Methods in HPV Surveillance: Experiences from a Population-Based Study of HPV Infection among Women in the San Juan Metropolitan Area of Puerto Rico

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This article describes the methodology of the first population-based study of human papillomavirus (HPV) infection among women aged 16-64 years residing in the San Juan Metropolitan Area of Puerto Rico (PR). The sample was identified through a complex sampling design of households. The sampling frame was selected in four stages, using census tracts maps from the Census Bureau. Women completed a face-to-face interview and a computer-assisted self-interview using the Audio CASI system, for the collection of demographic, clinical, and lifestyle variables, and sampling acceptability. Anal, cervical, and oral specimens were collected through self-collection methods for HPV DNA testing using a modified pool of MY09/MY11 consensus HPV L1 and human ß-globin amplification primers. Anthropometric measurements were taken using the Third National Health and Nutrition Examination Survey methodology. Blood samples were collected to create a bio-repository for future HPV-related studies. Fifty census tract blocks were randomly selected. We recruited 566 women, with a response rate of 83.4%. Response rates did not vary by age-group ($p>0.05$); although they varied by socioeconomic (SES) census block stratums ($p<0.05$), response rates were good (>75%) in all SES strata. All participants agreed to respond to the surveys and provide the requested anogenital and oral samples. Overall, more than 98% understood and more than 50% felt comfortable with the cervical, anal, and oral self-collection methods used. This article documents the feasibility of performing population-based studies for HPV surveillance in women in PR. [*P R Health Sci J 2015;34:117-127*]

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Human papillomavirus (HPV) infection is the most common sexually transmitted infection (STI) worldwide (1). HPV infection is associated with cervical, anal, vaginal, vulvar, penile, and oropharyngeal cancers (2). Given its burden, a well-organized program of surveillance among the Puerto Rican population should exist. Despite its world-wide burden and its relationship with multiple cancers, HPV is not a reportable infection, and information on the STI Surveillance system only focuses on genital warts, and is biased due to underreporting (3). Furthermore, the only annual surveillance survey in Puerto Rico (PR) is the Behavioral Risk Factor Surveillance System (BRFSS), which does not collect biological samples from participants. Surveillance surveys can be utilized as evidence for program planning, evaluation, and public policy. Surveys that collect biological specimens, such as the National Health and Nutrition Examination Survey (NHANES) (4) gather more accurate information about disease prevalence and distribution among those studied. In the US, NHANES has been responsible for describing the HPV burden and tracking changing infection prevalence estimates in the population (5-12). Unfortunately, NHANES is not conducted in PR and investigator-initiated population-based epidemiological studies that collect biological specimens are scarce.

Several population-based studies in PR have described the burden of various chronic (13-19) and infectious (20) diseases. These have collected biological specimens as well as demographic, clinical, and lifestyle data through personal and audio computer-assisted self-interview (ACASI) (20).

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Nonetheless, there have been no epidemiological studies of anogenital HPV infection conducted in PR, and no population-based incidence and prevalence estimates of HPV infection are available for this population. With the world-wide introduction of two prophylactic vaccines against high-risk HPVs causing cervical cancer (2), there is an urgency to determine the burden of HPV infection in the population before vaccine programs continue to be implemented on a widespread basis. Information on HPV burden prior to program expansion will allow a better assessment and understanding of the short-term and long-term effectiveness of this primary prevention strategy for cervical neoplasia and other related malignancies. In addition, it will allow for the description of the prevalence of specific HPV types in PR that are not currently included in the HPV vaccines available on the market. These studies are of interest in PR, as this population still has a low HPV vaccine uptake (21% and 11% of women and men aged 11-18 years, respectively, have completed the vaccination regimen) (21) and despite the legal mandatory health care-coverage of the vaccine established for girls aged 11-18 years since 2010 (Law #9 - January 20, 2010) and for boys since 2012 (Law #255 - September 15, 2012).

Aim

The aim of this article is to describe the methodology used in the first population-based study of HPV infection among women aged 16-64 years living in the San Juan Metropolitan Area of PR, using self-sampling collection techniques previously tested by our research team (22-24).

Methods

Planning and implementation

Sampling procedures

The study *HPV Infection in a Population-Based Sample of Puerto Rican Women* was a cross-sectional study whose primary aims were to describe the prevalence and correlates of anal and cervical HPV infection among women. The study was designed using a community-based random sample of women aged 16-64 years living in the San Juan Metropolitan Area of PR. The sampling methodology used was designed for a previous study of the metabolic syndrome among men and women residing in the San Juan Metropolitan Area of PR (19). The sample was identified through a cluster probability sampling design in four stages (25-26) of households in the area (1,070,719 inhabitants, of whom, 570,435 were women, according to the Census 2010), composed of the following seven municipalities: Bayamón, Carolina, Cataño, Guaynabo, San Juan, Toa Baja, and Trujillo Alto. The study population included non-institutionalized women aged 16-64 years residing in this area. Based on our resources and previous HPV estimate (23, 27), the minimum sample size required to detect a prevalence of HPV DNA of at least 17% in the cervix or in the anus (each site separately), with a precision level of 3%, was 600 women. The minimum sample size was estimated to provide an adequate statistical power (>80%) for the analysis of the association between cervical and anal HPV infection and risk factors when the prevalence odds ratio (POR) exceeded 1.50 and a 5% significance level. Assuming a 15% refusal rate and 15% of non-eligible population, it was estimated that approximately 780 women needed to be contacted.

