

PENILE ULCER DISEASE IN BANGKOK

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OBJECTIVE : To determine the clinical, epidemiologic, and microbiologic characteristics of male genital ulcer disease in Thailand.

BACKGROUND : In Africa (1-3) and Asia (4) genital ulcer disease accounts for 10-50% of all venereal disease. Studies by Sng and colleagues in Singapore found that *Hemophilus ducreyi*, the cause of chancroid, could be isolated from many of these ulcers (4). Recently selective media have become available which have made routine isolation of *H. ducreyi* possible (5,6). Using these improved isolation methods investigators have found that *H. ducreyi* can cause sporadic cases (7) and outbreaks of genital ulcer disease in temperate climates (8-10), and is an important cause of "travelers' venereal disease" among returnees from the tropics as well (11). The newer isolation methods were employed for the first time in the developing world in Kenya (12) where *H. ducreyi* was isolated from 60 of 97 patients seen with genital ulcers in Nairobi. The present study was undertaken to describe the etiology of genital ulcers in Thailand.

MATERIAL AND METHODS : From September 29 to October 30, 1982, men who came to the Bangrak VD Hospital outpatient clinic with penile ulcers were studied. Patients were interviewed by a trained health worker using a standardized questionnaire and examined by a clinic physician. Genital ulcers were gently cleaned with non-bacteriostatic normal saline (NNS). Material from the undermined edge of the ulcer was cultured with 3 sterile cotton swabs moistened with sterile NSS. One swab was used to inoculated the following primary isolation media :

1. Mueller-Hinton agar base (Difco) containing :
 - 5% chocolatized horse blood
 - 5% fetal calf serum (inactivated 30 minutes at 56°C)
 - 1% Isovitalex (BBL)
 - 3 g/ml of Vancomycin
2. Heart infusion agar base (Gibco) containing
 - 5% rabbit blood
 - 5% fetal calf serum (inactivated 30 minutes at 56°C)
 - 3 g/ml of Vancomycin

Immediately after inoculation, plates were incubated at 32-34°C in candle jars containing a 10% sodium azide saturated gauze-pad. Plates were examined at 48 and 72 hours for colonies characteristic of *H. ducreyi*. At 48 hours *H. ducreyi* appear as small, pale yellow, opaque colonies with a round feathery edge. At 72 hours, colonies are friable and not fixed to the media. Colonies with characteristic morphology and gram stain that were oxidase positive and catalase negative were defined as presumptive *H. ducreyi*. X factor requirement was determined by inoculating colonies grown overnight on rabbit blood agar to a minimal media containing GC base (Difco) with glutamine 0.01%, glucose 0.1%, and cysteine 0.05%, and bovine albumin 0.1% overlaid with the XV factor disc. Colonies requiring X factor for growth were sent to Dr. Peter Piot, Institute of Tropical Medicine, Belgium for confirmation, serotyping and antimicrobial sensitivity testing.

The second swab was smeared onto a glass slide for Gram stain. Smears were examined microscopically for *H. ducreyi* defined as Gram negative coccobacilli or short rods arranged in parallel rows or a "school of fish" pattern. Spirochetes were identified using dark field microscopy.

A third moistened swab taken from an ulcerated genital lesion was placed in transport medium containing 1% bovine albumin in HBSS with penicillin (250 µg/ml), streptomycin (200 µg/ml), and fungizone (1 µg/ml), and frozen at -70°C. Isolation of viruses was attempted by inoculating 0.1 ml onto a confluent monolayer of Vero cells. Cells were washed once with M199 containing 2% calf serum, supplemented with 2% inactivated calf serum, penicillin (50 µg/ml), streptomycin (100 µg/ml), kanamycin (100 µg/ml), and fungizone (0.5 µg/ml) then overlaid with 1 ml of the same medium.

The cells were maintained at 37°C with changes of medium every 3-5 days and observed daily for cytopathic changes (CPE). The monolayers were observed for a minimum of 14 days or until cell degeneration occurred. Cultures which showed 3-4+ CPE were harvested by freezing at -70°C and thawing at 37°C three times. The presence of herpes simplex virus was confirmed by indirect immunofluorescent staining using a commercial anti-human globulin fluorescein conjugated by Progressive Laboratories, Inc. Washington, DC.

