Etiology of Genital Ulcer Disease in Dakar, Senegal, and Comparison of PCR and Serologic Assays for Detection of *Haemophilus ducreyi*

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We used PCR assays to determine the etiology of genital ulcers in patients presenting to a sexually transmitted disease clinic in Dakar, Senegal, and evaluated the ability of two PCR tests (*groEL* and *recD*) and two serological tests (adsorption enzyme immunoassay [EIA] and lipooligosaccharide [LOS] EIA) to detect current *Haemophilus ducreyi* infection. We found that in this population, *H. ducreyi*, *T. pallidum*, and herpes simplex virus HSV DNA were detected in 56, 15, and 13% of 39 genital ulcer specimens, respectively, and *H. ducreyi* DNA was detected in 60% (3 of 5) of samples from ulcerated bubos. Among 40 consecutive patients with genital ulcer disease and with sufficient sample for both PCR assays, the *recD* and *groEL H. ducreyi* PCR assays were 83% concordant, with the *recD* PCR assay detecting six (15%) additional positive specimens and the *groEL* assay detecting one (3%) additional positive specimen. Compared to PCR, the adsorption EIA and LOS EIA tests had sensitivities of 71 and 59% and specificities of 57 and 90%, respectively, for the diagnosis of current *H. ducreyi* infection. While these differences in specificity could be due either to previous infection with *H. ducreyi* or to the detection of cross-reacting antibodies, only 6% of patients from a nearby family planning clinic gave a positive reaction in both the adsorption EIA and LOS EIA assays, indicating that cross-reacting antibodies are not prevalent among clinic attendees in this city. Our studies indicate that the adsorption EIA detects both current and past infection, while the LOS EIA assay is more specific for current infection with *H. ducreyi* in this population.

Haemophilus ducreyi is a common cause of genital ulcer disease (GUD) in Morocco, Kenya, and Lesotho, where it has been shown to cause 49 to 62% of GUD in sexually transmitted disease (STD) clinic populations (29). In other developing countries, the prevalence of this pathogen in patients with GUD is much lower, ranging from 3% in Peru, to 11% in Tanzania, to 23% in the Dominican Republic, to 26% in the Central African Republic (29). Chancroidal disease is uncommon in the United States but is recognized as a significant cause of GUD in several outbreaks in inner cities of the United States, where it is typically associated with individuals who exchange sex for drugs or money and/or use crack cocaine (13, 18).

Besides *H. ducreyi*, other pathogens that cause GUD are *Treponema pallidum*, herpes simplex virus (HSV), and *Chlamydia trachomatis*, which cause syphilis, herpes, and lymphogranuloma venereum, respectively. Because clinical diagnosis is an unreliable means of distinguishing between these GUD etiologies (7, 11, 13, 14), laboratory tests are needed to ensure correct diagnosis and treatment. Early recognition and treatment is particularly important given the association of GUD, particularly chancroid, with the heterosexual transmission of human immunodeficiency virus (HIV) (23, 27, 30). The traditional strategy for managing GUD has relied on individual patient diagnosis and treatment. However, in resource-poor settings, syndromic management, in which treatment is selected based on the most common etiology of a clinical syndrome in a particular locale, can be utilized effectively (16, 26).

The use of PCR has revolutionized the laboratory diagnosis of chancroid because alternative methods of diagnosis such as Gram stain and culture are inaccurate and insensitive, respectively (4). Advantages of PCR include the ability to transport samples off-site for testing, as well as its increased sensitivity relative to culture, presumably due to the difficulty in growing this fastidious organism (18–20). The reliability of the PCR-based tests for *H. ducreyi* is demonstrated by the high concordance (>95%) of different PCR tests performed on the same samples (18, 20). Disadvantages include the high cost and the time between collection of the specimen and laboratory result, particularly if the PCR assay is performed off-site.

Several PCR-based tests for *H. ducreyi* have been developed, including those directed against genes for *groEL* (22) and an *H. ducreyi*-specific PCR assay that amplifies an undefined 1.1-kb target sequence (17). In addition to these assays, Orle et al. (20) have developed a multiplex PCR (M-PCR) assay which simultaneously detects *H. ducreyi*, *T. pallidum*, and HSV in a single specimen. Unfortunately, this M-PCR assay is not commercially available, although detailed procedures for the performance of this assay have now been described (21).