Based on a previous sampling frame (19), four sampling stages were done, using the census tracts maps of the San Juan metropolitan area from the Census Bureau, Planning Board Office of PR. Information about the population aged 16-64 years, median age, number of occupied households, and median value for all owner-occupied households was gathered to define the sampling frame (28). The first stage was a random selection of 50 block groups using a systematic design of originally 829 block groups, arranged by block mean household value (<$50,000 and ≥ $50,000). In the second stage, a single block was randomly selected from each block group, and the amount of households was determined. In stage three, blocks were divided into segments of 12-16 consecutive households, and one was randomly selected. Finally, in stage four, one eligible female from each selected household was randomly selected for the interview process, controlling the desired number of participants by established age groups (16-34, 35-49, and 50-64 years). These sampling methods have been successfully used in previous studies by our research team (18-20).

Participant recruitment

This study was approved by the Institutional Review Board of the Medical Sciences Campus (MSC), University of Puerto Rico (UPR). To enhance participation, we implemented strategies used in our previous population-based studies (18-20). For example, community leaders were contacted to request their support. In addition, an introductory brochure was delivered to potential participants a week ahead of the scheduled visit. The brochure described the nature of the study, the methods for data and biological specimen collection, confidentiality measures, and potential benefits. It also contained contact information for those who were interested in participating or needed additional information about the study.

At the time of the household visit, the field manager and the research assistant were transported to the sites by an official vehicle provided by the PR Clinical and Translational Research Consortium (PRCTRC). As a strategy to reduce refusal rate, inconformity, and information bias, all recruiters were females. For each visit, they wore uniforms and identified themselves with their official identification cards of the University of Puerto Rico Comprehensive Cancer Center (UPRCCC). During the visits, they carried with them copies of the introductory brochure, answered questions about the study, and collected the household composition information (name, sex, age, phone, address, type of household, and availability). A maximum of three recruitment visits were performed during weekdays at different times of the day (daytime and evenings) and during

Saturday mornings. In every occupied household visited, the field manager listed all women aged 16-64 years, and invited one woman per household to participate. A random sampling was used with a weighting factor in each age group for every selected household to ensure the desired balance. Women were not eligible to participate if they were HIV-positive, pregnant, and/or were cognitively or physically impaired. Eligibility was determined by the study coordinator using the information provided by the potential participant. Replacement subjects were selected when the originally contacted women were not eligible or when no contact was made after three consecutive visits. These replacements were selected randomly from adjacent households. A maximum of eight positive contacts were made on each segment visit.

Appointments were scheduled for participants who agreed to take part in the survey. Interviews were scheduled according the availability of the participants. Thus, women who worked outside their home at daytime were considered and the interviews were performed during weekdays evenings or Saturdays mornings. Phone reminders were performed one day prior to the appointment. Additionally, text messaging proved to be useful for appointment reminders, when participants did not answer their phones. At the scheduled visit, participants were asked to sign an informed consent and complete all study procedures at their homes. All women were also asked to donate blood for future use in HPV-related studies. In addition, participants were asked if they agreed to be contacted in the future to receive invitations to participate in future studies in this area. In case the potential participants felt unsure about participating, these study clarification alternatives were provided: 1) If they agreed, they were asked to provide their phone numbers, so that one of the study investigators could contact them to further explain study procedures and answer/clarify potential questions about the study; 2) If they preferred not to provide their phone numbers, additional contact information of the study investigators was provided; 3) Participants who preferred to perform the study outside their homes were invited to come to the clinical facilities of the PRCTRC, located at the MSC-UPR; 4) For increased adherence of participants to the appointments, rescheduling options were made available.

Instrument development

The data collection instruments used in this study were similar to those used in our previous epidemiologic study of hepatitis C and other viral infections (29-31). After modification of the instrument for inclusion of additional variables relevant to HPV infection and women's health, these instruments were also previously tested and used in our pilot study on anogenital HPV infection (23-24). It consisted of two parts: a face-to-face interview and a computer-assisted self-interview, using ACASI. The computer interview, which collected sensitive behavior information, has been successfully used by our research team and others (20, 29-31) to collect this type of data. Information related to sexual orientation was collected using the Best Practices for Asking Questions about Sexual Orientation on Surveys, developed by the *Sexual Minority Assessment Research Team* (32). Additionally, information about the perceived social environment in the participants' neighborhoods was collected using *PhenX Toolkit* (33). HPV sampling acceptability questions were also included, using as a model those developed by Dzuba et al. (34) and used in our previous pilot study (24).

