An Investigation of Genital Ulcers in Jackson, Mississippi, with Use of a Multiplex Polymerase Chain Reaction Assay: High Prevalence of Chancroid and Human Immunodeficiency Virus Infection

Kristen J. Mertz, Judith B. Weiss, Risa M. Webb, William C. Levine, Joel S. Lewis, Karina A. Orle, Patricia A. Totten, Julie Overbaugh, Stephen A. Morse, Mary M. Currier, Martin Fishbein,* and Michael E. St. Louis

Division of STD Prevention, National Center for HIV, STD, and Tuberculosis Prevention, and Division of AIDS, STD, and Tuberculosis Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; Roche Molecular Systems, Alameda, California; Mississippi State Department of Health, Jackson; Departments of Medicine and Microbiology, University of Washington, Seattle

In 1994, an apparent outbreak of atypical genital ulcers was noted by clinicians at the sexually transmitted disease clinic in Jackson, Mississippi. Of 143 patients with ulcers tested with a multiplex polymerase chain reaction (PCR) assay, 56 (39%) were positive for Haemophilus ducreyi, 44 (31%) for herpes simplex virus, and 27 (19%) for Treponema pallidum; 12 (8%) were positive for organ. Of 136 patients tested for human immunodeficiency virus (HIV) by serology, 14 (10%) were HIV-seropositive, compared with none of 200 patients without ulcers (P < .001). HIV-1 DNA was detected by PCR in ulcers of 6 (50%) of 12 HIV-positive patients. Multivariate analysis indicated that men with chancroid were significantly more likely than male patients without ulcers to report sex with a crack cocaine user, exchange of money or drugs for sex, and multiple sex partners. The strong association between genital ulcers and HIV infection in this population highlights the urgency of preventing genital ulcers in the southern United States.

The number and percentage of persons with AIDS infected through heterosexual contact are increasing in the United States [1]. In 1993, the South had the highest percentage (42%) of AIDS cases associated with heterosexual contact [1]. The South also historically has had the highest rates of reported bacterial sexually transmitted diseases (STDs) in the country [2]. Factors that contribute to high rates of traditional STDs, such as poverty, barriers to timely and effective clinical care, and the quality of the public health infrastructure, may be contributing to this emerging heterosexual human immunodeficiency virus (HIV) epidemic. The synergistic effect of other STDs, especially ulcerative STDs, in increasing the transmission probability for HIV infection may also be an important reason for the increase in heterosexually acquired HIV [3–5].

At the STD clinic in Jackson, Mississippi, in September 1994, clinicians reported seeing patients with genital ulcers characteristic of chancroid, some of whom were HIV-positive. Chancroid, however, is often difficult to distinguish on clinical grounds alone from herpes simplex virus (HSV) and syphilis [6–9], the two most common ulcerative diseases in the United States. Microbiologic testing at the clinic for genital ulcer pathogens was limited; only the rapid plasma reagin (RPR) card test for syphilis was available. Neither darkfield microscopy for detecting Treponema pallidum nor culture for identifying Haemophilus ducreyi was available; culture for HSV was rarely done because of cost considerations. Using a multiplex polymerase chain reaction (M-PCR) assay, which from a single ulcer swab specimen detects specific DNA sequences from H. ducreyi, T. pallidum, and HSV [10], we conducted an investigation to determine the relative contribution of each organism to genital ulcers in Jackson, to identify risk factors associated with genital ulcer disease, and to assess the association of genital ulcers with HIV infection.

Methods

Study population. From 20 October 1994 to 3 May 1995, we collected ulcer specimens and did routine serologic testing for syphilis and HIV-1 for all patients with genital ulcers at the only public STD clinic in Jackson. Patients were enrolled only once in the study. We conducted 15-min interviews for most ulcer patients; some were not interviewed because no interviewer was available, and patients seen at the clinic >30 days after their ulcers appeared were not interviewed to avoid possible recall bias, though they were tested by M-PCR and serology.

For each case interviewed, we selected 2 controls of the same sex and age group (15–19, 20–24, 25–29, 30–39, and ≥40 years) from clinic patients with no genital ulcers; no self-reported history of chancroid, syphilis, or herpes; and no sex partners with these...
diseases. If the most recently registered clinic patient met the demographic and clinical criteria, the patient was invited to participate; otherwise, we waited for a new registrant. Controls were tested for syphilis by the RPR test and for HIV-1 following the usual clinic protocol, and those with reactive RPR tests were excluded from the study because of the likelihood of current or past syphilis infection.

