Design and results of the USA-Mexico Border Human Papillomavirus (HPV), Cervical Dysplasia, and *Chlamydia trachomatis* Study

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ABSTRACT

Objective. *Mexico has one of the highest mortality rates of invasive cervical cancer in the world. This is particularly true for the states in northern Mexico that border on the United States of America. In addition, Hispanics in the United States have higher rates than do non-Hispanics in the country. Therefore, a binational team was formed to focus on this problem and to determine the risk factors and prevalence of cervical dysplasia and human papillomavirus (HPV) infection, the sexually transmitted disease (STD) known to cause cervical cancer.* Chlamydia trachomatis *infection, a common STD and potential HPV cofactor, was also assessed.*

Methods. *Research was conducted in 1997 and 1998 in the border region of two states, Arizona in the United States and Sonora in Mexico, applying a cross-sectional study of women attending clinics for routine gynecologic care. Clinical measurements included Pap smears, HPV infection by both polymerase chain reaction (PCR) and Hybrid Capture (HC), and* C. trachomatis *status by HC and enzyme-linked immunoassay (EIA). A total of 2 436 women were enrolled (mean age 33.3 years ± 10.3 years).*

Results. *The overall prevalence of abnormal cytology was 9.3%, with a significant difference in the prevalence in Mexico (11.4%) vs. the United States (6.6%). Of the participants, 14.5% of them tested positive for HPV by PCR, with no significant difference between the two countries, in spite of a lower behavioral risk profile for the Mexican women. Overall prevalence of* C. trachomatis *was found to be greater by HC than by EIA (8.2% vs. 3.0%), and in Mexico higher by both methods.*

Conclusions. *An important accomplishment of the project was the implementation of a quality control program for Pap smear collection, which resulted in a significant reduction in inadequate smears in Mexico. Despite numerous potential logistical barriers, the binational team successfully conducted a large-scale study in the border area and developed an infrastructure for future research.*

Key words

Human papillomavirus, cervical cancer, *Chlamydia trachomatis*, United States of America, Mexico.

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Invasive cervical cancer comprises 11.6% of all cancers among women worldwide and is the second most common cancer that they experience. Latin America has one of the highest cervical cancer incidence rates in the world, with an age-adjusted incidence of 40.8/100 000, far above the age-adjusted incidence of 9.5/100 000 in North America (1). Cervical cancer remains one of the most important health problems in developing countries such as Mexico. It is also a major concern among minority populations in the United States of America, whose incidence of cervical cancer (17.1/100 000) is significantly elevated in comparison to the rate for non-Hispanic whites in the country (7.5/100 000) (2).

Mexico has one of the highest invasive cervical cancer incidence and mortality rates in the world, with one death from cervical cancer approximately every 2 hours (3), a mortality rate of 5.2/100 000 (4), and an incidence rate of 45/100 000.[8] In general, higher cervical cancer mortality rates in Mexico are found among those states with the lowest socioeconomic status. While not among the poorer states in Mexico, the states in northern Mexico that border the United States have a significantly higher cervical cancer mortality rate than does Mexico City (3). As with other minority populations in the United States, Hispanics suffer disproportionately from invasive cervical cancer, with higher incidence and mortality rates than among non-Hispanic whites in the country (2). While the exact incidence of cervical cancer in the United States-Mexico border area is unknown, the existing data suggest that the rate is higher than that for the interior of both countries, respectively.

Evidence from epidemiological studies conducted around the world clearly indicates that human papillomavirus (HPV) infection is a cause of cervical cancer (5). Strong and consistent associations have been noted for oncogenic HPV types and invasive cervical cancer. While HPV infection is the most common sexually transmitted infection, relatively few women develop clinical lesions or cervical cancer. These data suggest that HPV is a necessary but insufficient cause of cervical cancer. Prevalence of specific types of HPV is known to vary in different regions of the world, as do the factors that may inhibit or promote HPV carcinogenesis (5). To date, no studies of HPV infection and other sexually transmitted diseases, such as infection with *Chlamydia trachomatis*, a potential HPV cofactor, have been conducted in the United States-Mexico border area.

The border states of Arizona in the United States and Sonora in Mexico have a history of more than 30 years of collaboration on public health issues through two commissions, the United States-Mexico Commission and the Sonora-Arizona Commission. In addition, there has been a long history of collaboration among other academic and nongovernmental organizations in Arizona and Sonora. This collaboration has been very important in terms of building strong working relationships around common issues that impact both states. In the past 6 years additional efforts have resulted in the identification of some specific common health issues for research and intervention, with STDs and cervical cancer being priorities.

In 1995 the Cervical Cancer Binational Technical Team was formed to focus specifically on research related to cervical cancer and *C. trachomatis*. To address the need for information regarding the prevalence and risk factors for cervical dysplasia and cancer, the group designed and implemented a study in the Arizona-Sonora border area. In this manuscript we describe the design, methods, and results of the USA-Mexico Border Human Papillomavirus (HPV), Cervical Dysplasia, and *Chlamydia trachomatis* Study. In a previous publication (6) a description of the binational collaboration process is provided.