Staff training

An interviewer's training manual was created to facilitate the training of the female data collectors on study procedures. The manual provided instructions on interview procedures essential for the administration of the interviews, collection of biologic specimens and anthropometric measurements, and strategies that could be used when encountering difficulties. All research staff completed online courses (Human Subjects Protection, HIPAA, GCP training) sponsored by the Collaborative Institutional Training Initiative (CITI Program).

Data collection procedures

The data collection methods consisted of the following:

1. *Informed consent*: The informed consent was signed by all eligible participants before participating in the study. Participants consented to provide cervical, anal, and oral samples for HPV determination, donate blood for HPV-realted studies, and be further contacted for future studies in this area.
2. *Face-to-face and ACASI*: The primary methods for data collection were a face-to-face interview and the ACASI system. The data collection was supervised and monitored by the study coordinator. To monitor completeness and accuracy, the data manager was responsible for periodically screening the data. The face-to-face interview collected information on risk factors for HPV infection and cervical, anal, and oral squamous intraepithelial lesions, including demographic characteristics, reproductive history, contraceptive use, history of STIs and immunosuppressive disorders, history of Pap smear screening, gynecologic procedures to the cervix, history of anogenital lesions, use of corticosteroids, alcohol consumption, oral health, smoking, physical activity, and nutrition. In addition, seven items were used to assess self-sampling acceptability, based on five-item acceptability Likert scales. These items measured comfort (1=very uncomfortable, 5=very comfortable), pain (1=a lot of pain, 5=no pain), privacy (1=lacked privacy, 5= very private), and embarrassment (1=a lot of embarrassment, 5=no embarrassment). In addition, we evaluated their perceived understanding of sampling (1=did not understand, 5=understood completely), if they liked collecting their samples in their home (1=did not like at all, 5=liked a lot), and if they would like to collect the samples at home if the samples arrived through the mail (1=would not like at all, 5=would like a lot). For each item,

overall acceptability was defined as a response of 4 or 5 on the Likert scales. In addition, this instrument also included a question that was intended to determine whether a given patient preferred the clinician- or self-sampling method for retrieving HPV samples ("Which of the following methods for retrieving cervical samples did you prefer?"), based on five-item acceptability Likert scale (1=prefers a lot that the doctor takes the sample, 5=prefers a lot to take the sample yourself). The five items evaluated were regrouped into three (preferred self-sampling, neutral, preferred clinician sampling). A next question followed on the reasons for this preference, the answer to which included all the reasons that applied (*Why did you prefer this method?*: "Because you…1) felt less embarrassment, 2) felt more comfortable, 3) felt more confident that the sample would be done properly, and 4) spouse/partner would prefer that method). Similar questions were included for the anal and oral sample-collection method.

Information on sexual behavior, condom use practices, and drug use was collected through the ACASI system. Participants used a laptop computer to directly answer these questions. The interviewer was present to provide assistance, if warranted. The questions were recorded in sound files and the respondent was able to listen to the questions, as well as read them on the computer. Touch screen attachments to the computer monitors enabled participants to mark responses on the screen without the use of a keyboard, thus, not requiring computer use proficiency.

3. Collection of biological specimens and anthropometric measurements:

Anogenital and oral samples

The collection of anal, cervicovaginal, and oral specimens was performed upon completion of the study interviews. Samples were collected following previously tested self-collection methods (23-24). A collection kit that included the necessary materials for the collection of the anogenital specimens [(1 Dacron™ swab, 1 cytobrush, 2 pairs of gloves, 2 vials containing 10 mL of Scope, Sample Transport Medium (STM) (Digene Corp. Gaithersburg MD), 1 collection cup with 10mL of Scope and 1 translucent sealable plastic bag for disposal of sampling material)] and the oral specimen (10 mL bottle of Scope and a 50 mL collection container). Data collection containers were clearly identified with the participant's initials, study identification number, and collection date. In addition, a verbal explanation and written instructions in Spanish (including a diagram of the female genital anatomy) for self-collection were given by the study staff to each participant, to facilitate the specimen collection (Figure 1 and Figure 2).

- Anus - While wearing latex gloves, the women were instructed to remove one Dacron swab from its sterile package, and moist it in tap water before inserting it one inch into the anal canal. Women were then instructed to apply gentle pressure to the walls of the anal canal, and to remove the swab with spiral motion over a 10-second period. Then, women had to place the swab into the open separate 5-mL vial, close the bottle tightly, and hand it in to the study coordinator.
- Cervix - Women were instructed to remove a second long-handled, sterile Dacron swab for the cervical specimen

We ask you to personally take a vaginal sample and an anal sample to assess the presence of the Human Papilloma Virus, a virus that some people have in their cervix and anus. After finishing the discussion, please let me know if you have any questions.

