Haemophilus ducreyi Causing Chronic Skin Ulceration in Children Visiting Samoa

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Chancroid is a sexually transmitted infection associated with genital ulceration and lymphadenopathy caused by *Haemophilus ducreyi*. Localized skin infections, in the absence of genital lesions, have not been previously reported. We report 3 cases of lower limb ulceration in children caused by *H. ducreyi* and postulate that *H. ducreyi* may be a previously unrecognized cause of chronic skin ulceration.

Haemophilus ducreyi, a fastidious gram-negative bacillus, is the etiological agent of chancroid, an ulcerative genital disease associated with inguinal lymphadenitis, mainly seen in developing countries [1]. *H. ducreyi* is a strict human pathogen, and there is no known animal or environmental reservoir. Nonsexual transmission of *H. ducreyi* has not been described. We describe 3 children with chronic lower limb ulceration from whom *H. ducreyi* was recovered.

Case reports. Patient 1 was a 9-year-old girl who had recently returned from Samoa and presented to her general practitioner in February 2005 with painful ulcers on both legs. Three 1–2-cm discoid hypergranulated ulcers were present around her knees. She was given oral flucloxacillin, with minimal improvement. An intramuscular injection of 450 mg of benzathine penicillin was administered, resulting in resolution of the ulcers.

Ulcer exudate was cultured before the administration of the flucloxacillin. The Gram stain revealed neutrophils, but no organisms were seen. There was no growth after 48 h on tryptic soy agar with 5% sheep blood (Fort Richard Laboratories [FRL]), incubated at 35°C aerobically, and on colistin-nalidixic acid agar (FRL) and brain-heart infusion vancomycin-kanomycin agar (FRL), incubated at 35°C anaerobically. Additional swab specimens obtained before the administration of penicillin were cultured onto the aforementioned media and onto chocolate agar (FRL) and incubated at 35°C in 5% CO_2. After 72 h, a tiny gram-negative coccobacillus grew on the chocolate agar only. The isolate was weakly oxidase positive and catalase negative. No identification was obtained with the RapID NH (Remel).

Sixteen S rDNA PCR and sequencing were performed using previously described primers [2]. A BLAST search of the National Center for Biotechnology Information database revealed 100% sequence identity with *H. ducreyi* strain ATCC33921 (accession number, AY513483). Additional phenotypic testing was performed; the organism required haemin (X factor), but not nicotinamide adenine dinucleotide (Y factor), for growth and did not excrete porphyrin.

Patient 2, a 6-year-old girl, presented to her general practitioner in February 2006, 1 week after returning from Samoa, with a 2-month history of a painful ulcer on her right lower calf. Another child staying where she had stayed in Samoa also had a leg ulcer. Our case patient's general practitioner treated her with flucloxacillin for 1 week, with minimal benefit, and she was referred to a hospital for further treatment of a tender 3–4-cm ulcer with a raised undermined border on the right lower calf.

Gram stain of the ulcer exudate revealed moderate numbers of neutrophils and occasional gram-negative bacilli. The swab specimen was inoculated onto MacConkey agar (FRL) and colistin-nalidixic acid agar (FRL) and incubated at 35°C aerobically; the specimen was also inoculated onto tryptic soy agar with 5% sheep blood (FRL) and incubated at 35°C in 5% CO_2. No pathogens were isolated. Another swab specimen was obtained and, in addition to the aforementioned media, it was inoculated onto supplemented GC agar base (Becton Dickinson) containing 1% hemoglobin and incubated at 35°C in CO_2 for 7 days. After 72 h, a gram-negative coccobacillus grew on the GC agar. The isolate underwent 16S rDNA PCR and sequencing as described above. The sequence revealed 100% sequence identity with *H. ducreyi.* The patient was treated with 600 mg of azithromycin suspension, resulting in resolution of the ulcer.

Patient 3, a 5-year-old girl, presented to the hospital in June 2006. She had developed 3 painful ulcers on her right lower leg while visiting Samoa 2 months earlier. Three children staying

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where she had stayed also had leg ulcers. The patient's general practitioner prescribed oral flucloxacillin, followed by oral erythromycin; the latter was prescribed 2 days before admission. Gram stain of the exudates revealed a moderate number of neutrophils, but no organisms were seen. The swab specimens were inoculated onto routine media and onto chocolate agar, as outlined for patient 1. A gram-negative coccobacillus was isolated. Sixteen S rDNA PCR and sequencing confirmed the identification of the isolate as *H. ducreyi.* The patient was treated with 500 mg of azithromycin suspension, resulting in resolution of her ulcer.

