MYCOBACTERIUM ULCERANS INFECTION (BURULI ULCER): FIRST REPORTED CASE IN A TRAVELER

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Abstract. A chronic, painless sore developed over a 2-month period on the left calf of a Canadian man traveling for 8 months in Africa. A presumptive diagnosis of a Mycobacterium spp. infection was made despite initially negative biopsy and culture results, after failure of several courses of anti-bacterial antibiotics. Mycobacterium ulcerans was eventually isolated and the lesion progressed despite treatment with multiple anti-mycobacterial agents. The lesion finally responded to wide and repeated excision, aggressive treatment with anti-mycobacterial antibiotics, and split-thickness skin grafting. The isolation and treatment of this unusual organism are discussed.

In 1897, Sir Albert Cook described a range of unusual and chronic necrotizing skin ulcers in Africans. More than 50 years later, very similar lesions were reported in Australian farmers around Bairnsdale. The current term commonly used for these ulcers (Buruli ulcer) dates from a description of lesions in Rwandan refugees living in the Buruli County of Mengo, Uganda. The organism responsible for these lesions was eventually identified as Mycobacterium ulcerans. In the past 2 decades, hundreds of cases have been described in central Africa (in the former Zaire), and small case series have originated in a number of countries in West Africa (Benin, Ghana, Gabon, Ivory Coast--11), establishing a broad area of endemcity in the equatorial regions. Other tropical and subtropical foci have been reported in Southeast Asia, the Pacific islands, Australia, and Central America. It is now generally accepted that M. ulcerans has an as yet unidentified environmental reservoir associated with slow flowing or stagnant waters in tropical and subtropical regions.

To date, almost all of the reported cases of M. ulcerans have occurred in inhabitants of endemic regions. To our knowledge, only 3 cases have been diagnosed in North America since the 1960s: a Peace Corps volunteer who contracted the disease in Nigeria, a Nigerian immigrant who developed a skin ulcer 6 weeks after immigrating to the United States, and an expatriate who had lived in Bolivia for many years. Lack of familiarity with this unusual skin ulcer in North America, combined with difficulties in isolating the responsible microorganism, can result in significant delays in the diagnosis and treatment of Buruli ulcer, as illustrated below. We report a case of M. ulcerans infection in a Canadian traveler and discuss methods for the isolation and treatment of this organism.

CASE REPORT

The patient was a 36-year-old man who presented to the McGill Center for Tropical Diseases on June 1, 1994 with a painless furuncle-like lesion on the posterior aspect of his left calf. The lesion had been present for approximately 6 weeks. The patient was a journalist who had traveled extensively in Africa from September 1993 to April 1994. His trip had begun in northern Africa, proceeding southward to West Africa (Mali, Ivory Coast), then eastwards to central Africa (Zaire, Uganda) and finally finished in Kenya and Ethiopia. He had swum and waded in fresh water lakes in Zaire and Uganda and had been in direct contact with domestic animals (e.g., dogs, mules).