METHODOLOGY

Study design

A cross-sectional survey was conducted from January 1997 to June 1998, recruiting women aged 15 years and older living in three pairs of contiguous Arizona-Sonora border communities and areas near them: Douglas-Agua Prieta, Nogales-Nogales, and Yuma-San Luis Río Colorado. In addition, Tucson, Arizona, and Hermosillo, Sonora, were included as they are the major city centers closest to the border in each of the respective states.

Laboratory measurements included HPV infection by polymerase chain reaction (PCR) and Hybrid Capture, *C. trachomatis* status by enzyme-linked immunoassay (EIA) and Hybrid Capture, and cytology results. In addition to the clinical measurements, study participants completed a risk factor questionnaire that assessed reproductive, sexual and medical histories, and demographic data.

Population

The study population consisted of women aged 15 years or older receiving routine gynecological care in border-area locations of Arizona and Sonora. The participants in Arizona were recruited from women attending family planning clinics at community health centers operated by nonprofit groups and at clinics operated by the local affiliate of the national nonprofit Planned Parenthood organization. The four Arizona locations were: Douglas, Bisbee, and Sierra Vista in Cochise County; Nogales in Santa Cruz County; Yuma in Yuma County; and Tucson in Pima County. The Arizona clinics in our study served primarily Hispanic women in the eligible age range for this study.

The Sonora, Mexico, participants were recruited from public health clinics that are part of that state's Secretariat of Public Health system. The Sonora locations were: Agua Prieta, Nogales, San Luis Río Colorado, and Hermosillo;

also included was Cananea, which is approximately equidistant from Agua Prieta and Nogales.

The three Arizona-Sonora border sister city areas we selected all have unique characteristics. The Nogales-Nogales area is one of the most important border crossing points for commerce between the United States and Mexico, and Nogales, Mexico, has over 60 *maquiladora* plants, where imported parts are assembled into products for export. The San Luis Río Colorado and Yuma area is the site of the largest agricultural production and farm worker population at the Arizona-Sonora border. Finally, the Douglas-Agua Prieta region is the smallest and most rural of these three sister city areas; it is an important mining area. The research group agreed that in order to have a representative sample from the Arizona-Sonora border all three areas needed to be included. Additionally, Hermosillo and Tucson were selected as similar large urban centers in the two states that are heavily influenced by their proximity to the border.

Participant eligibility

Participants were women who self-referred to community health centers and Planned Parenthood clinics in Arizona, and public health clinics in Sonora, for routine gynecological care. We invited women to participate in this study who were: a) 15 years of age or older, b) residents of the Arizona-Sonora border areas or of Hermosillo or Tucson, c) not pregnant, and d) still having menstrual periods (e.g., no hysterectomy).

Participant recruitment

Four bilingual female interviewers in Arizona and four female interviewers in Sonora worked in the participating study clinics. The interviewers worked closely with the clinics' staff members to identify all women who had made appointments for routine gynecological care each day, and who met the study eligibility age criteria. When meeting the client, the interviewer explained to her the nature of the study and asked about her willingness to participate. Following the routine Pap smear, each participant would have to complete a questionnaire, have a second cervical smear taken for HPV analyses, and have a third smear taken for *C. trachomatis* determination. If the client was interested, the research interviewer carefully explained the study, and the participant completed an informed consent form that had been approved by the institutional review boards of the University of Arizona, the Arizona Department of Health Services, the Secretariat of Public Health of Sonora, and the Colegio de Sonora. The number of women approached for participation, the number refusing to participate, and their reasons for not participating were kept in a log updated daily by each research interviewer.

Study protocol

The study participants completed an interviewer-administered questionnaire that assessed established risk factors for HPV infection, *C. trachomatis* infection, and cervical cancer. Experience from extensive focus group sessions and piloting of the questionnaire among the target population indicated that women were most comfortable and most willing to provide detailed information on sensitive topics if there was a trusting relationship with the interviewer. To facilitate this rapport, the questionnaire was administered by the interviewer in the language that the respondent chose, either Spanish or English.

After the Pap smear was obtained for routine clinical purposes, two additional samples of exfoliated cervical cells were obtained, for the analyses of HPV DNA and for *C. trachomatis* detection.

By mail, we sent all the study participants notification of their HPV and cytology results, with an accompanying explanation of these results and recommendations for scheduling of future Pap smears. Women were also notified if they tested positive for *C. trachomatis.*

In Mexico the Secretariat of Public Health of Sonora provided follow-up on positive *C. trachomatis* results.

Cytology

Development of a common scheme for reporting/recording Pap smears: pilot validation study. Before we began the main body of our research, we worked to determine the degree of concordance in cytology reporting between pathologists in Mexico and the United States. This was especially important given that the two countries utilize different diagnostic reporting schema. This preliminary step was a way to discover any patterns of diagnostic discordance, and, as needed, develop a consensus on them.

