

## Chancroid in Sheffield

### A report of 22 cases diagnosed by isolating *Haemophilus ducreyi* in a modified medium

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**SUMMARY** The causative organism of chancroid, *Haemophilus ducreyi*, is generally considered to be very fastidious and its isolation, maintenance, and detailed study very demanding. In this study a modified medium was developed, which allowed the organism to be isolated more frequently than previously would have been expected. Twenty-two cases of chancroid were confirmed by the isolation of *H ducreyi* in 160 patients with genital ulceration examined over a one-year period. The cases were apparently unrelated, and in only five was there a history of recent sexual contact abroad. Concurrent infection with other sexually transmitted diseases was present in 18 (81·8%) patients, and in 14 (63·6%) both *H ducreyi* and herpes simplex virus were isolated from the same genital ulcers. Thus, these findings indicate that chancroid is underdiagnosed in England and that *H ducreyi* may frequently occur as a secondary invader of damaged genital skin and mucosa.

#### Introduction

Chancroid is endemic in many tropical and subtropical areas, but its reported incidence falls as standards of living and hygiene improve. In the United Kingdom it is the least commonly diagnosed of the three designated venereal diseases and is mainly seen in merchant seamen and businessmen returning from endemic areas. The incidence of chancroid in the past 50 years has declined at a faster rate than even that of syphilis.

Soft sore or chancroid was clearly differentiated from the hard or Hunterian chancre by the mid-nineteenth century, but it was not until 1890 that Ducrey<sup>1</sup> reported the appearance of characteristic bacilli in stained films prepared from soft sores. Although he failed to culture the organism he reported that it produced typical lesions when inoculated into the skin of the patient's forearm and that the organisms from these lesions could be passed serially.

In 1900 the organism was obtained in pure culture

by Bezancon *et al*<sup>2</sup> using agar to which 20% whole rabbit blood had been added. In the years that followed many workers described their results using various forms of blood-containing media.<sup>3-9</sup>

Until recently the view held by both clinicians and bacteriologists has been that *H ducreyi* is an uncommon cause of genital ulceration in indigenous patients in Britain (table I).

Routine laboratory methods for the isolation of *H ducreyi* have been unreliable in the past; confirmation of the clinical diagnosis of chancroid has largely depended on the microscopical examination of stained smears from genital ulcers, a technique which lacks sensitivity and specificity. In some patients confirmation has been sought by the histology of biopsy specimens or by auto-inoculation studies.<sup>10</sup>

In his taxonomic study of the genus *Haemophilus* Kilian<sup>11</sup> examined nine strains of *H ducreyi* and commented that they gave very poor growth on all

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TABLE I *Reported cases of chancroid due to Haemophilus ducreyi in Britain from 1975 to 1979*<sup>25</sup>

1975	1976	1977	1978	1979
76	59	49	57	49

media (none of these strains was freshly isolated). Tan *et al*<sup>12</sup> reported on 500 cases of "clinical chancroid" seen in Singapore in one year; 278 (56%) of these were confirmed bacteriologically. The high frequency of positive results was considered to be due to an improved culture method by inoculating swabs from lesions into 10 ml of the patient's serum, which had been inactivated at 56°C for 30 minutes. These liquid cultures were filmed after incubation for 48 hours at 37°C and reported as giving a positive result if typical Gram-negative bacilli were present. Sub-culture from the cultures to a solid medium was unsuccessful.

Although Tan and his co-workers reported that 100 control patients had negative results for *H ducreyi* Koo *et al*,<sup>13</sup> using the same technique, reported 29 positive results in a group of 300 asymptomatic prostitutes. None of these patients had ulcerative lesions although the organism was present in the cervix or vagina or both.

Hammond *et al*<sup>14</sup> described an improved culture technique by means of which they grew *H ducreyi* from eight of 16 suspected cases. Sottnek *et al*,<sup>15</sup> using various complex media, were able to isolate 17 strains in about six months from dark-ground-negative genital lesions.

Before looking for the organism in clinical material we investigated possible media for the growth of *H ducreyi* using NCTC strain No 10945 as test organism. Once reliable results were obtained we examined specimens taken from patients with ulcerative genital lesions attending the special clinic in Sheffield.

### Part I—materials and methods

#### MEDIA

The starting point was Difco GC base plus 2.0% defined supplement,<sup>16</sup> to which was added additional proteose peptone (Difco 3) to double the original concentrations. The composition of the base agar was: proteose peptone (Difco), 30.0 g; corn starch, 1.0 g; potassium phosphate dibasic, 4.0 g; potassium phosphate monobasic, 1.0 g; sodium chloride, 5.0 g; Bacto agar, 10.0 g; and distilled water, 1 litre. The ingredients were dissolved in water and sterilised by autoclaving at 15 lb/in<sup>2</sup> (121°C) for 15 minutes.