In view of the isolation of *H. ducreyi,* additional history was obtained and further examination was undertaken for all 3 children by the Child Protection Unit. There was no history of urogenital symptoms, of a change in behavior, or of a disclosure of sexual abuse. No inguinal lymphadenopathy or genital ulceration was found during examination. For the first patient, throat, vaginal, and rectal swab culture results were negative for *Neisseria gonorrhoeae* and *Chlamydia trachomatis;* nucleic acid amplification testing (ProbeTech, Becton Dickinson) of a urine sample was negative for *N. gonorrhoeae* and *C. trachomatis;* a vaginal swab culture was negative for *Trichomonas vaginalis;* and the results of a serological examination for *Treponema pallidum,* herpes simplex virus type 2, and HIV were negative. For cases 2 and 3, the urine test results were negative by nucleic acid amplification testing for *N. gonorrhoeae* and *C. trachomatis,* and serological examination results for *T. pallidum* were negative.

All 3 of the isolates had negative results when tested for β-lactamase production with the chromogenic cephalosporin nitrocefin (Becton Dickinson), and the MIC of penicillin for all 3 isolates, as determined by Etest (AB Biodisk), was 0.16 mg/L.

Discussion. Chancroid is traditionally considered to be a sexually transmitted infection associated with inguinal lymphadenopathy and genital ulceration [1, 3]. The prevalence of chancroid in the Western Pacific is unknown. It has been reported in Papua New Guinea [6, 7]. Recent surveys of sexually transmitted infections in antenatal populations in Pacific nations have analyzed rates of chlamydia, gonorrhea, syphilis, and HIV infection, but not of chancroid [4, 5]. *C. trachomatis* infection was endemic among the pregnant women surveyed, but the rates of syphilis were low, suggesting that sexually transmitted infections causing genital ulceration may be uncommon [4, 5, 8]. We report 3 cases of lower limb ulceration in children who developed lesions caused by *H. ducreyi* while visiting Samoa or soon after their return to New Zealand. Phenotypic identification was difficult, but 16S rDNA PCR and sequencing confirmed the identification of all 3 isolates as *H. ducreyi.*

Human-to-human transmission of *H. ducreyi* is primarily by sexual means alone [1]. However, nongenital skin lesions can occur in patients with chancroid as a result of auto-inoculation [3], and human experimental models involving inoculation of the dermis of the forearm have demonstrated the ability of *H. ducreyi* to infect the skin [9, 10]. History and examination findings did not reveal that the infection in these children was acquired by sexual contact. Lower-limb ulceration was described in other household members; thus, close contact between the children may have resulted in contamination of skin with wound exudate, allowing *H. ducreyi* to enter via breaks in the epithelium.

The recommended treatment for chancroid is azithromycin or ceftriaxone. Plasmid-mediated β-lactamase production is well described [11], and *H. ducreyi* is considered inherently resistant to both tetracyclines and penicillin [12]. However, none of these isolates contained a β-lactamase; 1 patient who received penicillin responded well. Penicillin has been used successfully in the past [13]. Empirical treatment directed at the common causes of skin infection in tropical environments, *Staphylococcus aureus* and *Streptococcus pyogenes,* was not effective; treatment with flucloxacillin was unsuccessful for all 3 cases [14]. There is limited information about the rates and etiology of tropical ulcers in Pacific countries, a disease neglected in studies since the 1980s [15].

These observations warrant further investigation of the etiology of chronic skin ulcers in children in Samoa and, perhaps, in other areas of the Pacific. The culturing of chronic ulcer exudates for fastidious organisms, such as *H. ducreyi,* is not routine, even in developed countries, but it should be considered for children coming from the Western Pacific region. Presumptive identification can be made on the basis of growth on chocolate agar, only after $\geqslant$72 h of incubation in CO_2, and on a requirement for haemin but not for nicotinamide adenine dinucleotide. The appropriate treatment is penicillin, either orally or intramuscularly. Epidemiological studies should be undertaken to determine the likely mode of transmission and reservoir for *H. ducreyi* among this population.

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