He had first noticed the lesion (which was initially thought to be an insect bite) during the last month of his trip. However, instead of resolving, the lesion had painlessly progressed to become an indurated subcutaneous nodule and subsequently a small, centrally crusted ulcer over a 3-4 week period. The patient had remained otherwise well and had no other complaints when he was seen for a screening examination (week 6 of the lesion). At that time, the lesion was of no importance to the patient and the diagnosis of a healing furuncle was entertained. No treatment was initiated. By June 29 (week 10), the lesion had enlarged to become a 2-cm ulcer with central crusting and an indurated periphery. He remained well and had no regional adenopathy. A 7-day course of cloxacillin had no impact on the lesion and a needle aspirate of the eschar border was negative for both Leishmania amastigotes (Leishmania donovani) bodies and acid fast bacilli (AFB). On July 22 (week 13), a 3-mm punch biopsy specimen of the indurated peripheral tissue revealed mixed bacterial flora (3+ coagulase-negative Staphylococcus spp., 2+ viridans streptococci, 2+ diphtheroids, and 2+ Peptostreptococcus magnus), but Ziehl-Neelsen staining for AFB was negative. The histology revealed moderate, acute, reactive inflammation with no evidence of granuloma formation or stainable microorganisms. Ultrasound examination showed involvement only of the epidermis and subcutaneous tissue with no fluid collection. Despite a 10-day course of metronidazole (250 mg three times a day) and doxycycline (100 mg twice a day), the lesion continued to enlarge slowly. By week 17, there was a 2 x 3 cm eschar-covered ulcer with a seropurulent discharge in the center of a 6 x 10 cm area of erythematous, “peau d’orange” induration. The lesion was not warm or tender (Figure 1A). Rare AFB were detected in smears made from a punch biopsy specimen taken from the edge of the lesion (Ziehl-Neelsen stain), but histologic examination revealed only an acute ulcer with secondary vasculitis and numerous Gram-positive and Gram-negative bacteria. Special stains revealed no fungi or AFB and there was no evidence of granuloma formation. Fungal cultures were negative. Bacterial cultures continued to produce a heavy mixed growth. A tuberculin unit (purified protein derivative) skin test result was negative. Despite the rarity of stainable AFB in biopsy material, a presumptive diagnosis of a cutaneous mycobacterial infection was enter-
tained with *M. marinum*, *M. ulcerans*, and *M. tuberculosis* as the most likely etiologic agents. The patient was started on rifampin (600 mg/day), ethambutol (1.2 gm/day), and trimethoprim/sulfamethoxazole (160 mg/800 mg three times a day).

During the first 3 weeks of anti-mycobacterial therapy, the ulcer continued to increase in size. In fact, the initiation of therapy appeared to accelerate the pace of the infection with a doubling of the diameter of the surrounding induration during this period. The patient also noted some discomfort for the first time with tightness and itching in the indurated tissue surrounding the central lesion. By week 20, the central ulcer/eschar was 5 × 5 cm in diameter and the total area of erythema and induration was 14 × 18 cm.

The same medications were continued through weeks 21–25 during which time the lesion grew more slowly but new leg pain developed on standing. Smears of the serous exudate were now strongly positive for AFB (Ziehl-Neelson stain). In week 26, 6 punch biopsy specimens (3–6 mm) from the center to the periphery of the lesion showed nonspecific ulceration with granulation tissue, fat necrosis, and underlying dense fibrous tissue. Once again, the histologic stains for AFB were negative. Clarithromycin (500 mg twice a day) and ciprofl oxacin (750 mg twice a day) were added to the initial therapy with 3 drugs and he was admitted to hospital for extensive debridement in week 27. Repeated debridement continued for a month, with the ulcer reaching a maximum size of 11 × 15 cm surrounded by 3–4 cm of indurated and undermined but viable skin (Figure 1B). In week 29, mycobacterial cultures from a biopsy specimen became positive for the first time after 4 weeks in culture. In week 31, most of the inflamed skin surrounding the central ulcer was excised and a split thickness skin graft was applied. The histology of this excised material revealed (for the first time) a diffuse granulomatous inflammation extending into the subcutaneous tissues. Two weeks later (week 33), a more radical excision of the erythematous skin surrounding the lesion was performed and a second split thickness skin graft was applied. Again histologic examination revealed granulomata. At this time, the skin surrounding the skin grafts appeared normal and the patient remained on therapy with rifampin, ethambutol, trimethoprim/sulfamethoxazole, clarithromycin, and ciprofl oxacin. Although healing of the grafts progressed uneventfully, smears of the exudate remained AFB positive (fluorochrome positive, Ziehl-Neelson negative) until week 35. At this time, the patient developed mild paraesthesias of the hands and lower extremities and the treatment with ethambutol was stopped. Nerve conduction studies demonstrated a mild, large and small fiber neuropathy affecting the lower limbs. These uncomfortable acral paraesthesias progressed slowly over a 3-month period until treatment with ciprofl oxacin and trimethoprim/sulfamethoxazole were also stopped. Treatment with clarithromycin and rifampin was continued for a total course of 18
months (Figure 2). In week 44, local heat with a thermometer-controlled heating pad (40°C; 1 hr twice a day) was added to the treatment regimen and continued for 6 months. At present, he remains well after 4 years of follow-up with no evidence of local recurrence.