Several serological tests have been developed for the detection of antibodies to *H. ducreyi*, including the lipooligosaccharide (LOS) enzyme immunoassay (EIA), which uses purified *H. ducreyi* LOS, and the adsorption EIA, which uses whole-cell

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H. ducreyi antigens plus adsorption of the serum with different *Haemophilus* whole-cell extracts (1, 12). Because these EIA assays presumably use different target antigens, they should detect different *H. ducreyi*-specific antibodies in patient sera. In addition, these serologic assays might differ in their ability to detect previous infection with *H. ducreyi* or may detect antibodies that result from exposure to cross-reacting antigens.

The current study was performed in Senegal to determine the etiology of GUD in this country and to determine the sensitivity and specificity of serological tests relative to PCR for the detection of chancroidal disease. We tested genital ulcer specimens by PCR and found that chancroid was the most common cause of GUD in Dakar, Senegal. We first compared the sensitivity of two different *H. ducreyi* PCR assays to detect this organism in ulcers and bubos and then used these PCR results as a reference standard for comparing the ability of the adsorption EIA and the LOS EIA to identify those individuals with current *H. ducreyi* infection. In addition, serological test results from patients attending an STD clinic were compared to those of a low-risk family planning clinic population. The results of these studies will help us to evaluate the utility of using serological tests both for the diagnosis of current *H. ducreyi* infection and for screening populations for the prevalence of past or present infection with this organism.

MATERIALS AND METHODS

Strains and plasmids. *H. ducreyi* CIP542 (type strain) was obtained from the stock culture collection at the Centers for Disease Control (CDC). Strains 35000 and R018 are *H. ducreyi* isolates from Winnipeg and Kenya, respectively, and are maintained at St. Boniface Hospital, Winnipeg, Manitoba, Canada. The recombinant plasmid pHD1A, which contains insert DNA derived from strain CIP542 by cloning, was originally selected by its homology to *H. ducreyi* but not to other organisms in the development of an *H. ducreyi*-specific PCR assay (17). This plasmid, along with subclones of this plasmid, pHDH1A4S and pHDprobe, were generously supplied by Steve Johnson (CDC, Atlanta, Ga.). Plasmid pHDH1A4S, a subclone of pHD1A, contains sequences homologous to primers SJ1A and SJ2A and the 1.1-kb sequence between these two primers, as shown in Fig. 1. Plasmid pHDprobe is an *Hae*III subclone of pHDH1A4S containing a 707-kb insert internal to the SJ1A and SJ2A primer sequences (Fig. 1). All plasmids were obtained through subcloning whole-cell DNA from *H. ducreyi* CIP542 and not by PCR amplification to avoid errors which might be introduced into the sequence by the latter technique.

Plasmid isolation, DNA sequencing, and DNA analysis. To determine the target DNA amplified by the *H. ducreyi* PCR described by Johnson et al. (17), termed *recD* PCR in the current paper, DNA was sequenced from the insert in plasmid pHDH1A4S and pHDprobe (Fig. 1). Plasmids used for sequencing were purified by using Wizard Minipreps DNA Purification System (Promega, Madison, Wis.). DNA sequencing was performed by using the DNA dye terminator cycle sequencing kit from Perkin-Elmer (Foster City, Calif.) according to the manufacturer's directions. The resulting template was purified from unincorporated primers by using CentriSep spin columns as described by the manufacturer (Princeton Separations, Adelphia, N.J.) and then subjected to automated sequencing at the Fred Hutchinson Biotechnology Center (Seattle, Wash.). Sequences from both DNA strands of pHDH1A4S were analyzed, and the products of different sequencing reactions were compared by using Genepro (Bainbridge Island, Wash.). DNA and amino acid homology searches were performed on the sequence of the full-length PCR product by using BLAST (3).

Patient population and sample collection. Consecutive patients with GUD reporting to the STD clinic at the Public Health Institute in Dakar, Senegal, from May to September 1992 were included in this study. The patients were a subset of a larger study of 880 patients tested for genital human papillomavirus and STD infection among adults presenting to the STD clinic between February 1990 to June 1994. Informed consent was obtained, and the study was approved by the Human Subjects Committee at the University of Washington and the Minister of Public Health of Senegal. After patients were enrolled in the study, a genital ulcer specimen was obtained for analysis by PCR and 10 ml of blood was obtained by venipuncture for the serological assays. The patient's age, sex, and duration of symptoms were noted on a standardized questionnaire. Five patients with ulcerated inguinal bubos were also sampled as described below.