RESULTS :

Isolation Rates : From September 29 to October 30, 1982, 2931 male patients were at Bangrak VD outpatient department; 1649 (56%) of these patients were seen previously and 1282 (44%) were seen for the first time. Of the new patients 248 (19%) had penile ulcers, 120 of which were selected for study. *Hemophilus ducreyi* was isolated from 45 (38%), *Herpes simplex* from 14 (12%) and *Neisseria gonorrhoea* from 2 (2%) genital ulcers in this group of patients. *H. ducreyi* and *Herpes simplex* were isolated from the same ulcer in 2 patients. Syphilis was detected by dark field in one patient who also had an elevated VDRL.

Epidemiologic aspects of chancroid ulcers : The patients from whom *H. ducreyi* was isolated were a mean of 22 years (range 16-38), 82% were unmarried and most lived in Bangkok. Approximately 10% of patients had a previous history of penile ulcer and 7% had a history of gonorrhoea. None had

been previously diagnosed as having herpes or syphilis. In these respects they did not differ from the other patients in the study.

Clinical aspects : Patients with *H. ducreyi* ulcers came to the clinic a mean of 12 days after the ulcer was first noticed; 35% of the patients had a single ulcer, 56% had 2-4 ulcers and 9% had more than 4 ulcers. The ulcers were a mean of 8 mm in diameter. Ulcers were located on the prepuce or the coronal sulcus under the prepuce (69%), the frenulum (18%), shaft (9%), or glans (5%). Two-thirds of the *H. ducreyi* ulcers had irregular borders, significantly more compared to 48% of the ulcers from which *H. ducreyi* was not isolated ($p \leq 0.05$, X²). The ulcers were usually tender, non-indurated, and tended to bleed easily. The ulcers were not associated with systemic symptoms such as fever. Unilateral inguinal adenopathy was present in only 11% of patients, bilateral was not detected in any.

Microbiologic aspects : The previous use of antimicrobials thought to be active against *H. ducreyi* was just as frequently used in patients from whom *H. ducreyi* was isolated as other patients. *H. ducreyi* was isolated more often from media containing horse blood; 42 of 45 (93%) than in media containing rabbit blood; 34 of 45 (76%). Gram stain of the ulcer base was diagnostic in 62% of patients from whom *H. ducreyi* was isolated and in only 1% of patients from whom *H. ducreyi* was not isolated.

Table 1. Bangrak V.D. hospital penile ulcer study.

	<i>H. ducreyi</i> culture			
	Positive		Negative	
	#	%	#	%
Number of patients	45	38	75	63
Age of patient (mean)	22 (16-38)		23 (15-44)	
Originally from Bangkok	18	40	21	28
Originally from Provinces	27	60	54	72
Years in Bangkok (mean)	11.7		14.9	
Married	8	18	37	15
Single	37	82	64	85
Married, with wife at home	7	16	7	9
V.D. history :				
Previous penile ulcer	11*	24	9	12
Gonorrhoea	7	16	6	7
Present ulcers :				
Duration of ulcer in days (mean)	12		13.2	
Received BAC, TCN, KM, SUL	33	57	25	48
Received PCN, CHLORO, OTHER	12	43	27	52
Diameter of ulcer (mean)	8.27		7.59	
Number of ulcers (mean)	2.58*		3.15	
one ulcer	16	36	26	35
two ulcers	12	27	15	20
three ulcers	7	16	9	12
four ulcers	6	13	12	16
more than 4	4	9	13	17

Location of ulcer :

prepuce	31	69	51	71
frenulum	8	18	7	10
shaft	4	9	6	8
glans	2	4	8	11

Tenderness (0-4+)

0	1	2	4	5
1	6	13	11	15
2	14	31	29	39
3	21	47	26	35
4	3	7	5	7

Induration	6	13	1	1
Purulent base	44	98	68	91
Regular border	15	33	39	52
Irregular border	30	67	36	48

Inguinal nodes

Right only	4	9	3	4
Left only	1	2	7	9
Both sides	0	0	5	7
None	40	89	60	80

Table 2. Etiologic agents isolated from male genital ulcers.

	Positive		Negative	
	#	%	#	%
<i>Hemophilus duoreyi</i>	45	38		
Isolation in media containing :				
Horse blood	42	93		
Rabbit blood	34	76		
Gram stain positive for <i>H. duoreyi</i>	28	62*	1	1
Gram stain negative for <i>H. duoreyi</i>	17	38	74	99
Dark field positive	0	0	1	1
VDRL reactive	3	7	4	5
Positive GC culture	1	2	2	3
Negative GC culture	27	60	40	53
Not cultured	17	38	33	44
Herpes simplex virus (culture)	2	4	12	16

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