Although a presumptive diagnosis of a mycobacterial skin infection was made approximately 3 months after the patient first presented, this impression was based on the evolution of the lesion and the presence of rare AFB in biopsy smears. A tentative bacteriologic diagnosis was only obtained at 6 months. Despite our initial failure to coax the organism to grow on conventional solid media, liquid culture specimens were sent to the Quebec Public Health Laboratory for analysis by high-performance liquid chromatography (HPLC). The HPLC profile suggested that the offending organism was either closely related or identical to *M. ulcerans*. Confirmation of the diagnosis of *M. ulcerans* was finally obtained by nucleotide sequence analysis of the 3’terminal region of 16S rRNA (performed by the Laboratory Center for Disease Control Mycobacteriology Laboratory, Ottawa, Ontario, Canada).

### MICROBIOLOGY

**Initial processing.** Each tissue specimen received by the laboratory was homogenized, decontaminated with NaOH-n-acetyl-cysteine and inoculated into 6 BACTEC 12B vials (liquid media; Becton Dickinson Diagnostic Instruments, Sparks, MD) and 6 Lowenstein-Jensen (L-J) slants (BBL solid media; Becton Dickinson Microbiology Systems, Cockeysville, MD). Two different enrichment substrates used separately and in combination were added to the liquid media and to the L-J slants immediately prior to inoculating specimens: 1) 0.1 ml of POES-PANTA (polyoxyethylene stearate with polymyxin B, amphotericin B, naladixic acid, trimethoprim, and azlocillin: Becton Dickinson Microbiology Systems) and 2) 10% lysed sheep blood cells (LSB; Oxoid, Nepean, Ontario, Canada).

A total of 12 cultures were established (6 BACTEC and 6 L-J slants) for each specimen. One set of vials and L-J slants was incubated at 30°C and the other at 35°C. Liquid cultures were tested weekly using the BACTEC TB-460 (radiometric) system to identify growth defined as a growth index (GI) $\geq 10$. The L-J slants were inspected weekly for growth. Microscopy was performed for each homogenized and decontaminated specimen by fluorescence (Auramine O; Sigma Fine Chemicals, St. Louis, MO) and confirmed by Ziehl-Neelsen staining (basic fuchsin; Anachemia, Montreal, Quebec, Canada). Ulcer aspirate smears and smears obtained from culture vials with a GI $\geq 400$ were also subjected to Ziehl-Neelsen staining.

**Reprocessing and subcultures.** Liquid samples in which no growth was detected after 12–18 weeks were concentrated, washed in sterile distilled water, and inoculated into fresh BACTEC 12B vials with both enrichment substrates added (0.1 ml of POES-PANTA and 10% LSB). Incubation was continued at the same temperature (30°C or 35°C). Samples in which growth was detected were subcultured by inoculating pairs of fresh 12B vials and L-J slants (with or without 10% LSB) and incubating at 30°C and 35°C.

**Antibiotic sensitivity testing.** Actively growing cultures of *M. ulcerans* (GI $\geq 999$ on 2 consecutive weeks) were used to seed 4 fresh POES-supplemented BACTEC 12B vials (1 ml/vial). The high GI inoculum was selected arbitrarily due to the very slow growth of *M. ulcerans*. These vials were tested weekly until a GI of approximately 400 was achieved at which time isoniazid (0.4 μg/ml), rifampin (2.0 μg/ml), or streptomycin (6.0 μg/ml); all antibiotics from Bec-
ton Dickinson Microbiology Systems) were added to individual vials. The fourth vial served as a control. All vials were cultured at 30°C and tested at various intervals in different assays (3–7 days). The antibiotics were added to the vials only once.