Genital ulcer and ulcerated bubos were cleaned with gauze that had been soaked with sterile saline and then a sterile swab was applied to the base of the ulcer or ulcerated bubo to obtain the sample. Swabs were then placed in 1 ml of Specimen Transport Medium (Digene Diagnostics, Inc., Silver Spring, Md.) and frozen at −20°C until analysis. At the same visit, venous blood was drawn. Serum

was removed after centrifugation and frozen at −20°C until serological testing could be performed.

Sera from age-matched patients reporting to a nearby family planning clinic served as controls. These patients were expected to have a low risk of chancroid infection compared to the STD clinic population.

***H. ducreyi* PCR.** Two separate PCR assays for *H. ducreyi* were performed: the *groEL* PCR (22) and a PCR which amplifies an unidentified 1.1-kb fragment specific for *H. ducreyi* (17), both with modifications described in detail elsewhere (29) and summarized below. The designation of the latter PCR assay (17), previously designated S-PCR (18) due to its use of Southern blotting, is confusing since both the *groEL* PCR and S-PCR assays in the present study used Southern blots. Thus, in the present study we will designate the assay described by Johnson et al. (17) as the *recD* PCR, based on the homology of the amplified product to the *recD* gene (see below).

For the *groEL* PCR assay, samples were analyzed in two different laboratories. For the 36 samples tested in one laboratory (The Wadsworth Center, Albany, N.Y.), 300 µl of the patient sample was treated with 20 µg of proteinase K per ml for 2 h at 55°C and heated for 10 min at 95°C. DNA from this sample was then purified by adsorption to glass milk (Geneclean II Kit; Bio 101, Inc., La Jolla, Calif.) and suspended in 40 µl of sterile water. Next, 20 µl of this sample was added to each reaction mixture, and the PCR amplified products were analyzed by Southern blotting as described previously (22). Ten samples were amplified by *groEL* PCR in a second laboratory (University of Washington, Seattle). In these samples, prior to PCR, 200 µl of the sample was treated with proteinase K, ethanol precipitated, and suspended in 50 µl of Tris-EDTA buffer. Two PCR reactions were performed, one with 2 µl and the other with 5 µl of patient sample. The *H. ducreyi* amplicons from these reactions were detected by placing 10 µl of the PCR reaction product onto a nylon filter and then hybridizing to a biotin-labeled probe which was subsequently detected by using strepavidin and a chemiluminescent substrate (Amersham/Pharmacia, Piscataway, N.J.). In this sample set, all patients that were positive with a 5-µl sample were also positive with a 2-µl volume sample.

For the *recD* PCR, 200 µl of patient specimen was treated with proteinase K, ethanol precipitated, and then suspended in 100 µl of distilled H$_2$O as described earlier (29). Two PCRs were performed on each treated patient sample, one with 2 µl and one with 10 µl of the treated patient specimen. These two concentrations of patient sample were selected to achieve the optimal amount of patient sample that was not inhibitory to the PCR reaction. The primers and thermocycler settings used in this PCR were performed as described by using primers SJ1A and SJ2A (17). Then, 20 µl of each PCR-amplified specimen was analyzed on agarose gels, followed by Southern blotting and hybridization to a DNA probe internal to the PCR product, plasmid pHDprobe. The 707-bp insert in this plasmid was amplified by PCR, purified, radiolabeled with ^{32}P, and then used to probe Southern blots of PCR-amplified patient specimens as described previously (29). All samples that were positive with a 10-µl sample were also positive with a 2-µl volume sample. All samples that were positive were analyzed again with a second sample preparation, amplification, and detection. Discrepant samples were processed a third time and were considered positive if they were positive in two of the three PCR assays.