Cervical Sampling

1. Relax and wash your hands.
2. Stand with your legs spread apart and slightly flexed, or with one foot resting on top of an elevated surface (toilet, bathtub, or stool).
3. Insert the brush into the vagina whilst maintaining the vaginal lips open. Try to position the brush directly into the vagina, without touching any part of your genitals.
4. Insert the brush as far into the vagina as possible, without letting go of the inferior part of the brush (as if it were a tampon).
5. Partly remove the brush (up until halfway out of the vagina) and insert it again up to the top of the vagina. Move the brush up and down five (5) times, and try to point the brush towards the cervix.
6. When the brush reaches the cervix, turn the brush three (3) times.
7. Remove the sample stick from the cervical canal. Open the container provided. Be careful not to spill the liquid inside the container. Place the sample stick inside the container, cotton part first, with caution not to touch around the container. Break the sample stick against the container border (only needs to be bent for it to break), and close the container.
8. Hand the container to the interviewer.

Anal Sampling

1. Relax and wash your hands.
2. Stand with your legs spread apart and slightly flexed, or with one foot resting on top of an elevated surface (toilet, bathtub, or stool).
3. Dampen the cotton part of the stick with sterile water.
4. Position the sample stick in the entrance of the anus and, applying a small amount of pressure, introduce the cotton part in the anal canal about one (1) inch.
5. Softly rotate the sampling stick applying a bit of pressure on the walls of the anal canal, performing a spiral movement for about ten (10) seconds.
6. Remove the sampling stick from the anal canal. Open the container provided. Be careful not to spill the liquid inside the container. Place the sampling stick inside the container, cotton part first, with caution not to touch around the container. Break the sampling stick against the container border (only needs to be bent for it to break), and close the container.
7. Hand the container to the interviewer.

* Instructions translated to English for manuscript purposes.

Figure 1. Instructions for the HPV cervical and anal self-exam*. Figure 1 presents the witten instructions provided to women for the self-collection of the anogetial HPV samples. These written instructions were accompanied by visual diagrams that exemplified study procedures and with verbal instrucctions provided to the participant by the study coordinator.

We ask you to perform a mouth wash with the mouthwash "Scope", and then spit out the mouthwash in a container, with the objective of testing the presence of the Human Papilloma Virus (HPV), a virus that some people have in their mouths. First, you will rinse your mouth with a mouthwash for five (5) seconds and then perform gargles for five (5) seconds. Repeat this process three (3) times and then spit out the mouthwash into a container. The interviewer will let you know when to perform each one of the steps. Do you have any questions?

1. Wash your hands
2. Rinse your mouth with approximately 10 mL of the mouthwash (or salt water) for five (5) seconds and follow with gargles for five (5) seconds. Repeat these steps three (3) times, following the time intervals guided by the interviewer:
   a. Rinse (5 seconds)
   b. Gargle (5 seconds)
   c. Rinse (5 seconds)
   d. Gargle (5 seconds)
   e. Rinse (5 seconds)
   f. Gargle (5 seconds)
3. Spit out the mouthwash (or salt water) into the container, without spilling any over the container's border.
4. Close the container immediately.
5. Hand the container to the interviewer.

*Instructions translated to English for manuscript purposes. Source: Instructions based on: HPV Rinse (2009-2010), National Health and Nutrition Examination Survey (NHANES), Center for Disease Control and Prevention, Atlanta, GA

Figure 2. Instructions for the HPV oral auto-exam*. Figure 2 presents the written instructions provided to the women for the self-collection of oral samples. These written instructions were accompanied by visual diagrams that exemplified study procedures and with verbal instrucctions provided to the participant by the study coordinator.

collection. Women were instructed to relax and insert the Dacron tip of the swab into the vagina, without touching the labia or urethra if possible. Using their thumb and two fingers, women were instructed to gently push the swab up into the vagina until physically it could not go any further, and then to pull the swab halfway out of the vagina, and then re-insert it. Once appropriately positioned, women were instructed to rotate the swab inside the vagina for three full rotations, keeping the swab as far into the vagina as possible. Afterwards, the swab was to be withdrawn from the vagina, holding the lips on the labia apart and taking care not to touch other portions of the genitals. If any irritation or discomfort was encountered, women were instructed to reduce the pressure of the swab inside the vagina, pull the swab out away from the cervix a bit, or stop the procedure completely. After the woman completed the sample collection, they were instructed to also insert the second swab immediately in a separate 5-mL vial, close it tightly, and hand it in to the study coordinator.

- Oropharyngeal cavity - An oral mouthwash method was used to collect information of oral HPV infection, using 2009-2010 NHANES HPV rinse methodology (35). If the participant had gum or dentures they were asked to remove them previous to the sample collection. Then, participants were asked to rinse/gargle with the mouthwash in their mouth for 30 seconds. Afterwards, they had to spit the mouthwash into the collection container, while trying not to spill any of the liquid, close it tightly, and hand it in to the study coordinator.
- Blood samples - Serum and plasma samples were collected for the development of a bio-repository for future HPV-related studies. Approximately 21 mL of blood (1 red top tube and 2 EDTA tubes) were collected from each participant who agreed to donate blood. Blood samples were collected by a health care professional (graduate nurse or medical technologist).
- Anthropometric measurements - Anthropometric measurements of study participants were taken according to the NHANES III Anthropometric Video Procedures (36). Waist and hip circumferences were determined in centimeters through the use of a measuring tape. A digital scale was used to measure body weight in pounds, and a portable stadiometer was used to determine height in centimeters.