RESULTS

Samples. Biopsy and debridement material was sent to the laboratory for attempted isolation a total of 8 times over a 5-month period: the first was a punch biopsy specimen from the edge of the lesion obtained prior to treatment (week 13) and the last was debridement tissue excised in week 33. Between weeks 33 and 42, an additional 4 aspiration/exudate samples were sent to the laboratory for AFB evaluation from slow-to-heal areas of the graft edge.

Primary isolations. No growth was ever detected from the primary specimens inoculated onto L-J slants despite a 6-month period of observation. In the liquid media, growth was first detected from a biopsy specimen 4 weeks after inoculation in the vial containing POES-PANTA plus LSB incubated at 35°C (GI = 17). Although growth in the duplicate vial incubated at 30°C was only detected 10 weeks after inoculation (GI = 20), the organism subsequently grew more rapidly at the lower temperature. No growth was detected in any vial with only 1 enrichment substrate for up to 18 weeks after primary inoculation at either 30°C or 35°C. However, reprocessing of the negative vials from this specimen with addition of both enrichment substrates resulted in detectable growth within 3–4 weeks. Again, the growth was consistently faster at 30°C than at 35°C. Ziehl-Neelsen–stained smears of the positive cultures revealed numerous AFB of moderate length, often forming slender cords resembling those of M. tuberculosis. The organism was isolated a second time from a biopsy specimen sent during week 21, 4 weeks after the initiation of antitycobacterial therapy. In this specimen, growth was only detected at 30°C 5 weeks after reprocessing the original specimen.

Subcultures. Upon subculture, the organism continued to grow more quickly in liquid media than on the solid media slants and there was a rapid loss of the requirement for enrichment substrates. Colonies on the slants were small, convex, rather dry, and a pale cream color. This appearance is in accordance with previous descriptions. Growth on the slants was very slow but was consistently faster at 30°C than at 35°C.

Antibiogram. Our pilot (unstandardized) antibiotic susceptibility protocol was carried out 4 times and reproducibly showed inhibition of M. ulcerans growth in the presence of rifampin or streptomycin. The GI in rifampin- and streptomycin-containing vials decreased from approximately 400 to <10 after 3–5 weeks of incubation compared with the control vials, which had GIs >999 within 2–4 weeks. In contrast, the organisms appeared to be resistant to isoniazid in this assay with continued growth indistinguishable from that observed in the control vials.

DISCUSSION

In many parts of the world the diagnosis of a Buruli ulcer continues to be made primarily on clinical and histologic grounds. Indeed, in endemic areas, a presumptive diagnosis based on the appearance of the lesion and the presence of acid-fast bacilli in exudates is relatively straightforward. However, the diagnosis can be greatly delayed in areas where the disease is not well known. Left untreated, this infection can lead to extensive disease and even amputation. As travel to and from regions of the world where M. ulcerans is endemic becomes more common, the need to recognize and rapidly identify infections caused by this microorganism will increase.

As illustrated by the current case, this organism grows very slowly, typically taking 6–9 weeks to form visible colonies at optimum temperatures (30–33°C). However, even under ideal conditions, growth is fastidious and often not seen at all using conventional solid media for mycobacteria. Primary isolation is rendered particularly difficult due to the small number of microorganisms present in lesion exudates and the relative sensitivity of M. ulcerans to routine decontamination procedures. Since M. ulcerans lesions are almost always contaminated with skin flora or super-infected, decontamination remains an important part of the isolation of this mycobacterium species. In recent years, advances in nucleic acid-based diagnostic techniques have created the potential for more rapid identification of M. ulcerans: for example by analysis of 3’-terminal region of the 16S rRNA gene sequence. However, these techniques typically require at least minimal growth in culture to achieve reasonable levels of sensitivity.