A stock solution of type III equine haemin (crystalline (pfs), Sigma) was prepared by dissolving 250 mg of haemin in 2 ml of 0.2 mol/l KOH in 47.5% ethanol and bringing it to a final volume of 10 ml with sterile distilled water.

After sterilisation of the modified base 2% defined supplement and 10 ml of haemin solution per litre was added aseptically (thus giving a final haemin

concentration of 250 µg/ml). Plates were poured and stored in the refrigerator at 4°C.

Initial observations were based on this medium which, after inoculation, was incubated at 33°C in an atmosphere of 5% CO<sub>2</sub> for 4-5 days.

#### COLONIAL APPEARANCE AND IDENTIFICATION

Typical *H ducreyi* colonies grown under the conditions described were about 1-3 mm in diameter and darkish brown in colour, contrasting clearly with other organisms grown from the same lesions. The most striking difference was in the extreme coherence of the colonies, which could be pushed intact across the plate. Indeed it was very difficult to pick such colonies off the medium or to make films from them having done so. This characteristic has been noted repeatedly in reports on this organism. Subcultures to blood-containing media tended to give somewhat softer colonies; this could be demonstrated by growth on fresh chocolate blood plates. Culture in liquid medium gave growth in clumps, which were often attached to the sides of the tube.

By microscopy the organism appeared as a Gram-negative bacillus, frequently occurring in clumps reminiscent of the cording of tubercle bacilli. They were not acid fast.

In our laboratory the organism conformed to Kilian's<sup>11</sup> account, with all biochemical tests giving negative results except the alkaline phosphatase test. Contrary to recent reports in America,<sup>15</sup> our strains were neither oxidase-positive nor β-lactamase-producers.

### Results

The medium gave consistently reliable results. It had an adequate shelf-life of three weeks at 4°C, thus obviating the need for the frequent preparation of fresh medium. With small inocula we had no difficulty in isolating typical *H ducreyi* from other contaminating genital organisms.

### Part II—patients and methods

The normal routine testing of patients who present with genital ulceration to this department of genitourinary medicine includes repeated dark-ground microscopy of serum from ulcers from *Treponema pallidum*, tissue culture of swabs from lesions for isolation of herpes simplex virus, repeated serological tests for syphilis (rapid plasma reagin, *T pallidum* haemagglutination assay, and fluorescent treponemal antibody-absorption tests) over a three-month period, and examination of acute and convalescent sera for antibodies to herpes simplex virus. All patients are routinely examined for the presence

of other sexually transmitted diseases (STD). Details of the methods of testing and diagnostic criteria are given elsewhere.<sup>17</sup>

In this study 160 patients who presented in the 12-month period beginning 1 March 1980 also had swabs from their genital ulcers cultured for the isolation of *H ducreyi* on the haematin-containing medium described above.

## Results

### *H. DUCREYI* ISOLATION RATE

*H ducreyi* was isolated from swabs taken from genital ulcers in 22 of 160 (13.8%) patients tested. The cases were apparently unrelated (table II).

### DEMOGRAPHIC DATA

All patients were exclusively heterosexual. The male-to-female case ratio was 1.75:1 (table III).

### RECENT SEXUAL HISTORY

In the four weeks before the onset of symptoms 14 patients reported only one, and eight patients two or

more, sexual contacts. In five patients infection was acquired outside Britain—two in the Caribbean and one each in Nigeria, Greece, and Thailand. The other 17 patients denied either travel abroad or sexual intercourse with a partner who had been abroad during the preceding six months. One man and one woman had apparently been infected in London and Leeds respectively. The other 15 (68.2%) patients had acquired their infections in Sheffield.

### CLINICAL DATA

#### Past history of STD

Thirteen (59.1%) patients admitted or were known to have had STDs on previous occasions.

#### Incubation period and symptoms

Twenty-one patients complained of symptoms related to painful genital ulcers; these had developed within a week of their most recent sexual contact in 17 patients and within 14 days in another four. One patient was referred with a three-month history of persistent bleeding from a large deep indurated vaginal ulcer and denied any sexual contact for four months.

#### Clinical signs

These showed a marked variation. Four patients had single and 18 multiple ulcers. The size of individual ulcers varied between 2 mm and 2 cm. They were invariably tender with a purulent or necrotic base. An accompanying unilateral or bilateral inguinal lymphadenitis was present in 11 (50%) patients; in no case was there suppurative of lymph nodes.

#### Concomitant STDs

Herpes simplex virus (HSV) was isolated from the same genital ulcers as *H ducreyi* in 14 (63.6%) patients. In three other patients with negative culture results for this virus, two had a confirmed history of recurrent genital herpes and one had a concurrent herpes simplex ophthalmia and developed culture-positive genital herpes three months later when further sexual exposure was denied.