Laboratory investigation. For all STD clinic patients with one or more genital ulcers, multiple swabs were collected from the base of the largest ulcer after moistening the ulcer with saline. A swab for the M-PCR was agitated in 1 mL of Amplicor Specimen Transport Medium (STM; Roche Diagnostic Systems, Branchburg, NJ) and then discarded, a swab for an independent Southern-based *H. ducreyi* PCR and for an HIV-1 PCR was agitated in 1 mL of 50 mM Tris-HCl buffer (pH 7.8) containing 1 mM EDTA and 1 mg of sodium chenodeoxycholate and then discarded, a swab for M-PCR was placed in chlamydia EIA transport medium (EIA-TM; Baxter Diagnostics, Bartels Division, Issaquah, WA), and a swab for *H. ducreyi* culture was inoculated onto chocolate agar containing 10% fetal bovine serum and heart infusion agar with 5% rabbit blood, both supplemented with 1% IsoVitaleX (Becton Dickinson Microbiology Systems, Cockeysville, MD) and 3 µg/mL vancomycin.

All 3 specimens for PCR were stored at −20°C, transported frozen, and thawed before testing. First-swab specimens for M-PCR were aliquoted, diluted, and tested in duplicate as described previously [10], except that 10 U of Taq polymerase (AmpliTaq; Perkin-Elmer, Norwalk, CT) was used. PCR inhibition was analyzed by use of an internal control plasmid. Confirmatory PCR assays for each of the three organisms, which target different gene sequences, were also done on all specimens [10]. Specimens for M-PCR in EIA-TM were diluted 2-fold with STM, incubated for 10 min at room temperature, and then diluted and tested by M-PCR as described previously [10]. From specimens for the independent Southern-based *H. ducreyi* PCR, 200 µL of the sample was extracted first with phenol and then with chloroform, precipitated with ethanol, resuspended in 100 µL of distilled water, and analyzed as described previously [11] except that the MgCl₂ concentration was increased to 4.0 mM and PCR products were detected on Southern blots. Samples for this assay were extracted and amplified in duplicate on 2 separate days and scored as positive only if *H. ducreyi* DNA was detected in two separate analyses. Specimens that tested negative for β-globin DNA by a β-globin PCR assay [12] were excluded from the analysis, as were specimens for which volume was insufficient for two extractions.

Using the same specimens as for the Southern-based *H. ducreyi* PCR, we tested for the presence of HIV-1 gag DNA following a nested PCR protocol for patients who were HIV-1–seropositive and a sample of those HIV-1–seronegative [13]. The conditions and primers for PCR amplification were as described previously [13], except that 2.5 mM MgCl₂ was used in the first-round reaction. Samples were tested at up to 8 µL in a 25-µL first-round reaction volume.

Inoculated growth media for *H. ducreyi* were incubated for 48–72 h between 33° and 35°C in a candle jar placed in a portable incubator at the clinic. Any visible growth was then transferred by swab to a freezing medium (trypticase soy broth containing 15% glycerol), which was stored at −20°C, transported frozen, and then thawed and plated on chocolate agar containing 10% fetal bovine serum and 1% IsoVitaleX. Presumptive isolates of *H. ducreyi* were confirmed by typical colony morphology and Gram’s stain, requirement for X but not V factor, and whole cell homology to the *H. ducreyi* type strain CIP 542 by the taxonomic spot blot test [14]. Plate dilution MICs were determined both for antibiotics to which *H. ducreyi* strains are variably resistant (penicillin, tetracycline, kanamycin, and chloramphenicol) and for those used in currently recommended treatment regimens (ceftixime, erythromycin, and azithromycin) [15].

Serologic testing for syphilis, by RPR, and for HIV-1, by an EIA (HIV Ab-1; Abbott Laboratories, Chicago) with confirmation by Western blot (Immunoblot; Bio-Rad Laboratories, Richmond, CA), were done. Sera with reactive RPR results were tested by the microhemagglutination assay for antibodies to *T. pallidum* (MHA-TP), and if nonreactive by MHA-TP, they were subsequently tested by the fluorescent treponemal antibody absorption (FTA-ABS) test.