We were able to isolate M. ulcerans only in liquid media (BACTEC 12B) and growth was observed only when the vials had been supplemented with 2 enrichment substrates (POES-PANTA and LSB). Although the first vial in which growth was detected was incubated at 35°C, M. ulcerans subsequently grew more quickly at 30°C. Parenthetically, the extremely slow growth of this organism means that detection in the BACTEC system may be delayed by too frequent testing of the vials (i.e., depletion of the released radiolabeled CO₂). Growth on slants was seen only after subculture of the organism from liquid media. Again, the fastest growth on solid media was observed at 30°C. To our knowledge, there are only 2 other reports in the literature mentioning the use of liquid media in the isolation and manipulation of M. ulcerans. In a small Australian series, liquid cultures were apparently used to isolate M. ulcerans in children. Unfortunately, no details are provided in this report on the culture conditions. A group from Belgium has subsequently used the BACTEC system to evaluate the effects of different decontamination methods and enrichment substrates on the viability of several strains of M. ulcerans. This latter study demonstrated that standard decontamination methods using NaOH markedly inhibit the growth of M. ulcerans and suggested that milder decontamination with HCl may give better results. In accordance with our findings, the addition of enrichment substrates stimulated the growth of at least one of the African strains tested.

Our preliminary data from this single isolate suggest that the BACTEC 12B liquid media may be used to perform drug susceptibility testing for M. ulcerans. To our knowledge, this has not been successfully done before. The results in our limited testing are in accordance with previous reports suggesting that M. ulcerans is usually resistant to isoniazid but...
sensitive to rifampin and streptomycin.\textsuperscript{4,13,22} We did not perform susceptibility testing for clarithromycin, but a recent study suggests that at least some isolates are susceptible to this drug \textit{in vitro}.\textsuperscript{23} However, the relevance of sensitivity testing for \textit{M. ulcerans} is uncertain since \textit{in vitro} susceptibility to a wide variety of agents in non-standardized assays has not been predictive of clinical response (reviewed by Josse and others\textsuperscript{3} and Goutzamanis and Gilbert\textsuperscript{5}).

The most instructive aspect of this case is the re-affirmation that aggressive surgical excision of tissue is crucial to the treatment of this infection.\textsuperscript{24,25} Individuals trained in infectious diseases and microbiology in the developed world have an almost visceral resistance to the notion that a superficial bacterial infection can still require treatment by surgery. In this case, the extent of tissue involvement appeared to accelerate with increasingly aggressive anti-mycobacterial therapy (perhaps as a result of enhanced immune responses to dead or damaged mycobacteria), and the decision to perform wide excision was forced in the absence of a definitive microbiologic diagnosis. It is possible that the total area of tissue involvement could have been reduced if surgery had been performed at the initial presentation.\textsuperscript{8,24} Although relatively slow growing, \textit{M. ulcerans} lesions have the potential to cause serious disfigurement and can even lead to amputation if there is circumferential involvement of an extremity.\textsuperscript{4,5} Deep invasion and involvement of tendons and bone has been reported rarely,\textsuperscript{5,26} but the relative roles of \textit{M. ulcerans} and other super-infecting bacteria in these complicated lesions has not been established.

Although \textit{M. ulcerans} is a disease that predominantly affects inhabitants of endemic areas in tropical and sub-tropical countries, Buruli ulcer should be considered in the differential diagnosis of any chronic skin ulcer in a patient who has lived in\textsuperscript{16} or traveled to an endemic region within the preceding year. The diagnosis requires a high index of suspicion and is based on clinical appearance, the presence of AFB in biopsy specimens, and histologic findings of granulomatous, non-necrotizing panniculitis. Bacteriologic confirmation may be obtained using liquid media supplemented with enrichment substrates, but definitive therapy should not be delayed until such confirmation is available. Early surgical excision\textsuperscript{25,26} and local heat\textsuperscript{18,26} may be the best combination therapy for these unusual infections. The role of antimycobacterial drug therapy remains to be defined.

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REFERENCES

1. Cook A., 1897. \textit{The Mengo Hospital Notes for the Year 1897.} Kampala, Uganda: Makerere College Medical School Library.