4. Completion: After completion of the study procedures, participants received educational material on HPV and HPV vaccination and a monetary compensation ($40.00) for their time and effort. Only participants whose cervical samples were positive for cervical infection with a high risk HPV type were further notified. A letter notifying their cervical test results as well as recommending a medical evaluation or consultation was sent to these participants. A medical evaluation or consultation with the study's gynecologist was arranged if they wished to discuss any concerns.

Research staff and Data management

One field coordinator and nine research assistants (six graduate and three undergraduate students) worked on recruitment, data collection, and data entry. For the biological specimens, one laboratory coordinator, two certified nurses, and six laboratory assistants (five undergraduate students and a volunteer) worked on sample collection, delivery, processing, and storage.

Data monitoring

The main objective of the data management approach was to provide the systematic means for recording, editing, and retrieving data for study management, tracking of biological specimens, and analyses. Completed interviews were processed by the study coordinator every week. Data were entered in a computer system; management and editing were performed using commercial computer software, Epi-Info 6.04d (CDC, Atlanta, GA), to detect errors in the computer file. Screen forms were designed for data entry, entry of recruitment log forms, laboratory results, and other processed data. The data were entered directly from the data collection forms. All data collection forms were reviewed for errors and completeness, and entered after the coding process was completed. Double

data entry procedures were used to reduce errors. The data manager was responsible for maintaining and merging data files containing interview and laboratory data into a master file. Weekly backup copies of the master file were performed and stored. Participants were assigned a study identifier for confidentiality and data linkage purposes. Data were transferred into the statistical software Stata version 13 (Stata Corp LP, College Station, TX) for data monitoring, data analysis, and production of reports and manuscripts. None of the databases contained personal identifying information.

Biological sample processing

After collection, specimens were transported by the study coordinator from the participant's households to the UPRCCC laboratory. At the UPRCCC, biological samples were frozen and stored at -70°C, and shipped on dry ice to Dr. Palefsky's laboratory at the University of California San Francisco (UCSF) for HPV DNA testing and typing. HPV DNA testing for cervical and anal samples was performed using a modified pool of MY09/MY11 consensus HPV-L1 primers as well as primers for amplification of the human ß-globin gene. PCR products from positive samples were typed by dot-blot hybridization using 40 individual type-specific probes.

For oral mouthwash samples, within 24 hours of sample collection, the tube containing the exfoliated cells was centrifuged, the supernatant discarded, and the cell pellet re-suspended in 1 mL of STM (Qiagen). These were then frozen in liquid nitrogen and stored at -70°C at the UPRCCC for future assessment of oral HPV infection. Nonetheless, 15 oral samples were sent to UCSF for HPV testing, in order to evaluate sample adequacy.

Scientific accomplishments

Study population

Data collection was performed from August 2010 to May 2013, sampling 50 of the total 829 census block groups in the San Juan Metropolitan Area. A total of 1,176 households were visited; of these, 746 were initially contacted and 67 refused to give information upon initial contact (eligibility unknown); a total of 679 women were determined to be eligible for the study (Table 1). Of eligible women, 566 consented to participate in the study, yielding a response rate of 83.4%. The age distribution of the participants was 32.0% for the 16-34 age group, 35.5% for the 35-49 age-group, and 32.5% for the 50-64 age-group. Response rates did not vary by age group ($p>0.05$); however, they did vary by socioeconomic census blocks ($p<0.05$), being high (>75%) in low, middle, and high strata (Table 2). Pertaining to other variables evaluated, the majority of women were born in Puerto Rico (88.7%), and had achieved high school education (83.9%). The annual family income of the participants was mostly lower than $20,000 (58.8%) and 50.4% had private healthcare coverage, 40.3% had public coverage and 9.3% did not have healthcare coverage (data not shown).

Face-to-face and ACASI

Participants spent an average of 30 minutes (range: 25-35 minutes) on the face-to-face interview and 20 minutes (range: 15-25 minutes) on the ACASI. All women participated in both interviews.

Biologic and Anthropometric data collection

All women (n=566) agreed to provide the cervical, anal, and oral samples, and to have anthropometric measurements taken. One woman was unable to provide the cervical and anal samples due to handicapped status (muscular dystrophy) and another because of cross contamination of samples (the cervical brush and the anal swab were placed in the same collecting tube), leaving 564 (99.6%) samples suitable for further HPV testing. Of all the oral samples collected, two were not processed due to unforeseen problems during sample management, also leaving 564 (99.6%) samples for further testing. Of the 566 women recruited, 541 agreed to provide the optional blood samples. However, 10 of these women were unable to provide the blood samples (serum and plasma) due to several causes (e.g. vein not found, nausea, dizziness) and 6 of the plasma samples were eliminated because of unexpected problems during the processing time. A total of 531 (93.8%) and 525 (92.7%) serum and plasma samples, respectively, were collected.