Clinical and epidemiologic evaluation. Information on the clinical characteristics of ulcers was recorded by a clinician for each ulcer patient. Information on demographics, sexual behavior, and substance abuse was collected during the interview; patients with genital ulcers were asked about their behavior during the month before they noticed their lesions, and controls were asked about their behavior during the month before enrollment.

Statistical analysis. M-PCR results were used to determine the prevalence of *H. ducreyi*, *T. pallidum*, and HSV. The χ² test statistic was used to compare the clinical characteristics of patients with single infections of *H. ducreyi*, *T. pallidum*, HSV, and no identified etiology. The Cochran-Mantel-Haenszel test statistic was used to compare demographic information and risk behavior, controlling for age group, for men with single infections of *H. ducreyi*, *T. pallidum*, or HSV who were interviewed and all male controls, and for all female ulcer patients who were interviewed and all female controls. A multivariate logistic regression model for men with *H. ducreyi* only and all male controls, adjusting for age group, was constructed by backward selection, beginning with variables significant in the univariate analysis. The variable for having a casual partner was excluded because of intercorrelation with other variables. The number of female patients was too small for multivariate analysis. We calculated a population-based annualized incidence for chancroid in the City of Jackson based on the number of cases detected by M-PCR at the STD clinic during the 6.5-month study period and the US census estimate of the population for 1994.

Results

Laboratory investigation. Between 20 October 1994 and 3 May 1995, ulcer swab specimens were tested by M-PCR for 143 patients (111 men, 32 women) at the STD clinic in Jackson. Forty-seven specimens (33%) were positive for *H. ducreyi* alone, and 9 (6%) were positive for *H. ducreyi* in addition to other organisms, for a total of 56 (39%) positive for *H. ducreyi* (table 1). Based on this number of PCR-proven *H. ducreyi* infections, the estimated annual incidence for chancroid in Jackson was 54 cases/100,000 population. Thirty-nine specimens (27%) were positive for HSV alone, and 5 (3%) were
positive for HSV and other organisms, for a total of 44 (31%) positive for HSV. Sixteen specimens (11%) with a single detectable pathogen and 11 (8%) with multiple pathogens were positive for *T. pallidum*. Twenty-nine specimens (20%) were negative for all three organisms. Results of the confirmatory PCR tests targeting different gene sequences were identical to the M-PCR results (table 2). The most common pathogen among men was *H. ducreyi*, detected in 47 (42%) of 111 specimens (table 1). For women, HSV was the most common identified pathogen, detected in 15 (47%) of 32 specimens.

Of 143 patients tested by M-PCR, 136 were tested for HIV or were considered positive on the basis of a previous test. Of these, 14 (10%) were HIV-positive, 1 had an indeterminate result, and 121 were negative (table 3). Ulcer patients who were positive for *H. ducreyi* by M-PCR had a higher prevalence of HIV infection than did ulcer patients who were negative for *H. ducreyi* (13% vs. 8%), although this finding was not statistically significant ($P = .4$). Of 107 men with genital ulcers who were tested for HIV, 12 (11%) were positive. Of 29 women with ulcers who were tested for HIV, 2 (7%) were positive; both had *H. ducreyi* detected by M-PCR. Of the 201 control patients from the STD clinic without genital ulcers or a history of ulcers, 200 (99%) were tested for HIV, and none were HIV-positive ($P < .001$ compared with ulcer patients).

Of ulcer specimens from 12 HIV-seropositive patients tested for HIV-1 gag DNA by PCR, 6 (50%) were positive. Among HIV-seropositive patients, those with detectable HIV-1 DNA in the ulcer specimen included 2 of 5 positive for *H. ducreyi* only by M-PCR, the 1 patient with *T. pallidum* only, 1 of 2 with HSV only, and 2 of 3 with unknown ulcer etiology; the patient with all three ulcer pathogens detected did not test positive for HIV-1 DNA. HIV-1 DNA was not detected in any of the 9 ulcer specimens from HIV-seronegative patients.