One hundred Pap smear slides collected in Mexico were rescreened in a blinded fashion by a pathologist from the University of Arizona. The diagnoses of the Pap smear slides were recorded per criteria as outlined in the Bethesda System for reporting cervical/vaginal cytologic diagnoses (7), and included evaluation of specimen adequacy. Evaluation of the specimen adequacy included: 1) amount of well-preserved and well-visualized squamous epithelial cells, 2) presence and amount of the endocervical/transformation zone component, 3) the degree (if any) of obscuring blood or inflammation, and 4) presence of air-drying artifact. With respect to specific diagnoses, a simplified set of five categories was established to reflect the need for clinical follow-up. This was done to derive data regarding diagnostic concordance between the two countries. The diagnostic categories were: 1) normal/inflammation, 2) atypical squamous cells of undetermined significance (ASCUS)/atypical glandular cells of undetermined significance (AGUS), 3) low-grade squamous intraepithelial lesion (HPV effect/mild dysplasia), 4) high-grade squamous intraepithelial lesion (moderate dysplasia/severe dysplasia/carcinoma in situ), and 5) squamous cell carcinoma. The degrees of diagnostic agreement/disagreement were reported numerically from 0 to 4,

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with 0 representing complete diagnostic agreement and 4 indicating maximum disagreement.

Through the process of validation, several findings were made. First, 70% of the Mexican specimens were not optimal due to the lack of endocervical cells on the smear. These were categorized as "satisfactory but limited by absence of endocervical cells" per the Bethesda System criteria for specimen adequacy. This finding clearly pointed to a need for standardization of specimen collection between the United States and Mexico. Upon further discussion, it was determined that in Sonora, the public health clinics collected cells for Pap smears using a tongue depressor that had been broken in half. Specific sampling of the endocervix was not performed. Thus, prior to onset of the study, health care professionals in Mexico at the clinic study sites were trained by members of the Department of Obstetrics and Gynecology at the University of Arizona and the Social Security and Services Institute for Government Employees Hospital, Hermosillo, Sonora, Mexico, in the technique of Pap smear collection as established in the United States. This technique included sampling of the ectocervix using a wooden Ayre spatula and sampling of the endocervix with a cytobrush.

Pilot validation study results. Of the 100 Pap smear slides collected in Mexico, 35 of them were discordant in their reading by the Mexican pathologists and the United States pathologist. Of these 35, 23 of them (65%) were a consequence of differences in reporting of HPV in Mexico and in the United States. Specifically, in the United States HPV, or koilocytotic atypia, is categorized under the rubric of low-grade squamous intraepithelial lesion (LGSIL). In Mexico, however, if HPV is the only abnormal finding (with no concomitant squamous dysplasia), the smear is reported as normal, with a comment stating the presence of viral changes. This discordance was considered a semantic issue rather than discordance based upon true disagree-

ment between actual cytologic observations. However, given the fact that HPV analyses of the study participants were planned, the decision was made to include HPV as part of the diagnosis rather than as a part of a comment.

Of the remaining 12 discrepant cases, 6 of them had a discordance of 1. Of these 6, 1 was diagnosed as mild dysplasia by Mexico and as ASCUS by the United States. Another of the 6 was reported as ASCUS by the United States and normal by Mexico. The remaining 4 of the 6 specimens with 1 degree of discordance were reported as moderate dysplasia by Mexico and as LGSIL by the United States. Of the 6 remaining cases with a discordance greater than 1, 5 of the cases had a discordance of 2 and the other case had a discordance of 3. With the discordance of 2, 4 were diagnosed as mild dysplasia by Mexico and as normal/inflammation by the United States. The other case was reported as severe dysplasia by Mexico and as ASCUS by the United States. The case with a discordance of 3 was reported as moderate dysplasia by Mexico and as normal by the United States. This most-discordant specimen was reviewed in depth, and it was found to be markedly air-dried and thus not optimal for cytologic evaluation.

Overall, the degree of agreement between the two countries with respect to cytologic diagnoses was good, with a kappa value of 0.73. Due to discordance noted above with the low-grade lesions, additional collaborative sessions were held to achieve as much consensus as possible between the two countries. Particular emphasis was directed at outlining criteria for the categories of ASCUS and AGUS, neither of which are used in the cervical intraepithelial neoplasia (CIN) grading system, which was developed before the Bethesda System.

An additional finding stemming from the pilot validation study was the observation that 70 of the 100 Pap smear slides from Mexico lacked an endocervical component. This was of particular significance since the transformation zone is the site wherein cervical dysplasia and neoplasia are thought to

arise. As such, its absence in a significant number of the smears highlighted several concerns with respect to the Pap smear techniques in Mexico: 1) the need for appropriate tools to sample the cervix, 2) the need for training of health care workers in the proper collection of Pap smears, and 3) the possibility that a significant number of preneoplastic lesions may have been missed due to inadequacy of sampling.