HSV and *T. pallidum* PCR. Samples (2 and 5 µl) of each patient specimen treated with proteinase K and ethanol precipitation for the *recD* PCR were used for the HSV and *T. pallidum* PCR assays. For HSV DNA detection, primers HSVgB2a-1 and HSVgB2a-2 were used to amplify a 342-bp fragment of the glycoprotein B2a gene of both HSV-1 and HSV-2 as described elsewhere (9). Specific HSV PCR products were detected by dot blot hybridization as described above for the *H. ducreyi* samples but with the biotinylated oligonucleotide probe HSVgB2a-P (9).
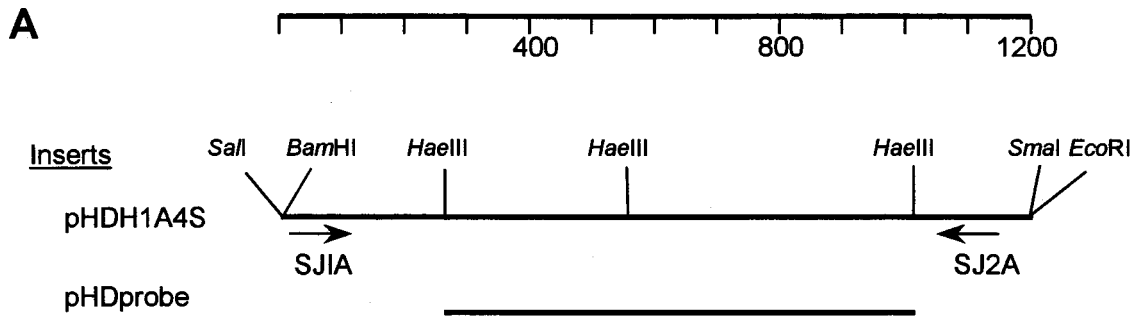
For *T. pallidum* DNA detection, primers 47-1 and 47-2 were used to amplify a 658-bp fragment of the gene encoding the 47-kDa membrane immunogen (6). In these assays, the *T. pallidum* PCR products were detected by dot blot hybridization by using a biotinylated probe consisting of a 496-bp fragment internal to the amplified DNA (6).

β-Globin PCR. Amplification of human β-globin gene, with detection of a 256-kb PCR product on a 7% acrylamide gel, was used to analyze the adequacy of the sample and to detect inhibitors in the PCR assay as described earlier (5) with slight modifications (29).

***H. ducreyi* serology.** The *H. ducreyi* adsorption EIA with whole-cell preparations from *H. ducreyi* 35000 and sorbents of *H. influenzae*, *H. parainfluenzae*, and *H. parahemolyticus* was performed as previously described (12) with minor modifications (8). The LOS EIA was performed as described earlier (1) using two separate assays, one using LOS purified from strain 35000 the other using LOS purified from strain R018.

RESULTS

Sequence of *H. ducreyi* PCR amplicon. The sequence amplified by the *H. ducreyi* PCR described by Johnson et al. (17) was determined from plasmids pHDH1A4S and pHDprobe and was found to be homologous to the *H. influenzae recD* gene (Fig. 1B). This homology was most striking in three areas:

A

B Region 1

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H.duc:   518 TTCCCTGAACCAACAACGCAAATCGATTGGCAAAAAATTGCCGTAGCAACTGCACTACGC 577
             IIIIIIIII  I         III  IIIIIIIIIIII I II II II II II  I
H.inf:  8049 TTCCCTGAAAAACAGGAAAAAACAGATTGGCAAAAAGTGGCAGTGGCGACAGCGATTAAA 8108

H.duc:   578 AAAAAATTCAGCTTAATTTCTGGTGGCCCCGGTACTGGCAAAACGACGACGGTCTCTAAA 637
             I    III      I III I II IIIII II II II IIIII IIIII II  I
H.inf:  8109 AGTCCATTTTCGATTATTACAGGCGGCCCAGGAACAGGAAAAACCACGACAGTTACGCGT 8168

H.duc:   638 TTATTAGCCGCGTTACAAGAA 658
             IIIII   II  IIIIIIII
H.inf:  8169 TTATTGCTCGTTCTACAAGAA 8189
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Region 2

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H.duc:   697 ATTGGTTGCGCCCACAGGCAAAGCAGCCGCTCGCTTAAAAGAATCCATTACACA 750
             IIIIIIIIIIII II IIIII II II   IIII II  IIII II IIII I I
H.inf:  8216 ATTGGTTGCGCCGACTGGCAAGGCTGCATCTCGTTTGGAAGAGTCGATTAAAAA 8269
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Region 3

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H.duc:   848 TATCATAAACAAAACCCACTACATTTAGATTTATTAATTGTTGATGAAGCTTCAATGATA 907
             III I I  II IIIII  I II II III IIIII IIIIIIIIII I IIIIIIII
H.inf:  8391 TATAACAGCCATAACCCTTTGCAGTTGGATGTATTAGTTGTTGATGAAACATCAATGATT 8450

H.duc:   908 GATTTATTTATGATTGAAAAACTGATGAATGCATTAAAACCAACAACCCGTTTGATTATG 967
             IIIIII     IIIIII I IIIIII II IIIIIIIIII II   III II II III I
H.inf:  8451 GATTTACCAATGATGGCAAAACTTATCAATGCATTAAAGCCCGAAACTCGATTAATTTTA 8510