Of the 143 ulcer patients, 47 (33%) had reactive RPR tests (table 3). Of 27 patients with *T. pallidum* detected by M-PCR, 23 (85%) had reactive RPR tests. Of these 23, 19 were confirmed by MHA-TP or FTA-ABS (1 was negative and 3 were not tested). Of 47 ulcer patients with reactive RPR tests, 24 (51%) were negative for *T. pallidum* by M-PCR; 11 of these 24 patients tested positive for *H. ducreyi* and 6 for HSV, and 7 had no identified etiology. Of these 24, 21 were confirmed by MHA-TP or FTA-ABS (2 were negative and 1 was not tested).

The high frequency of chancroid as detected by M-PCR on first-swab specimens in STM was confirmed by results of the independent Southern-based PCR for *H. ducreyi* and the M-PCR on specimens in EIA-TM (table 2). Isolation of *H. ducreyi*, however, was less successful; 3 isolates of *H. ducreyi* were recovered, all from patients positive for *H. ducreyi* by M-PCR. All 3 isolates were susceptible [16] to all antibiotics tested with the following MICs (µg/mL): penicillin, ≤1; tetracycline, 1;
kanamycin, 4; chloramphenicol, 1; ceftriaxone, <0.004; erythromycin, 0.06; and azithromycin, 0.015.

Clinical and epidemiologic investigation. Single infections with *H. ducreyi*, *T. pallidum*, and HSV could not be reliably distinguished by clinical presentation (table 4). A high proportion of patients with each of the diseases had ulcers that were described by the patients as painful or by a clinician as tender, purulent, or ragged; these characteristics were less common for ulcers with no identified etiology. Of the 20 ulcers with all five characteristics of a classic chancroidal ulcer (pain, tenderness, ragged border, purulence, and tender inguinal adenopathy), 13 (65%) tested positive for *H. ducreyi* by M-PCR; however, these 13 represented only 25% of the 53 ulcers testing positive for *H. ducreyi* for which complete clinical information was available (including 9 ulcers coinfected with *T. pallidum* or HSV). No single characteristic had a positive predictive value of >50% for *H. ducreyi*.

Of 143 ulcer patients tested, 10 had ulcers of >30 days’ duration and thus were not interviewed, 1 refused to be interviewed, and 29 attended the clinic when no interviewer was available. The 103 ulcer patients interviewed had overall M-PCR results similar to those of all 143 ulcer patients tested. Two hundred one patients without ulcers were interviewed as controls; the study was terminated before the remaining 5 controls were identified, although each case had at least 1 control. Of the 201 controls, 70% had other symptoms of STDs, including 55% with urethral discharge.

The majority of ulcer patients and controls were African-American (92%), unmarried (89%), and employed (64%). No patients reported intravenous drug use during the preceding month, although 6 ulcer patients (6%) and 8 controls (4%) reported injecting drugs in the past. Only 2 patients with ulcers (2%) and 2 controls (1%) reported having a sex partner of the same sex during the preceding month.

Compared with the 155 male controls, men testing positive for *H. ducreyi* by M-PCR were significantly more likely to have had a casual partner, exchanged money or drugs for sex, used crack or other form of cocaine, or met a casual partner on the street, or had unprotected sex with a casual partner (table 5). Male syphilis patients also were more likely than male controls to have used crack or to have had a sex partner who used crack. Male HSV patients were more likely than male controls to have had a sex partner who used marijuana; for most characteristics, however, men with HSV were similar to controls. Male ulcer patients with no identified etiology were more likely than male controls to have exchanged money or drugs for sex, used crack or other form of cocaine, had a partner who used crack or other form of cocaine, or met a casual partner on the street (data not shown).

In the multivariate analysis comparing male chancroid patients with male controls, having a partner who used crack or other form of cocaine (odds ratio [OR], 26.8; 95% confidence interval [CI], 4.6–156), exchanging money or drugs for sex (OR, 5.5; 95% CI, 1.2–24.4), and having multiple partners (OR, 3.8; 95% CI, 1.1–13.3) were significantly associated with *H. ducreyi* infection. Of male patients with chancroid, 66% had one or both of the first two characteristics.