Common scheme coding system. The final cytological coding system that we developed for our study combined elements of both the Bethesda System and the CIN system. The diagnostic categories included specific diagnoses and evaluation of specimen adequacy (Table 1).

TABLE 1. Binational coding system for Pap smear results, Cervical Cancer Binational Technical Team, Sonora, Mexico, and Arizona, United States, 1997–1998

Diagnosis	Code
Normal	0
Inflammation	1
Atypical squamous cells of unknown significance (ASCUS)	2
ASCUS favor inflammation/reactive	3
ASCUS favor dysplasia	4
Atypical glandular cells of unknown significance (AGUS)	5
AGUS favor inflammation/reactive	6
AGUS favor dysplasia	7
Low-grade squamous intraepithelial lesion	8
Cellular changes consistent with HPV	9
Mild squamous dysplasia	10
High-grade squamous intraepithelial lesion	11
Moderate squamous dysplasia	12
Severe squamous dysplasia	13
Carcinoma in situ	14
Squamous cell carcinoma	15
Adenocarcinoma	16
Inadequate specimen	
Satisfactory but limited	17
Unsatisfactory	18
Satisfactory but limited/negative	19
Satisfactory but limited/inflammation	20
Satisfactory but limited/ASCUS	21

Collection of study pap smears. Following the completion of the pilot validation study and the development of a common scheme, cervical cytology specimens for the main part of our study were collected using the Ayre spatula to sample the ectocervix, and a cytobrush to sample the endocervix/transformation zone. Healthcare workers in each of the clinic sites received training in the proper collection technique from members of the Department of Obstetrics and Gynecology of the University of Arizona.

Review of study pap smear results. All Pap smear specimens collected from the four sites in the United States were read initially by pathologists in the laboratories with which the particular clinics were affiliated. These laboratories included: PathNet (Van Nuys, California), LabCorp (Phoenix, Arizona), SonoraQuest Laboratories (Phoenix, Arizona), Kino Community Hospital (Tucson, Arizona), Tucson Medical Center (Tucson, Arizona), and Smith-Kline Beecham (San Jose, California). One study pathologist (Janine Feng, University of Arizona, Department of Pathology) reread the cytology slides from the United States in a blinded fashion, and those diagnoses were reported for the study using the system developed collaboratively.

Pap smears from women enrolled in Mexico were initially read by cytologists in the Sonora State Laboratory of the Secretariat of Public Health. Another study pathologist (EMBG) then rescreened these Pap smears, adhering to the cytology coding system developed for this study (Table 1).

The two study pathologist then both reviewed all of the abnormals and 10% of the normals from both countries. Quality assurance data from the two countries found interrater agreements of 46.3% (Mexican review of United States slides, kappa = 0.292) and of 61.7% (United States review of Mexican slides, kappa = 0.523) if all 21 categories delineated in the final, common, binational coding system were used. When the system was condensed to six categories, the interrater agreement increased to 57.3% and 80.1% (kappas of 0.418 and 0.729), respectively. The major areas of disagreement were in the diagnoses of low-grade lesions and in ASCUS/AGUS categories. Good interrater agreement was noted for the high-grade lesions.

HPV cervical cell collection and detection

Cervical cells were collected for HPV analysis from the ectocervix and endocervix using a Cytosoft cytology brush and immediately suspended in 0.6 mL ViraType Diagnostics Sample Transport Medium (Digene Corporation, Gaithersburg, Maryland, United States). Following collection, samples were maintained at 4 °C for a maximum of 2 weeks, until being transported on ice to the University of Arizona laboratory. Samples were then maintained at –70 °C until analyzed.

HPV DNA analyses in exfoliated cervical cell samples were conducted using the Hybrid Capture 2 System (Digene Corporation, Gaithersburg, Maryland, United States) and polymerase chain reaction (PCR) from samples obtained during the gynecological examination. All specimens were analyzed by Digene Hybrid Capture 2 technology using the high-risk probe, which is designed to detect oncogenic HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. The Hybrid Capture 2 technology is a signal amplified hybridization antibody capture microplate assay (8). It utilizes chemiluminescent detection in which the presence of HPV is detected by light emitted and then measured as relative light units (RLUs). An RLU positive control ratio of ≥ 1 is regarded as HPV-positive for one or more of the above-mentioned oncogenic HPV types. Specimens with RLU cutoff value ratios < 1 are considered "negative" or "nondetected." The sensitivity of the test is 1 picogram of viral DNA per 100 µL. To ensure that samples were accurately classified, those samples testing with an RLU ratio of 1.0–2.0 were retested two subsequent times by Hybrid Capture. Only samples that had RLU ratios greater than 1.0 on two of the three tests were classified as HPV-positive by the Hybrid Capture method. The low-risk probe was not utilized in this study, and so nononcogenic HPV types were not detected using the Hybrid Capture 2 method.