Biological sample adequacy

Among samples collected and processed, the rate of women capturing high-quality samples of the cervix and anus through self-collection (as determined by a positive ß-globulin result) was 100% and 95.1%, respectively. A pilot testing of 15 oral samples showed that ß-globulin was detected in 100% of the samples.

Acceptability of HPV self-collection methods

More than half of the participants indicated that they felt comfortable with the self-sampling collection methods used; a lower percentage reported comfort for anal sampling (49.2%), compared with cervical (57.4%) and oral sampling (89.9%) (Table 3). A higher percentage mentioned that they felt no pain during anal (76.1%), cervical (82.7%), and oral (99.3%) sampling. For all three sampling methods, most women (>98%) felt they understood the instructions on the self-sampling, more than 95% approved having the interview and self-sampling performed at home, and more than 89% accepted the option of taking samples if they arrived by mail.

Analysis also revealed that participants preferred self-collection of samples to physician-collected samples for future sampling of cervical (64.6%, $p<0.001$), anal (71.9%, $p<0.001$), and oral (65.7%, $p<0.001$) sites (Table 4). Reasons for method of preference, which included measures of comfort, embarrassment, pain, privacy, and partner's preference, are presented in Table 4. For all sampling methods (cervix, anus, and oral), the participant's partner's sampling preference was

Table 1. Age distribution of the target population and study sample

Age group (years)	PR Population (Census 2010) N (%)	SJMA* Population (Census 2010) N (%)	Households' Residents N (%)	Eligible Residents N (%)	Recruited Residents N (%)
16-34	496,456 (39.7)	143,521 (39.2)	392 (39.8)	222 (32.7)	181 (32.0)
35-49	385,122 (30.8)	111,742 (30.6)	301 (30.5)	242 (35.6)	201 (35.5)
50-64	368,553 (29.5)	110,509 (30.2)	293 (29.7)	215 (31.7)	184 (32.5)
Total	1,250,131 (100.0)	365,772 (100.0)	986 (100.0)	679 (100.0)	566 (100.0)

*San Juan Metropolitan Area

Table 2. Age groups and Socioeconomic strata according to recruitment status.

	Not-recruited n (%)	Recruited n (%)	Total n (%)	P-value
Age Group (years)				*>0.1*
16-34	41 (36.3)	181 (32.0)	222 (32.7)	
35-49	41 (36.3)	201 (35.5)	242 (35.6)	
50-64	31 (27.4)	184 (32.5)	215 (31.7)	
Total	*113 (100.0)*	*566 (100.0)*	*679 (100.0)*	
Socioeconomic Strata				*<0.0001*
High	56 (49.5)	171 (30.2)	227 (33.4)	
Middle	29 (25.7)	205 (36.2)	234 (34.5)	
Low	28 (24.8)	190 (33.6)	218 (32.1)	
Total	*113 (100.0)*	*566 (100.0)*	*679 (100.0)*	

the only factor that had no influence over the participants' preferences. A higher proportion of those who preferred self-sampling did so because they felt less embarrassed and more comfortable, although a higher percentage of those who preferred clinician-sampling did so because they felt that the samples would be more appropriately taken (p<0.0001).

Data weighting

Since the samples were taken using a complex sampling design of households, additional data were collected to construct a weighting factor to produce unbiased prevalence estimates of HPV infection among participants. These data include the selection procedures and the acceptability of the participants in the study for each block of households. This weighting factor was normalized using the following expression:

$$w_i = \frac{1/(f_1 * f_2 * f_3)}{\overline{w}}$$

where f_1 was the selection probability for each participant, f_2 was the rate of participation in each block, f_3 was the post-stratification adjustment based on the age and sex distribution of the Census 2010 in the San Juan Metropolitan area, and was the mean final weight for the entire sample (37).

Significance

This article documents the feasibility of performing population-based studies for HPV surveillance in women in Puerto Rico. When comparing the socioeconomic indicators of the study participants with those expected in PR, the annual family income (<$20,000) was comparable with the median income of the island's ($14,400) population according to Census 2010 (38). Educational attainment of at least high-school was higher (83.9%) than that of women in PR aged 18 and older according to the BRFSS data (69.5%) (39), although this was expected as data from the PR Community Health Profile show higher educational attainment in San Juan (40). A comparable proportion (9.3%) did not have healthcare coverage compared with the Census 2010 and the 2011 Puerto Rico Community Survey (5-9%) (40,41).

Similar to results from our other population-based studies in PR (19-20), we achieved high response rates among eligible participants. In addition, all women provided anal, cervical, and oral samples. This is higher than the 85%-88% of cervical samples provided by 2003-2006 and 2007-2010 NHANES participants (42-43), although comparable to the percentage of participants (>98%) that provided cervical samples in the Hawaiian and American Samoa HPV studies (44-46). For anal samples, the response rate was also much higher in our study

Table 3. Women's perception of HPV self-collection methods (n=566).