Of 32 women with genital ulcers, 23 (72%) were interviewed. Of these, 14 had HSV only detected by M-PCR, 3 had *T. pallidum* only, 1 had *H. ducreyi* only, 2 had multiple infections, and 3 had no detectable organisms. Their behavioral characteristics were very similar to those of male ulcer patients with only HSV detected by M-PCR. A higher percentage of women with ulcers than of female controls reported having a casual sex partner (35% vs. 20%; *P* = .15), having unprotected sex with a casual partner (26% vs. 9%; *P* = .05), or taking money or drugs for sex (13% vs. 0; *P* = .01).

**Table 4.** Characteristics of genital ulcers for patients with single or no identified etiology by PCR.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><em>Haemophilus ducreyi</em></th>
<th><em>Treponema pallidum</em></th>
<th>HSV</th>
<th>No etiology identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2 ulcers</td>
<td>28/47 (60)</td>
<td>7/16 (44)</td>
<td>25/39 (64)</td>
<td>12/29 (41)</td>
</tr>
<tr>
<td>Largest ulcer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Painful by patient’s history</td>
<td>36/46 (78)</td>
<td>11/15 (73)</td>
<td>30/38 (79)</td>
<td>15/27 (56)*</td>
</tr>
<tr>
<td>Tender‡</td>
<td>34/46 (74)</td>
<td>8/15 (53)</td>
<td>23/38 (61)</td>
<td>11/28 (39)*</td>
</tr>
<tr>
<td>Purulent base</td>
<td>34/46 (74)</td>
<td>9/15 (60)</td>
<td>21/38 (55)</td>
<td>10/27 (37)*</td>
</tr>
<tr>
<td>Ragged border</td>
<td>32/45 (71)</td>
<td>11/15 (73)</td>
<td>23/38 (61)</td>
<td>16/29 (55)</td>
</tr>
<tr>
<td>Size ≥1 cm</td>
<td>28/45 (62)</td>
<td>8/14 (57)</td>
<td>16/39 (41)</td>
<td>9/28 (32)*</td>
</tr>
<tr>
<td>Inguinal adenopathy</td>
<td>29/47 (62)</td>
<td>13/15 (87)</td>
<td>17/39 (44)</td>
<td>10/28 (36)*</td>
</tr>
<tr>
<td>Tender inguinal adenopathy</td>
<td>20/28 (71)</td>
<td>8/12 (67)</td>
<td>7/16 (44)</td>
<td>4/9 (44)</td>
</tr>
<tr>
<td>Unilateral adenopathy</td>
<td>17/29 (59)</td>
<td>9/12 (75)</td>
<td>12/17 (71)</td>
<td>6/10 (60)</td>
</tr>
<tr>
<td>Circumcised (men only)</td>
<td>11/39 (28)</td>
<td>3/12 (25)</td>
<td>11/24 (46)</td>
<td>6/23 (26)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of patients with characteristic/no. of patients for whom information is available (%). HSV, herpes simplex virus.

* P < .05, compared with *H. ducreyi*.

† P < .05, compared with HSV.

‡ Score of 2 or 3 on scale of 0 (not at all tender) to 3 (extremely tender).