HPV detection was also conducted using PCR with all the cervical cell samples collected. Genomic DNA was extracted following standard techniques. (9, 10). In brief, 50-µL aliquots were digested with 5 µL Proteinase K for 1 h at 65 °C, followed by 5 M ammonium acetate and ethanol precipitation. The crude DNA pellet was dried and resuspended in 50 µL 10 mM Tris, pH 7.5. The DNA extracts were then stored at –80 °C until amplification. Specimens were tested for the presence of HPV by amplifying 5 µL of the DNA extracts with the PGMY09/11 L1 consensus primer system (9) and AmpliTaq Gold polymerase (Perkin-Elmer, Foster City, California). In brief, each amplification contained 10× PCR Buffer II, 25 mM $MgCl_2$, 200 µL (each) dCTP, dGTP, and dATP, 600 µL dUTP, 5 U of AmpliTaq Gold, 50 µM of PGMY09, 50 µM of PGMY09, 50 µM PGMY11, 50 µM of B_PC04, 50 µM of B_GH20, and 5 µL of the template. For eventual inclusion of uracil-*N*-glycosylase to prevent product carryover, dTTP was replaced with dUTP. To determine specimen adequacy, the GH20/PC04 human β-globin target was coamplified with the HPV consensus primers. For every 10 samples a negative control (H_2O) and a positive control (CaSki cells) were run to control for contamination and accuracy.

The samples were amplified using the GeneAmp PCR System 9700 (Perkin-Elmer, Foster City, California). The following amplification profile was used: 95 °C hot start for 9 min, 95 °C denaturation for 1 min, 55 °C annealing for 1 min, and 72 °C extension for 1 min for 40 cycles; this was followed by a 5-min terminal extension at 72 °C and a hold step at 4 °C.

HPV genotyping was conducted using the reverse line blot method (10) on all samples that were positive by PCR. This detection method utilizes

the HPV L1 consensus PCR products labeled with biotin to detect 27 HPV types. The HPV genotype strip contains 29 probe lines, 27 for individual HPV genotypes and 2 with concentrations of the β-globin control probe. The following types are detected: 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51 to 59, 66, 68, MM4, MM7, MM8, and MM9. Briefly, the PCR products labeled with biotin were denatured and added to the probe strip in a hybridization buffer. After strips were washed, streptavidin-horseradish peroxidase was added to facilitate detection of the various HPV types. After final wash, buffer was removed by vacuum aspiration, and strips were rinsed in 0.1 M sodium citrate. Color development was activated by incubation in a mixture of hydrogen peroxide in sodium citrate buffer and tetramethylbenzidine in dimethylformamide for 5 min on a rotating platform (70 rpm). Developed strips were interpreted and photographed for future reference. Strip interpretation was performed with a labeled overlay, with lines indicating the position of each probe relative to the reference mark.

The oncogenic HPV types associated with cervical dysplasia and cancer include 16, 18, 31, 33, 35, 39, 45, 51, 52, 55, 56, 58, 59, 68, 26, MM4, MM7, and MM9. The nononcogenic types detected are 6, 11, 40, 42, 53, 54, 57, 66, and MM8. Furthermore, there are five oncogenic HPV types (26, 55, 73, MM4, and MM7) detected by PCR that are not included in the Hybrid Capture 2 high-risk probe.

C. trachomatis cell collection and detection

Following cervical sampling for cytology and HPV analyses, specimens for *C. trachomatis* analysis were collected from the endocervical os or the cervical canal, using a cotton swab. The cell specimens were placed in transport medium and sent for analysis by enzyme-linked immunoassay (EIA).

C. trachomatis testing was originally conducted at the Arizona Department

of Health Services (ADHS) laboratory using the EIA method. During the first half of the study, analysis was performed using Chlamydia Microplate EIA (Sanofi Diagnostics Pasteur, Inc., Chaska, Minnesota, United States); during the second half of the study, Chlamydiazyme EIA (Abbott Laboratories, Inc., North Chicago, Illinois, United States) was used. Specimen collection, transport, and analysis were performed in accordance with the manufacturers' instructions. All positive results were confirmed within 48 h by the blocking assay procedure.

C. trachomatis testing was also conducted using the CT-ID Hybrid Capture II (Digene Corporation, Gaithersburg, Maryland, United States) test at the University of Arizona laboratory. Like the Hybrid Capture 2 test for HPV analysis, this test is a signal amplified hybridization microplate assay that utilizes chemiluminescence for qualitative detection of *C. trachomatis* DNA. In addition, the *C. trachomatis* probe contains a mixture specifically

chosen to eliminate cross-reactivity with DNA sequences from human cells, other bacterial species, and chlamydia species other than trachomatis. The endocervical and ectocervical specimens collected for HPV detection, described above, were used for this second *C. trachomatis* analysis.