Acceptability Questions**	HPV self-collection site		
	Cervix n (%)*	Anus n (%)*	Oral n (%)
Felt comfort			
+	324 (57.4)	278 (49.2)	509 (89.9)
-	241(42.6)	287 (50.8)	57 (10.1)
Felt no pain			
+	467 (82.7)	430 (76.1)	562 (99.3)
-	98 (17.3)	135 (23.9)	4 (0.7)
Felt enough privacy			
+	559 (98.9)	556 (98.4)	-
-	6 (1.1)	9 (1.6)	
Felt no embarrassment			
+	530 (93.8)	499 (88.3)	-
-	35 (6.2)	66 (11.7)	
Understood			
+	558 (98.8)	559 (98.9)	565 (99.8)
-	7 (1.2)	6 (1.1)	2 (0.2)
Would like to collect the sample at home			
+	545 (96.5)	541 (95.8)	559 (98.8)
-	20 (3.5)	24 (4.2)	7 (1.2)
Would like to collect the sample at home if it arrived by mail			
+	517 (91.5)	508 (89.9)	534 (94.4)
-	48 (8.5)	57 (10.1)	32 (5.6)
Prefers self-collection			
+	365 (64.6)	406 (71.9)	372 (65.7)
-	200 (35.4)	159 (28.1)	194 (34.3)

*n=565, one participant with muscular dystrophy could not take the anogenital samples.
**Data were collected using the Likert scale acceptability indices. For each item, positive acceptability (+) was defined as a response of 4 or 5 on the Likert scales, and neutral/negative acceptability (-) was defined as a response of 1 to 3 on the Likert scales.

Table 4. Reasons for sampling method preference, by preference of HPV sampling in the future (n=566).

	Method preferred for HPV sampling in the future			
	Clinician sampling n (%)	Neutral n (%)	Self-sampling n (%)	P-value*
Cervix† (n=565)	*(n=78, 13.8%)*	*(n=122, 21.6%)*	*(n=365, 64.6%)*	
Less embarrassment				
Yes	1 (1.3)	2 (1.6)	128 (35.1)	<0.001
No	77 (98.7)	120(98.4)	237 (64.9)	
More comfortable				
Yes	12 (15.4)	7 (5.7)	315 (86.3)	<0.001
No	66 (84.6)	115 (94.3)	50 (13.7)	
More confident sample would be properly taken				
Yes	73 (93.6)	8 (6.6)	34 (9.3)	<0.001
No	5 (6.4)	114 (93.4)	331 (90.7)	
Spouse/Partner would prefer that method				
Yes	0 (0.0)	0 (0.00)	6 (1.6)	>0.1
No	78 (100.0)	122 (100.0)	359 (98.4)	
Anus† (n=565)	*(n=65, 11.5%)*	*(n=94, 16.6%)*	*(n=406, 71.9%)*	
Less embarrassment				
Yes	2 (3.1)	1 (1.1)	171 (42.1)	<0.001
No	63 (96.9)	93 (98.9)	235 (57.9)	
More comfortable				
Yes	10 (15.4)	3 (3.2)	350 (86.2)	<0.001
No	55 (84.6)	91 (96.8)	56 (13.8)	
More confident sample would be properly taken				
Yes	59 (90.8)	5 (5.3)	36 (8.9)	<0.001
No	6 (9.2)	89 (94.7)	370 (91.1)	
Spouse/Partner would prefer that method				
Yes	0 (0.0)	(0.0)	5 (1.2)	>0.1
No	65 (100.0)	94 (100.0)	401 (98.8)	
Oral (n=566)	*(n=48, 8.5%)*	*(n=146, 25.8%)*	*(n=372, 65.7%)*	
Less embarrassment				
Yes	1 (2.1)	1 (0.7)	49 (13.2)	<0.001
No	47 (97.9)	145 (99.3)	323 (86.8)	
More comfortable				
Yes	10 (20.8)	10 (6.8)	322 (86.6)	<0.001
No	38 (79.2)	136 (93.2)	50 (13.4)	
More confident sample would be properly taken				
Yes	41 (85.4)	3 (2.1)	35 (9.4)	<0.001
No	7 (14.6)	143 (97.9)	337 (90.6)	
Spouse/Partner would prefer that method				
Yes	0 (0.0)	0 (0.0)	0 (0.0)	**
No	48 (100.0)	146 (100.0)	372 (100.0)	

*Fisher's exact test p-value, **P-value not calculated, ***Data were collected using the Likert scale acceptability indices; the five items evaluated were regrouped into three (preferred self-sampling, neutral, preferred clinician sampling). † n=565, one participant with muscular dystrophy could not take the anogenital samples.

than in the Hawaiian studies (34%-76%), although comparable to the study in American Samoa (99%). For oral samples the response rate from our study participants was similar (100%) to that reported by NHANES 2009-2010 (5).