H.duc:   968 CTTGGTGATAAAGATCAACTAGCCTCTGTTGAAGCGGGCGCAATTATGGGCGAATTGGGC 1027
             I II III I I   III IIIIIIIIIIII IIIII II I IIII IIIIIII
H.inf:  8511 TTAGGCGATCAGGCGCAATTAGCCTCTGTTGAGGCGGGGGCTGTATTGGGGGAATTGGCT 8570

H.duc:  1028 CATTTTATGACTCAAGGTTATAGTACAGCACATTGCACTGCTTTGAATGAATTAACCGGA 1087
             II III I IIIIII  IIIIIII  I III  I I   II   I I III II
H.inf:  8571 CAATTTGTTACTCAACCTTATAGTCATGAACAAGCCGCATACTTACTAGCAACAACAGGC 8630

H.duc:  1088 TACCAATTAAATATGATGAATCAAGTTCCAACGATTTGCGATTCATTATGTCATCTACAA 1147
             III II II I       II       I II I IIII IIIII IIIIII I
H.inf:  8631 TACAAAGTAGAAGGTTCCGATTGTTCAAATCCAATACGTGATTGCTTATGCCATCTAACA 8690

H.duc:  1148 AAAAGTTATCGCTTTGATGCTAATTCAGGCATCGGC 1183
             IIIII  II IIIIII   IIIIIIIIIII III
H.inf:  8691 GAAAGTCGCCGTTTTGATAAAGATTCAGGCATTGGC 8726
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FIG. 1. Characterization of insert in pHDH1A4S, the target sequence for the *H. ducreyi recD* PCR showing homology to *H. influenzae*. (A) Map of the insert of pHDH1A4S, showing the locations of *Hae*III sites and the target sequence for primers SJ1A and SJ2A. The location in the insert of the pHD probe used to detect the *H. ducreyi* specific PCR product is also shown. (B) Nucleotide comparison of sequences in the *H. ducreyi* amplicon and *H. influenzae* genomic sequences (GenBank accession number HIU32811). Numbers to the left reflect nucleotides numbered from the start of SJ1A (*H. ducreyi*) and as published for the *H. influenzae* genome (15). Vertical lines indicate homology in nucleotides from *H. ducreyi* and *H. influenzae*. The sequence of the *H. ducreyi* amplicon has been deposited in GenBank under the accession number AF090193.

region 1, positions 518 to 658 (61% identical); region 2, positions 697 to 750 (66% identical); and region 3, positions 848 to 1183 (66% identical). The amino acid homology of the putative RecD protein of *H. ducreyi* was 47% identical and 61% similar to *H. influenzae* RecD and 41% identical and 56% similar to *Escherichia coli* RecD. We thus renamed this *H. ducreyi*-spe-

cific PCR the *recD* PCR to distinguish it from the *groEL* PCR, which has a different target sequence.

While we easily identified the target sequence of SJ1A on plasmid pHDH1A4S, the target sequence of SJ2A was different from the published sequence (17) and was determined to be due to sequencing errors in the original study (Steve John-

TABLE 1. Detection of *H. ducreyi* DNA by PCR from bubos and ulcers compared to serologic tests from five patients with GUD

Patient	PCR results				Serology results	
	Bubo		Ulcer		LOS	Adsorption EIA
	groEL	*recD*	*groEL*	*recD*		
1[a]	−	−	+	−	−	+
2	+	+	−	+	+	+
3	+	+	−	NS	+	+
4	−	NS[b]	−	NS	−	+
5	+	NS	+	NS	+	+

[a] This was the only sample among the 42 tested by both PCR assays that was positive by the *groEL* PCR and negative by the *recD* PCR assay.
[b] NS, specimen was not sufficient.

TABLE 2. Etiology of genital ulcers in 39 consecutive patients tested for *H. ducreyi*, *T. pallidum*, and HSV by PCR[a]

Agent	No. (%)
H. ducreyi only[b]	19 (49)
T. pallidum only	3 (8)
HSV only	3 (8)
H. ducreyi plus *T. pallidum*	2 (5)
H. ducreyi plus HSV	1 (3)
HSV plus *T. pallidum*	1 (2)
PCR negative	10 (26)
Total	39

[a] Of 46 patients tested by *H. ducreyi* PCR, 7 did not have samples available for PCR testing by *T. pallidum* and HSV.
[b] Samples were considered positive for *H. ducreyi* if they were positive with either *H. ducreyi* PCR test in either the ulcer or the bubo sample.

son, personal communication). Thus, the first three bases of the target sequence are TTT rather than GCC, and there is an additional A in the middle of the published SJ2A primer sequence. The correct primer sequence should read TTT AGC CAG TG CGC CGA TGC C rather than GCC AGC CAG TGA CGC CGA TGC C. (The differences between the target sequence and the published sequence of primer SJ2A are underlined.) The sequence of SJ1A was correct as published.