RESULTS

Participant recruitment and response rates

A total of 2 626 women were approached for recruitment and were initially screened for eligibility for the study. There were 2 578 women identified in this manner who met the study eligibility criteria, and 2 436 of these women were enrolled in the study. The overall response rate was 92.8%. Reasons for nonparticipation are shown in Table 2.

Table 3 shows the number of completed questionnaires and clinical lab-

TABLE 2. Reasons for nonparticipation by women approached for study, Cervical Cancer Binational Technical Team, Sonora, Mexico, and Arizona, United States, 1997–1998

Status/Reason	No.	% of 2 626 women approached
Eligible		
Refused participation, no reason	113	4.3
"Survey too personal"	3	0.1
"Too old"	5	0.2
Bleeding, no sample taken	21	0.8
Ineligible		
Pregnant	21	0.8
Hysterectomy	8	0.3
Other	19	0.7

TABLE 3. Completed study results by country, Cervical Cancer Binational Technical Team, Sonora, Mexico, and Arizona, United States, 1997–1998

	Mexico	United States	Total
Questionnaire	1 399	1 037	2 436
HPV (Hybrid Capture)	1 304	1 015	2 319
HPV (PCR)	1 258	988	2 246
Cytology	1 257	980	2 237
Chlamydia (EIA)	1 033	947	1 980
Chlamydia (Hybrid Capture)	1 285	985	2 270

oratory results. Survey questionnaires were completed by 1 399 women from Sonora, Mexico, and 1 037 from Arizona, United States. There were fewer *C. trachomatis* analyses completed with EIA than with Hybrid Capture, for reasons that included problems with transport of samples across the border and arrival and analysis of samples beyond the 5-day window for analyses utilizing the EIA method. Similarly, cytology results were unavailable for 199 women. Reasons for lack of cytology results included: samples were not obtained at the clinical visit, samples were unavailable for retrieval in Mexico, and samples were inadequate for evaluation. Seventy-three fewer PCR HPV results were available compared to Hybrid Capture HPV results due to the following reasons: inadequate sample (18 samples), unevaluable PCR due to 1 or 0 globin bands or lack of double stranded DNA (19 samples), and samples being sent to a reference laboratory for other analyses and thus not available for PCR testing (36 samples).

Selected characteristics of participants

Selected demographic and health characteristics are provided by country of enrollment in Table 4. The mean age of participants was 33.3 years ± 10.3 years. Mexican women were significantly older than the women in the United States ($P < 0.001$). Of the women in the United States, just over three-quarters of them were Mexican-American. Overall, fewer than 30% of the women had completed high school, with the women in the United States reporting a higher level of education than the women in Mexico. Sexual and reproductive histories differed significantly between the two countries. Women in the United States reported a lower age at first intercourse and a higher number of male sexual partners during their lifetime. Mexican women had more live births per woman and slightly lower oral contraceptive use. While only 53% of the entire study population had been screened by Pap smear in the preceding year, 87% of them had received screening in the preceding 3 years.

Prevalence of HPV and chlamydia infections and abnormal cytology

Pap smears were collected from 2 237 of the 2 436 women enrolled in the study, 17 of which resulted in inadequate/unsatisfactory specimens. Diagnoses were therefore available for 2 220 women (91.1%). Table 5 shows the age-adjusted prevalence of abnormal cytology by country. To control for the statistical differences in ages of Mexican and United States women, the population distribution of the entire study sample was used as an estimate of the standard population. The overall prevalence of abnormal cytology in this population was 9.3%. Significant differences in the age-adjusted prevalence of abnormal cytology were found between Mexico (11.4%) and United States (6.6%).

Table 5 also shows the HPV infection rates as determined by the two independent laboratory testing procedures, Hybrid Capture and PCR. Using the Hybrid Capture high-risk probe, 15.5% overall were HPV-positive, with no significant difference between the countries. Using PCR, 14.5% overall were positive for any HPV type, with a nonsignificant higher rate in the United States (15.1%) than in Mexico (13.8%) ($P = 0.405$). Approximately 83% of the HPV infections observed were infections with oncogenic-type viruses. The prevalence of oncogenic HPV was higher in the United States (12.8%) than in Mexico (11.3%), but the difference was not significant. No significant differences in prevalence of nononcogenic HPV were observed by country.

As explained earlier, *C. trachomatis* infection was determined using two independent procedures, EIA and Hybrid Capture. Tremendous differences in *C. trachomatis* prevalence were found with the two methods. Using Hybrid Capture, 8.2% of study participants were positive for *C. trachomatis*, with a significantly higher prevalence noted in Mexico (9.7%) than in the United States (6.7%) when controlling for age ($P = 0.013$). In contrast, using EIA, only 3.0% of the participants overall were positive, with an age-adjusted prevalence of 4.4% in Mexico and of 1.3% in the United States.

DISCUSSION

This is the first study to examine prevalence of HPV, *C. trachomatis*, and cervical dysplasia among women living in the United States-Mexico border area. Despite numerous logistical barriers to working binationally, which we have previously discussed (6), we successfully recruited and interviewed 2 436 women. Through this effort we developed an infrastructure with which to conduct future epidemiological studies of sexually transmitted diseases and cervical cancer.

