcinogenicity of HPV and age group.

*\(^P\) values were calculated by Cox models using the WLW method to account for within subject correlation.

\(^a\) Confidence intervals in HPV clearance analysis were adjusted for within subject correlations using clustered Kaplan-Meier method.

Abbreviations: WLW, Wei Lin Weissfeld marginal model approach.

A: Incidence of oncogenic and non-oncogenic HPV infection.
B: Clearance of oncogenic and non-oncogenic HPV infection.
C: Incidence of any HPV infection, stratified by age group.
D: Clearance of any HPV infection, stratified by age group.
E: Incidence of oncogenic HPV infection, stratified by age group.
F: Clearance of oncogenic HPV infection, stratified by age group.
G: Incidence of non-oncogenic HPV infection, stratified by age group.
H: Clearance of non-oncogenic HPV infection, stratified by age group.
Figure 1. Kaplan Meier estimates of the incidence (per 1000 person months) and time to clearance (months)* of HPV infection among 1059 men from rural China, 2009-2013, stratified by carcinogenicity of HPV and age group.

* P values were calculated by Cox models using the WLW method to account for within subject correlation.

Abbreviations: WLW, Wei Lin Weissfeld marginal model approach.

A: Incidence of oncogenic and non-oncogenic HPV infection.
B: Clearance of oncogenic and non-oncogenic HPV infection.
C: Incidence of any HPV infection, stratified by age group.
D: Clearance of any HPV infection, stratified by age group.
E: Incidence of oncogenic HPV infection, stratified by age group.
F: Clearance of oncogenic HPV infection, stratified by age group.
G: Incidence of non-oncogenic HPV infection, stratified by age group.
H: Clearance of non-oncogenic HPV infection, stratified by age group.
Prevalence, Incidence, Clearance and Associated Factors of Genital Human papillomavirus Infection among Men: a population-based cohort study in rural China

Mengfei Liu, Zhonghu He, Chanyuan Zhang, et al.

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