Patient population. There were 46 patients with GUD analyzed in this study: 41 men and 5 women. The average age of the patients was 28 years, with a range of 21 to 53 years. The mean duration of the ulcer was 34.5 days, with a range of 2 to 174 days.

***H. ducreyi* PCR.** Of the 46 genital ulcer samples analyzed by *H. ducreyi* PCR, 2 were negative for the β-globin PCR product and 4 had insufficient sample for analysis by both tests. Among the 40 specimens analyzed by both the *recD* and *groEL* PCR assays, 14 (35%) were positive in both PCR tests and 19 (48%) were negative in both PCR tests. Of the samples that gave divergent results, six (15%) were positive in the *recD* PCR assay only and one (3%) was positive by the *groEL* PCR assay only. Thus, the two PCR-based tests were 83% concordant. Although samples were tested using the *groEL* PCR assay in two laboratories, the agreement between samples tested with the *recD* PCR assay versus those tested with the *groEL* PCR assay was the same for both laboratories. In the *recD* PCR assay, testing samples in duplicate with two separate sample preparations revealed three more positive samples that were subsequently confirmed by a third sample prep and PCR assay. Of these three additional positive specimens which were discovered by duplicate analysis of specimens, two were negative by the *groEL* PCR assay and one was positive.

Bubo specimens were obtained from five patients with genital ulcers and were analyzed by both *H. ducreyi* PCR assays when the amount of patient specimen was sufficient for analysis (Table 1). In these tests, *H. ducreyi* DNA was detected in both the genital ulcer and bubo in two patients, in the bubo but not the genital ulcer in one patient, and in the ulcer but not the bubo in one patient. No *H. ducreyi* DNA could be detected in either the bubo or ulcer specimens from the one remaining patient. For further analysis, the etiology of the genital ulcer was considered to be *H. ducreyi* if either the bubo or genital ulcer was positive by either *H. ducreyi* PCR assay.

Prevalence of genital ulcer pathogens among consecutive patients in Senegal. Sufficient sample was available to test for the presence of HSV and *T. pallidum* by PCR in 39 of the genital ulcer specimens (Table 2). *T. pallidum* was detected in six (15%) specimens, either as a sole pathogen and as a co-pathogen with *H. ducreyi* and HSV. In contrast, *H. ducreyi* was

detected in 22 (56%) specimens, as the sole pathogen in 19 (49%) specimens and as a coinfection with *T. pallidum* or HSV in 3 (8%) specimens. HSV was detected in five (13%) specimens, as the sole agent in three (8%) specimens, and together with *T. pallidum* or *H. ducreyi* in two (5%) specimens. No etiologic agent could be detected in 10 (26%) specimens. *H. ducreyi* was the most common pathogen detected (76%; 22 of 29) in genital ulcer specimens with a defined etiology.

Comparison of serological tests for *H. ducreyi*. Serum specimens from 40 patients were analyzed by the two EIA tests to determine the antibody response to *H. ducreyi*. Initially, LOS from two strains, 35000 and R018, were tested for their reaction with patient sera. Seven serum specimens were positive in the R018 LOS EIA assays. These seven sera and an additional six serum specimens were positive with the 35000 LOS EIA. Thus, the results from the 35000 LOS assay were used in all subsequent analyses.

The adsorption EIA and the LOS EIA gave concordant results in 27 (68%) of 40 serum specimens studied; 11 sera (28%) were positive and 16 sera (40%) were negative in both EIA tests (Table 3). However, divergent results were obtained with 13 serum (33%) specimens; 2 serum specimens (5%) were positive in the LOS EIA and negative by the adsorption EIA assay, and 11 (28%) were positive in the adsorption-EIA and negative in the LOS EIA.