Even though Mexican women had an overall lower behavioral risk profile for STDs, they had significantly higher rates of *C. trachomatis* in comparison to United States women. There were similar rates of oncogenic HPV infection in both groups. As expected, given the high incidence of invasive cervical cancer in Mexico, significantly higher rates of abnormal cytology were observed in Mexico than in the United States. Studies such as these are important in understanding the factors associated with cervical cancer and the programs that are needed for this region to reduce the disproportionately high rates of the disease.

One of the more important findings to emerge from this study is the problem associated with the lack of appropriate tools for implementing the Pap smear in public health clinics in Sonora. Results from the pilot cytology validation study that we conducted before the main body of our research indicated that most Pap smears obtained under routine clinical conditions in Sonora were lacking endocervical cells and had to be considered inadequate. Similar results have been found by other studies evaluating the quality of the Pap smear screening program in Mexico, with one of the

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TABLE 4. Distribution of selected characteristics of participants by country, Cervical Cancer Binational Technical Team, Sonora, Mexico, and Arizona, United States, 1997–1998

Characteristic	Mexico (n = 1 399)		United States (n = 1 037)		Total (n = 2 436)		P value[a]
	No.	%	No.	%	No.	%	
Age (years)							< 0.001
15–19	26	2.0	107	10.5	133	5.7	
20–24	183	14.0	208	20.4	391	16.8	
25–34	519	39.6	347	34.0	866	37.1	
35–44	389	29.6	240	23.5	629	27.0	
45–79	195	14.9	120	11.7	315	13.5	
Mean (standard deviation)	34.5 (10.0)		31.6 (10.4)		33.3 (10.3)		< 0.001
Educational level							< 0.001
< Primary	237	18.7	35	3.5	272	12.0	
Primary completed	435	34.3	146	14.6	581	25.6	
Middle school completed	434	34.2	327	32.7	761	33.5	
High school completed	84	6.6	239	23.9	323	14.2	
> High school	79	6.2	253	25.3	332	14.6	
Race/Ethnicity							< 0.001
Mexican	1 399	100.0	0	0.0	1 399	57.8	
Mexican American	0	0.0	783	76.5	783	32.3	
White non-Hispanic	0	0.0	213	20.8	213	8.8	
Other	0	0.0	27	2.6	27	1.1	
Smoking history							< 0.001
Never	1 022	76.2	708	69	1 730	73.1	
Former	110	8.2	132	12.9	242	10.2	
Current	209	15.6	186	18.1	395	16.7	
Age at first intercourse							0.006
> 20	470	35.2	296	29.2	766	32.6	
18–19	330	24.7	242	23.9	572	24.3	
16–17	298	22.3	269	26.6	567	24.1	
6–15	239	17.9	206	20.3	445	18.9	
Total no. of male sexual partners							< 0.001
1	830	64.7	507	50.5	1 337	58.5	
2–3	365	28.5	269	26.8	634	27.7	
4–9	58	4.5	153	15.2	211	9.2	
10–100	30	2.3	75	7.5	105	4.6	
Mean (standard deviation)	2.1 (4.4)		3.2 (5.4)		2.6 (4.9)		< 0.001
Duration of oral contraceptive use (years)							0.024
0–1	540	38.6	349	33.7	889	36.5	
1–3	511	36.6	390	37.6	901	37.0	
≥ 4	347	24.8	297	28.7	644	26.5	
Mean (standard deviation)	2.6 (4.4)		2.8 (4.3)		2.7 (4.4)		0.347
Total number of live births							< 0.001
0	127	9.1	249	24.0	376	15.4	
1–2	562	40.2	436	42.0	998	41.0	
3–4	523	37.4	280	27.0	803	33.0	
5–6	125	8.9	53	5.1	178	7.3	
7–15	62	4.4	19	1.8	81	3.3	
Mean (standard deviation)	2.8 (2.0)		2.0 (1.7)		2.4 (1.9)		< 0.001
Pap smear in past year							< 0.001
No	528	50.8	385	42.1	913	46.7	
Yes	512	49.2	529	57.9	1 041	53.3	
Pap smear in past 3 years							0.020
No	153	14.7	102	11.2	255	13.1	
Yes	887	85.3	812	88.8	1 699	87.0	

[a] P value is for difference between Mexico and the United States.