Thirteen (59.1%) patients had an STD other than herpes genitalis. One man, thought to have Behçet's syndrome (on the basis of recurrent genital and oral ulceration, peripheral arthritis, and cutaneous lesions), had an associated non-specific urethritis. There were no cases of concomitant syphilis. In only four patients was *H ducreyi* the sole pathogen isolated.

### TREATMENT

In three patients the genital ulcers healed spontaneously (aided by saline bathing) before the diagnosis was made and antibiotic therapy started. Three

TABLE II Isolation rate of *Haemophilus ducreyi* in two consecutive studies. (Initial study, 1 March-30 September 1980; prospective study, 1 October 1980-28 February 1981.)

	Initial study*	Prospective study†	Total
No of patients examined	82	78	160
No of positive isolations	9 (11%)	13 (16.7%)	22 (13.8%)

\*Lesions cultured when medium was available

†All lesions cultured

TABLE III Demographic data on patients with positive isolation results for *Haemophilus ducreyi* (N=22).

Demographic data	No of patients
Sex	
Male	14
Female	8
Age	
Mean ± SD = 26.32 ± 7.65 years	
Range = 18-44 years	
Marital state	
Single	19
Presently or previously married	3
Ethnic group	
Caucasian	17
Negro	5
Occupational status	
Employed	15
Unemployed	3
Student	4
Method of referral	
Self	15
Sexual contact	2
Other clinician	5

patients were treated initially with sulphadimidine 2 g daily and 17 patients with co-trimoxazole two tablets twice daily for 1-3 weeks. One patient failed to respond to sulphadimidine but was successfully treated with doxycycline. Attempts to reisolate *H ducreyi* from genital swabs after treatment proved unsuccessful, and in all cases the genital ulcers and lymphadenitis resolved.

#### CONTACT TRACING

Attempts were made to trace and investigate all local contacts of patients with chancroid. Of 14 contacts seen three had genital ulceration; *H ducreyi* was not isolated from these or from the other 11 symptomless contacts.

#### Discussion

Despite being a large industrial city with a population approaching 600 000, Sheffield has a relatively low incidence of STDs. During the 1970s incidence rates for infectious syphilis and gonorrhoea were on average 1.7 and 162 per 100 000 population per year respectively. In the nine-year period preceding this study (1971-79) only five cases of chancroid were diagnosed in this department. The introduction of a new culture method for *H ducreyi* during 1980 resulted in 22 patients being diagnosed as having chancroid. The infections were apparently unrelated.

The male-to-female ratio of cases of 1.75:1 is low compared with many previous studies<sup>18</sup> but is similar to that in a recent epidemic of chancroid in Greenland.<sup>19</sup> Although the clinical picture was variable in our patients, the appearance of one or more painful superficial genital ulcers within a week of the last sexual contact was typical of chancroid. Suppurative lymphadenitis, sometimes resulting in the formation of a fistula, is a recognised complication in one-third to one-half of male cases but is less common in women.<sup>20</sup> The absence of these complications in our patients was possibly the result of early presentation and treatment. Sulphonamides, to which resistance has been described,<sup>21</sup> or co-trimoxazole<sup>22</sup> are the initial treatments of choice so that concomitant syphilis is not masked. A good response to doxycycline has also been described.<sup>23</sup>

Coexisting infection with *T pallidum* and *H ducreyi* in the same genital ulcer is well recognised<sup>24</sup>; genital herpes is regarded as a major differential diagnosis. In this study the finding that 14 patients harboured herpes simplex virus and *H ducreyi* in the same genital ulcers suggests that *H ducreyi* was a secondary bacterial pathogen in these cases. The same would appear to be true of the man with Behçet's syndrome.

That 15 patients apparently acquired their infections locally was perhaps the most surprising finding. Sheffield is not a major thoroughfare for international travel nor has it a large transient population; moreover, we have established good control over other STDs. Our results suggest that chancroid may be underdiagnosed in England. This will continue to be so as long as diagnosis depends on the insensitive technique of microscopical examination of smears and on negative test results for other pathogens. We believe this modified medium is both cheaper and simpler than those generally recommended and could form a useful basis for further studies in routine laboratories in which the isolation of *H ducreyi* would not normally be attempted.

Our study of the local prevalence of *H ducreyi* is continuing, together with the investigation of a selective medium containing vancomycin. With such a medium we hope to identify *H ducreyi* in contacts of cases and other symptomless clinic attenders. Confirmation of our findings is being sought independently by colleagues in other centres.

If our findings are confirmed our concept of the role of *H ducreyi* in the pathogenesis of chancroid will have to change; that the organism is sometimes, if not usually, a secondary invader of damaged genital tissue will need to be acknowledged.

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