§ P < .05, compared with *T. pallidum*.
Table 5. Behavioral characteristics of male patients with single infections of chancroid, syphilis, or genital herpes compared with controls during month before they noticed genital sore.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Chancroid</th>
<th>Syphilis</th>
<th>Herpes</th>
<th>Controls</th>
<th>Odds ratio* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex with &gt;=2 partners</td>
<td>19/29 (66)</td>
<td>3/8 (38)</td>
<td>5/20 (25)</td>
<td>46/155 (30)</td>
<td>5.1 (2.3–11.6)</td>
</tr>
<tr>
<td>Sex with casual partner</td>
<td>25/29 (86)</td>
<td>3/8 (38)</td>
<td>7/20 (35)</td>
<td>65/155 (42)</td>
<td>9.5 (3.5–25.9)</td>
</tr>
<tr>
<td>Unprotected sex with casual partner</td>
<td>21/29 (72)</td>
<td>2/8 (25)</td>
<td>5/20 (25)</td>
<td>42/155 (27)</td>
<td>7.1 (3.1–16.4)</td>
</tr>
<tr>
<td>Exchanged money/drugs for sex</td>
<td>15/29 (52)</td>
<td>1/8 (13)</td>
<td>1/20 (5)</td>
<td>6/154 (4)</td>
<td>23.1 (8.9–60)</td>
</tr>
<tr>
<td>Met partner on street</td>
<td>13/29 (45)</td>
<td>1/8 (13)</td>
<td>1/20 (5)</td>
<td>10/155 (7)</td>
<td>10.9 (4.3–27.8)</td>
</tr>
<tr>
<td>Used crack or cocaine</td>
<td>5/29 (17)</td>
<td>4/8 (50)</td>
<td>1/20 (5)</td>
<td>4/154 (3)</td>
<td>5.6 (1.3–23.0)</td>
</tr>
<tr>
<td>Drank alcohol</td>
<td>23/29 (79)</td>
<td>7/8 (88)</td>
<td>13/19 (68)</td>
<td>171/154 (76)</td>
<td>1.0 (0.4–2.7)</td>
</tr>
<tr>
<td>Sex partner used crack or cocaine</td>
<td>15/24 (63)</td>
<td>2/8 (25)</td>
<td>2/18 (11)</td>
<td>5/139 (4)</td>
<td>44.0 (14.8–131)</td>
</tr>
<tr>
<td>Sex partner drank alcohol</td>
<td>21/26 (81)</td>
<td>2/8 (25)</td>
<td>12/19 (63)</td>
<td>80/142 (57)</td>
<td>2.6 (0.9–7.2)</td>
</tr>
<tr>
<td>Sex partner used marijuana</td>
<td>5/21 (24)</td>
<td>2/6 (33)</td>
<td>7/17 (41)</td>
<td>17/133 (13)</td>
<td>2.5 (0.8–7.7)</td>
</tr>
<tr>
<td>Had “steady or main” sex partner</td>
<td>14/29 (48)</td>
<td>7/8 (88)</td>
<td>17/20 (85)</td>
<td>114/155 (74)</td>
<td>0.3 (0.1–0.8)</td>
</tr>
</tbody>
</table>

NOTE: Data are no. of patients with characteristic/no. of patients for whom information is available (%); “don’t know” responses were excluded from analysis.
* Odds ratio for male chancroid patients compared with male controls. CI, confidence interval.
² P < .05, compared with controls.

Discussion

In this investigation, a new research prototype PCR assay for genital ulcers documented a high incidence of chancroid at the public STD clinic serving a primarily heterosexual population in Jackson, Mississippi, where chancroid had not previously been reported or widely suspected. The estimated annual incidence of chancroid in Jackson was 180 times the reported incidence of 0.3 cases/100,000 population in the United States in 1994 [2]. These findings demonstrate that a high level of chancroid may exist in communities in the United States with delayed or perhaps no recognition. This is alarming, given the strong and consistent association of chancroid with HIV transmission [17–19], although not surprising, given the limited capacity of facilities serving STD patients to perform laboratory tests for chancroid [20].

According to previous studies and our own clinical data, chancroid is difficult to diagnose by clinical appearance [6–9]. It cannot be excluded by a reactive RPR, especially in settings of high syphilis morbidity [21]. At the time of our investigation, Mississippi had the highest rate of reported primary and secondary syphilis in the country: 72.4 cases/100,000 population in 1994 [2]. We found that more than one-third of ulcer patients with reactive RPR tests, typically considered diagnostic of syphilis, had H. ducreyi by M-PCR. A patient with a lesion due to chancroid or HSV may have a reactive RPR test because of previously treated or latent syphilis or because of coinfection with T. pallidum. Before the investigation, many ulcer patients were treated on the basis of RPR results; after an appreciable prevalence of chancroid was confirmed by M-PCR within several weeks of initiating this investigation, ulcer patients at the clinic were treated presumptively for both chancroid and syphilis [22, 23].