To determine the prevalence of *H. ducreyi* antibodies among the STD clinic population compared to a population at low risk for *H. ducreyi* infection, serology tests were performed on serum obtained from patients attending a family planning clinic, and these were compared to a subset of the STD clinic patients that could be age matched (Table 3). In this analysis, the family

TABLE 3. Comparison of the two *H. ducreyi* serology tests among the STD clinic patients and age-matched controls from the family planning clinic

Adsorption EIA	LOS EIA, no. (%) of patients in each category					
	Total cases[a] (n = 40)		Age-matched patients			
			Cases[b] (n = 31)		Controls[c] (n = 31)	
	+	−	+	−	+	−
+	11 (28)	11 (28)	8 (25)	9 (29)	0 (0)	2 (6)
−	2 (6)	16 (40)	2 (6)	12 (39)	2 (6)	27 (87)

[a] Consecutive cases with GUD reporting to the STD clinic.
[b] Cases that could be age matched with controls.
[c] Patients reporting to the family planning clinic.

TABLE 4. Comparison of PCR and serology in 38 consecutive patients with genital ulcers in Senegal[a]

Results of PCR[b]	Results of serologic tests, no. (%) of patients in each category			
	LOS EIA		Adsorption EIA	
	+	−	+	−
+	10[c] (26)	7[d] (18)	12[e] (32)	5[f] (13)
−	2 (5)	19 (50)	9 (24)	12 (32)

[a] Of the 46 patients analyzed in this study, 2 had GUD specimens that were not amplifiable and 6 did not have serum specimens available for serology testing.

[b] "PCR positive" was defined as positive in either of the PCR tests in either the bubo or the genital ulcer.

[c] Of the 10 patients that were PCR and serology positive, 8 were positive in both PCR tests and 2 were positive in the *recD* PCR test only.

[d] Of the seven patients that were PCR positive and serology negative, two were positive in both the *recD* and *groEL* PCR tests; one was positive in the *groEL* PCR test and negative in the *recD* PCR test; and four were positive in the *recD* PCR test and negative in the *groEL* PCR test.

[e] Of the 12 patients that were PCR positive, 8 were positive in both PCR tests, 3 were positive in the *recD* PCR test and negative in *groEL* PCR test, and 1 was positive in the *groEL* PCR test and negative in the *recD* PCR test.

[f] Of these five patients, three were positive for the *recD* PCR test and negative for the *groEL* PCR test and two were positive for the *groEL* PCR test and negative for the *recD* PCR test.

planning clinic population had a much lower prevalence of antibodies reactive to *H. ducreyi* (6% in each assay) than the age-matched STD clinic population (32% [10 of 31] with the LOS assay; 55% [17 of 31] with the adsorption EIA assay).

Comparison of *H. ducreyi* serology and PCR. To determine the sensitivity and specificity of the serologic tests for current infection with *H. ducreyi*, the results of the two serology tests were compared to those of the PCR from 38 patient samples analyzed with both serology and PCR tests (Table 4). In this analysis, patients were considered positive for current *H. ducreyi* infection if either of the PCR assays were positive by using either ulcer or bubo specimens. The sensitivity and specificity of the LOS EIA compared to PCR were 59% (10 of 17) and 90% (19 of 21); the sensitivity and specificity of the adsorption EIA were 71% (12 of 17) and 57% (12 of 21), respectively.

Patient analysis. Various factors were analyzed to determine the association with a positive EIA test in the patient population analyzed. There was no association with the duration of ulcer and positivity, either in the adsorption EIA or the LOS EIA (with either the 35000 or the R018 antigens). The median for duration of ulcer for patients with ulcerated bubos was the same as that for patients without bubos (14 days). Of the five patients with ulcerated bubos, four were men and one was a woman.

DISCUSSION

In this study, we determined the etiology of genital ulcers in Dakar, Senegal, and defined the sensitivity and specificity of two serological tests for the detection of current infection with *H. ducreyi* by using detection of *H. ducreyi* in ulcer samples by either of two PCR assays as a "gold standard." Among the GUD specimens from 39 patients analyzed by PCR assays, *H. ducreyi*, *T. pallidum*, and HSV were detected in 22 (56%), 6 (15%), and 5 (13%) patients, respectively. No pathogen was identified in 10 (26%) of these specimens, indicating a lack of sensitivity of the PCR assays, inadequacy of these samples for PCR, or alternative etiologies for GUD in these patients. Of the specimens from which a pathogen could be identified, *H. ducreyi* was the most common, being detected in 22 of 29 (76%) of the genital ulcer specimens.