Sample adequacy, as determined by the presence of ß-globulin in PCR, showed that 100% of our cervical samples and 95% of our anal samples were adequate for HPV typing, showing that our self-collection methods were appropriate, as had been shown in our previous study (23). Cervical samples results were comparable to previous studies (>99%), although our results for anal sampling were superior than the 73% - 90% reported by the Hawaiian and American Samoa studies (43-45). Although our oral samples have not been entirely analyzed, a pilot testing of 15 samples showed that ß-globulin was detected in 100% of the samples, suggesting that it is likely that the remaining samples were properly taken. Results from the NHANES also showed high adequacy of their oral samples (99.9% out of their 5,579 samples were ß-globulin positive).

The study also provides information on the overall acceptability of self-sampling methodologies from women of all age groups evaluated (16-64 years old). Our results are consistent with previous studies (23-24) and those of others (22) that have shown that self-sampling is an acceptable method for HPV collection. As in our clinic-based pilot study, more comfort and less embarrassment and pain were felt with self-collection. Similarly, in a study performed in Mexico, more than half of the women (68%) preferred cervical self-sampling methods because they were more comfortable and caused less embarrassment (35). Most women in our population-based study reported that they preferred self-collection methods, compared with our clinic-based study where most women preferred having a clinician collect cervical and anal samples (67% and 61%, respectively). The stronger preference for clinician collection within the clinic setting could be related to selection bias, as women who come to the clinic may have a stronger trust in their physicians and may be less likely to be interested in alternative sampling methods. Nonetheless, in both studies, the main reported reason for preferring clinician collection among women who reported this preference was that they felt that the samples would be more appropriately taken. A study performed among Hispanic, low-income, uninsured, and recently-screened women showed that although more than two-thirds (68%) of these women preferred the clinician-collected HPV test to self-collected sampling, self-sampling is acceptable in this population and may increase the likelihood of participation in cervical

cancer screening (47). Thus, education of women in primary health care settings about the validity, appropriateness, and quality of HPV self-collection would help more women select these methods in the future. Additionally, it was noted that women in our study accepted self-sampling performed at home and overall preferred themselves over a physician performing the sampling, for all samples tested (cervix, anal, and oral). Our self-sampling acceptability results are consistent with a previous study in PR and with results for other populations (23). The observed high acceptability of HPV self-sampling methods should permit high quality HPV infection surveillance in PR. It also opens the door towards surveillance by mail delivery, as the majority of participants approved the option of taking the HPV infection surveillance test via mail, a practice already proven effective by a study performed in Sweden (48). Although little is known about the acceptability of oral self-sampling in HPV studies, acceptability in our study of oral sampling was high, as most women reported comfort (90%) and no pain (99%), and 66% of them also preferred the self-sampling method.

Plans and Conclusion

This study will provide the first assessment of the burden of HPV infection among women living in the San Juan Metropolitan Area of PR, providing invaluable information on public health surveillance, essential for monitoring the impact of vaccination programs. These results will be presented in forthcoming publications. Given that our sampling frame included only women from the San Juan Metropolitan Area, a limitation is that our results are not generalizable to the total female population of PR. Nonetheless, the recruited study population is comparable in terms of demographics to the female population of women in PR. The methodological information presented is relevant for the development of future HPV-related studies in PR and development of prevention and intervention strategies to decrease the burden of HPV in PR. Our experience shows that the recruitment and data collection methodologies used in the study were effective, since a high response rate was achieved, quality of samples collected was excellent, and self-collection methods were acceptable for participants. Self-sampling methodologies are an effective approach to screening women for HPV infection. As previously suggested (49), self-sampling options should be considered in innovative programs that promote further screening of women who are not receiving regular cervical cancer and potentially other HPV-related screening.

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Resumen

Este artículo describe la metodología utilizada en el primer estudio poblacional realizado sobre infección con virus de papiloma humano (VPH) en mujeres de 16-64 años residentes del área metropolitana de San Juan, Puerto Rico (PR). El diseño de la muestra se basó en un diseño complejo de muestreo de viviendas. Las participantes completaron una entrevista cara-a-cara y una entrevista auto administrada asistida por computadora, donde se recopilaron datos personales y de la aceptabilidad de las muestras recolectadas. Especímenes anales, cervicales y orales fueron recolectados usando métodos de auto-colección, con el fin de realizar pruebas del ADN del VPH. Se tomaron medidas antropométricas usando la metodología del *Third National Health and Nutrition Examination Survey* y muestras de sangre fueron recolectadas para la creación de un bio-repositorio para futuros estudios relacionados a VPH. Cincuenta bloques censales fueron seleccionados aleatoriamente. Se reclutaron 566 mujeres, alcanzando una tasa de respuesta de 83.4%. La tasa de respuesta no varió entre grupos de edad ($p>0.05$) y, aunque esta varió entre los estratos socioeconómicos censales ($p<0.05$), fue adecuada (>75%) entre todos los estratos socioeconómicos. Todas las participantes aceptaron responder los cuestionarios y proveer las muestras anogenitales y orales solicitadas. Más del 98% entendió y más del 50% se sintió cómoda con los métodos de colección utilizados para las muestras cervicales, anales y orales. Este artículo documenta la factibilidad de usar estudios poblacionales para monitorear la ocurrencia de VPH en mujeres en PR.

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