The M-PCR test has been shown to be more sensitive than reference diagnostic tests [10, 24]. The reference test for chancroid, which is culture, is rarely available in clinical settings, and its sensitivity ranges from 0 to 84% [25]. In our study, we did not attempt to identify H. ducreyi on site because of lack of available expertise, but instead transported growth in freezing medium, which may have accounted for our limited ability to isolate H. ducreyi. In this outbreak, M-PCR picked up many more cases of chancroid than did culture. M-PCR results were confirmed by PCRs for H. ducreyi, T. pallidum, and HSV targeting different gene sequences; by an independent Southern-based H. ducreyi PCR; and by M-PCR testing on specimens from the same patient but placed in a different transport medium. A few more specimens in STM than in EIA-TM were positive, possibly because they were collected from patients first, and possibly because the EIA-TM specimens were diluted 2-fold relative to STM specimens, which may lower sensitivity. Male patients with no detectable ulcer organisms by M-PCR had behavioral characteristics similar to male chancroid patients, though the characteristics of their ulcers were significantly different. DiCarlo et al. [26] noted similar findings for men with genital ulcers in New Orleans. It is possible that many of the unidentified ulcers were chancroidal ulcers that were healing or for other reasons contained fewer organisms than did the M-PCR–positive ulcers. Thus, an even higher percentage of ulcer patients than we detected by M-PCR may have had chancroid, suggesting that empiric treatment is essential for controlling and preventing this disease.

Most important, genital ulcer patients in Jackson had a significantly higher prevalence of HIV infection than did other STD clinic patients without ulcers or a history of ulcers who were selected as controls (10% vs. 0). The limited number of cases of heterosexual AIDS in Mississippi in 1994 and the
absence of detected HIV infections among STD clinic patients without genital ulcers suggest that HIV infection was emerging within the population at risk for genital ulcers. Patients with chancroid and syphilis had behavioral characteristics placing them at high risk for HIV: Two-thirds of the men with chancroid exchanged money or drugs for sex or had sex with a crack user during the month before developing sores, and half of the men with syphilis used crack. High-risk behavior associated with both ulcers and HIV acquisition may explain the high prevalence of HIV among ulcer patients; however, recent studies have established that genital ulcers in general, and chancroid in particular, are independent risk factors for acquiring HIV [17–19]. Thus, HIV transmission associated with genital ulcers in general and chancroid in Mississippi in particular appears to be fueled by the synergistic effects of behavioral and biologic factors.

Our findings suggest that genital ulcers may be increasing the infectivity of HIV-infected patients in Jackson. HIV-1 DNA was detected by PCR in the ulcer exudate of half of HIV-seropositive ulcer patients. In another small study using a different PCR technique, HIV-1 DNA was detected in a majority of ulcer specimens from HIV-seropositive patients [27]. HIV has also previously been isolated from ulcer exudate by culture, although for only a small percentage of HIV-positive patients [28]. It is unclear whether detection of HIV-1 DNA means presence of infectious virus [29]; however, it is possible that in Jackson, delay in or lack of proper treatment (before presumptive dual treatment was routinely practiced) may have left HIV-positive ulcer patients infectious, both with ulcer-causing organisms and with HIV in the genital ulcer, for several weeks. Even with appropriate treatment, the ulcers of HIV-infected persons tend to heal more slowly than ulcers of persons without HIV infection [30].

Control of STDs, and ulcerative STDs in particular, has been advocated as a means of reducing HIV transmission, both in Africa and in the United States [30–33]. Recent data from a large-scale community-level trial of STD treatment in Tanzania provide the strongest evidence yet that improved STD treatment can have a sizable effect on reducing HIV incidence [34]. Thus, clinicians suspecting chancroid should report these cases to the local health department and take steps to confirm the diagnosis. Communities with documented infections of both H. ducreyi and T. pallidum should institute presumptive treatment for both organisms [23] and should expand partner notification activities to include patients with nonreactive syphilis serology results. More aggressive treatment and prevention measures for HSV are also needed, in view of the high percentage of ulcer patients with HSV and the notable percentage of HSV patients who are HIV-infected. Improved laboratory capacity, surveillance, and treatment of STDs, as well as other appropriate HIV prevention activities for persons at risk for genital ulcers, are essential components of HIV prevention in communities with high rates of ulcerative STDs, including much of the southeastern United States.

Acknowledgments

We are indebted to Valerie Grant, Debra Jones, Paul Byers, Frank Wood, and other members of the staff at the Mississippi State Department of Health (MSDH) STD clinic for specimen and data collection; to Tracey Hardy, Robert Kennedy, Gary Cobb, and Anna Shakarishvili of CDC and to Robert Hotelkiss, Lois Farris, Mike Cassell, Carol Langley, and Donald Grillo at the MSDH for contribution to the field investigation; to Martha Fears of CDC and Dora Norn at the University of Washington for laboratory support; and to Akbar Zaidi of CDC for statistical support.

References