We found that the two PCR assays, *recD* and *groEL*, were 83% concordant among the 40 patients analyzed by both methods. The agreement of these two PCR assays with each other is consistent with other studies in which the *recD* PCR assay compared well with the Roche Multiplex PCR test for genital ulcer pathogens, with greater than 95% concordance for *H. ducreyi* (18). Discordant specimens in our present study (six *recD* PCR-positive, *groEL* PCR-negative samples; one *recD* PCR-negative, *groEL* PCR-positive sample) could be due to differences in sample preparation, specificity of the primers used, sensitivity of detection by probes, possible variation within the sample, and duplicate sample processing for the *recD* PCR assay. Without the latter analysis, the *recD* and *groEL* PCR assays were more concordant (four *recD* PCR-positive, *groEL* PCR-negative samples; one *recD* PCR-negative, *groEL* PCR-positive sample). While we cannot rule out contamination as the cause of more positive samples in the *recD* PCR assay, this is unlikely since the samples were positive in two separate sample preparations and PCR amplifications.

The target sequence of one PCR assay for *H. ducreyi* (17) used here was sequenced and found to have homology with the *recD* gene of *H. influenzae*. Given its essential nature for housekeeping functions in bacteria, this gene might be expected to vary little between strains of the same organism, thus supporting its suitability for detection of all strains of *H. ducreyi*. We sequenced the original clone obtained from this organism and found that the target sequence for one of the primers, SJ2A, was different from the published sequence (17). However, in spite of these differences, this assay is very sensitive and specific for *H. ducreyi* DNA, both from isolated organisms and in patient specimens (17, 18, 28, 29).

Serological tests for *H. ducreyi* have been used to determine the prevalence of chancroid in several settings, including trucking company workers in Kenya (27%); female sex workers in Lagos, Nigeria (86%); and STD clinic patients in Jackson, Miss. (48 and 53% with two different EIA assays) (8, 10, 24). The availability of PCR testing for the detection of current infection with *H. ducreyi* provided an opportunity to reevaluate the sensitivity and specificity of these assays in Africa, in a way similar to an analysis in the United States (8).

In the current study, we chose the LOS EIA and the adsorption-EIA tests to detect antibodies to *H. ducreyi* in STD clinic patients because they presumably use two different target antigens (1, 12). We found that the sensitivity and specificity of these assays, compared to current infection with *H. ducreyi* detected by PCR, were 59 and 90% for the LOS assay and 71 and 57% for the adsorption EIA assay, respectively. These results are similar to those obtained in Mississippi (8), in which the LOS assay gave a slightly lower sensitivity (48 versus 53%) but a greater specificity (89 versus 71%) compared to the adsorption EIA assay based on current infection with *H. ducreyi* detected by PCR. As with a previous study (8), serologic assays for detection of current infection with *H. ducreyi* are insensitive compared to PCR. This low sensitivity could be due to a delayed immune response to *H. ducreyi* or to the use of antigens in the assay, which may differ between strains. Indeed, in our study, the assay with LOS from strain 35000 was more sensitive than a similar assay with LOS from strain R018. Similar results would be expected with adsorption EIA, since strain differences have also been observed in this assay (12, 25).

The low specificity of serologic tests relative to PCR could be due to several factors, including past infection with *H. ducreyi*, false-negative *H. ducreyi* PCR tests, or false-positive EIA tests. Because antibodies to *H. ducreyi* persist for many months postinfection (1, 2), antibodies to a previous infection with *H. ducreyi* may have been present in the PCR-negative,

serology-positive patients in our high-risk STD population. Additionally, because 26% of patients in this study had no pathogen detected, some of these patients might have had a false-negative *H. ducreyi* PCR test. Finally, antibodies that cross-react with antigens from other organisms might account for some of the EIA-positive, PCR-negative results. However, the low background reactivity of the serological tests in the family planning clinic patients (6% by both assays) is consistent with the interpretation that antibodies to *H. ducreyi* are not prevalent in the general population. Furthermore, patients attending STD clinics are also more likely to have had a past infection with other STD pathogens and perhaps other commensal organisms that might cross react in these assays, making interpretation difficult.

We conclude that, in our patient population, *H. ducreyi* caused the majority of GUD cases in Dakar, Senegal. In addition, our study indicates that serologic tests need further evaluation to determine their suitability to detect chancroid in a given community. The sensitivity and specificity of serologic assays may depend upon the strain of *H. ducreyi* used for detection and the patient exposure to cross-reacting antigens that might differ in different populations. Perhaps a more sensitive and specific serological test for *H. ducreyi* infection could be based on individual cellular components that are unique and conserved in this organism.

ACKNOWLEDGMENTS

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