TABLE 5. Age-adjusted prevalence (% and 95% confidence interval) of abnormal cytology, of HPV, and of *Chlamydia trachomatis,* by country, Cervical Cancer Binational Technical Team, Sonora, Mexico, and Arizona, United States, 1997–1998

	Overall		Mexico		United States	
	No.	%	%	95% CI	%	95% CI
Abnormal cytology	206	9.3	11.4	9.5–13.2	6.6	5.0–8.2
HPV						
Hybrid Capture	359	15.5	15.5	13.4–17.7	15.2	13.0–17.4
PCR						
All types	325	14.5	13.8	11.7–15.9	15.1	12.9–17.3
Oncogenic type	270	12.1	11.3	9.4–13.2	12.8	10.7–14.9
Nononcogenic type	42	1.9	1.8	0.9–2.7	2.2	1.2–3.1
Chlamydia trachomatis						
EIA	59	3.0	4.4	2.8–6.0	1.3	0.6–2.0
Hybrid Capture	185	8.2	9.7	7.8–11.5	6.7	5.2–8.2

Pap smear screening program has been that it focuses needless annual and biennial screening on low-risk groups and inadequately screens high-risk groups. Reflex HPV testing could provide an objective measure of risk from which to determine screening and postscreening guidelines.

Building on the results of this study, we are now evaluating risk factors for abnormal cytology, including a thorough examination of type-specific HPV infection and sexual and reproductive factors as they relate to the utility of HPV testing in this border population. Such information may be useful in identifying innovative methods of cervical cancer prevention, including an efficacious HPV vaccine.

studies (11) showing 64% of samples lacking endocervical cells, and cytopathology laboratories presenting overall false negative rates of 10% to 54%. We implemented a quality control program in our study that spanned the spectrum of obtaining, fixing, staining, transporting, and diagnosing smears. This program resulted in a dramatic reduction of inadequate smears in Mexico, from 70% to 9.8%, and a considerable improvement in the reliability of cytology readings. Our experience supports what others (3) have suggested should occur on a national level in countries faced with a high incidence and mortality of cervical cancer. While we were able to improve the quality of the Pap smear screening program in Sonora during the course of the study, repeated quality control measures would need to occur to ensure that these gains in precision and reliability were maintained under normal clinical conditions.

In this study we found an overall HPV prevalence of 14.5% by PCR and 12.1% infection with oncogenic HPV types among the participating women. There was no significant difference between Mexico and the United States in the prevalence of oncogenic HPV types. The prevalence of HPV infection in this study is similar to the rates reported for other Mexican populations (4) and for Mexican-American populations (12).

Recent studies suggest that given the high rate of infection in young women, HPV testing of the general population would not be useful clinically, but instead should be used as a method for triaging women presenting with ASCUS and LGSIL (13). This method of reflex HPV testing would have tremendous implications for the Pap smear screening interval currently recommended in Mexico and the United States. One of the criticisms directed toward the Mexican national

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RESUMEN

Diseño y resultados del estudio sobre los papilomavirus humanos (PVH), la displasia cervical y *Chlamydia trachomatis* en la frontera de Estados Unidos y México

Objetivos. México tiene una de las mayores tasas mundiales de mortalidad por cáncer cervical invasor, sobre todo en los estados del norte que tienen frontera con los Estados Unidos de América (EE.UU.). Además, los hispanos residentes en los EE.UU. tienen mayores tasas que los no hispanos de este país. Considerando esta situación, se formó un equipo de ambos países para analizar el problema y determinar la prevalencia y factores de riesgo de la displasia cervical y de la infección por papilomavirus humanos (PVH), una enfermedad de transmisión sexual que se sabe que causa cáncer cervical. También se analizó la infección por *Chlamydia trachomatis,* una enfermedad de transmisión sexual frecuente que podría constituir un cofactor de los PVH.

Métodos. La investigación, realizada en 1997 y 1998 en la zona fronteriza de los estados de Arizona (EE.UU.) y Sonora (México), consistió en un estudio transversal de 2 436 mujeres (media de 33,3 ± 10,3 años) que acudían a consultas ginecológicas de rutina. Se efectuaron frotis cervicales de Papanicolaou y pruebas de detección de la infección por PVH, mediante la reacción en cadena de la polimerasa (PCR) y la captación de híbridos (HC), y de la infección por *C. trachomatis,* mediante HC e inmunoanálisis enzimático (EIA).

Resultados. La prevalencia global de exámenes citológicos anormales fue del 9,3%; la diferencia entre México (11,4%) y EE.UU. (6,6%) fue significativa. Un 14,5% de las participantes fueron positivas para los PVH en la PCR, sin que hubiera diferencias entre los dos países, y esto a pesar de que las mujeres mejicanas tenían menores riesgos relacionados con la conducta. La prevalencia global de *C. trachomatis* fue mayor con la HC que con el EIA (8,2% frente a 3,0%) y fue mayor en México que en EE.UU. con ambos métodos.

Conclusiones. Un importante logro de este proyecto consistió en la puesta en marcha de un programa de control de la calidad de la recolección de los frotis de Papanicolaou que dio lugar a una notable reducción de los frotis inadecuados en México. A pesar de numerosas posibles barreras logísticas, el equipo de los dos países consiguió realizar con éxito un estudio a gran escala en el área fronteriza y desarrollar la infraestructura para futuras investigaciones.