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Medical Imagery

Buruli ulcers in a Spanish aid worker after a stay in Peru

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ABSTRACT

Buruli ulcer (BU) is a chronic and destructive infection of the skin and soft tissues caused by *Mycobacterium ulcerans*. Recently, population flows have triggered the appearance of several sporadic cases of BU in non-endemic countries. This represents a significant diagnostic challenge for clinicians and microbiologists. We describe the first case of BU imported to Spain. The patient was a Spanish woman who had stayed 5 months in the jungle of Peru.

Case report

The case patient was a 32-year-old Spanish woman, a resident of León (Spain), who was a biologist with no significant disease history. On April 23, 2014, she was admitted with a poorly healing ulcer on the posterior-internal side of the left arm, which exhibited worsening signs and increasing size, despite several topical agents having been applied since March 10, 2014. On that date, an ulcer of 12 cm in diameter with irregular edges and a necrotic base was observed (Figure 1A). Between July 15, 2013 and December 17, 2013, the patient had travelled in Peru as part of an international cooperation programme for the conservation of the Andean titi monkey of San Martín (Moyobamba) and had visited the Department of Loreto (Iquitos). Two months before the appearance of the ulcer, she had exhibited profuse nocturnal sweating of unknown origin for 15 days, which was self-limited.

Upon admission, the patient was afebrile and in good general condition. The patient exhibited a 12-cm ulcer with a necrotic base and red-violaceous and oedematous undermined edges that had also progressed to ulceration (Figure 1B). Biochemical and haematological tests were normal. Serology against HIV was negative, and Mantoux testing was negative, an unusual result for Buruli ulcer (BU) patients. Histological analysis of the lesion revealed superficial and deep interstitial and perivascular dermatitis with the presence of microabscesses. Microbiological studies with Giemsa and auramine stains were negative, as were PCR for *Leishmania* sp. and cultures for bacteria and mycobacteria at 30 °C and 37 °C for 12 weeks.

After receiving several treatments without improvement, the patient was referred to the Tropical Diseases Unit of the Hospital Ramón y Cajal (Madrid, Spain) on May 5, 2014, where a second biopsy was performed, which was cultured. While the biopsy was being incubated, the diagnostic possibility of pyoderma gangrenosum was raised; thus, oral steroid treatment was initiated. As there was no response (Figure 1C), the case was re-evaluated on May 20, 2014 by reviewing the previous anatomopathological samples, and a third cutaneous biopsy of the inflammatory zone was taken. Ziehl–Neelsen and Fite–Faraco stains were positive for biopsies #1 and #3. The previous negative microbiological test results may have been a consequence of sampling error. Moreover, after 30 days of incubation of the second biopsy in Löwenstein–Jensen medium at 30 °C, five non-chromogenic colonies of an acid–alcohol-resistant bacillus (AARB) grew. The colonies were initially identified as *Mycobacterium marinum* both by base pair sequencing of the 16S rRNA gene and by Bruker matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Nevertheless, the clinical evolution was not characteristic of *M. marinum* and it was suspected that it might be *Mycobacterium ulcerans*, a species that cannot be discriminated from *M. marinum* by these techniques. Consequently, the third biopsy and histological sections of the first biopsy were sent to the Mycobacteria Reference Centre of Asturias (Oviedo, Spain) for the detection of *M. ulcerans* by PCR of the insertion sequence IS*2404*, and the result was positive for both samples. The species was confirmed as *M. ulcerans* by Genotype Mycobacterium CM/AS (Hain Lifescience, Nehren, Germany) and typed by multilocus variable number tandem repeat analysis (MLVA) at the Mycobacteria Reference Centre of Asturias. In addition, whole genome sequencing (WGS) was performed at the microbiology laboratory of Monash University (Clayton, Australia).

The initial treatment consisted of extensive surgical debridement (Figure 1D) and a combination of three antibiotics: rifampicin 600 mg/day, clarithromycin 500 mg/12 h orally, and streptomycin 1 g/24 h intramuscularly. Streptomycin was discontinued at 4 weeks due to toxicity. Nine months of treatment with

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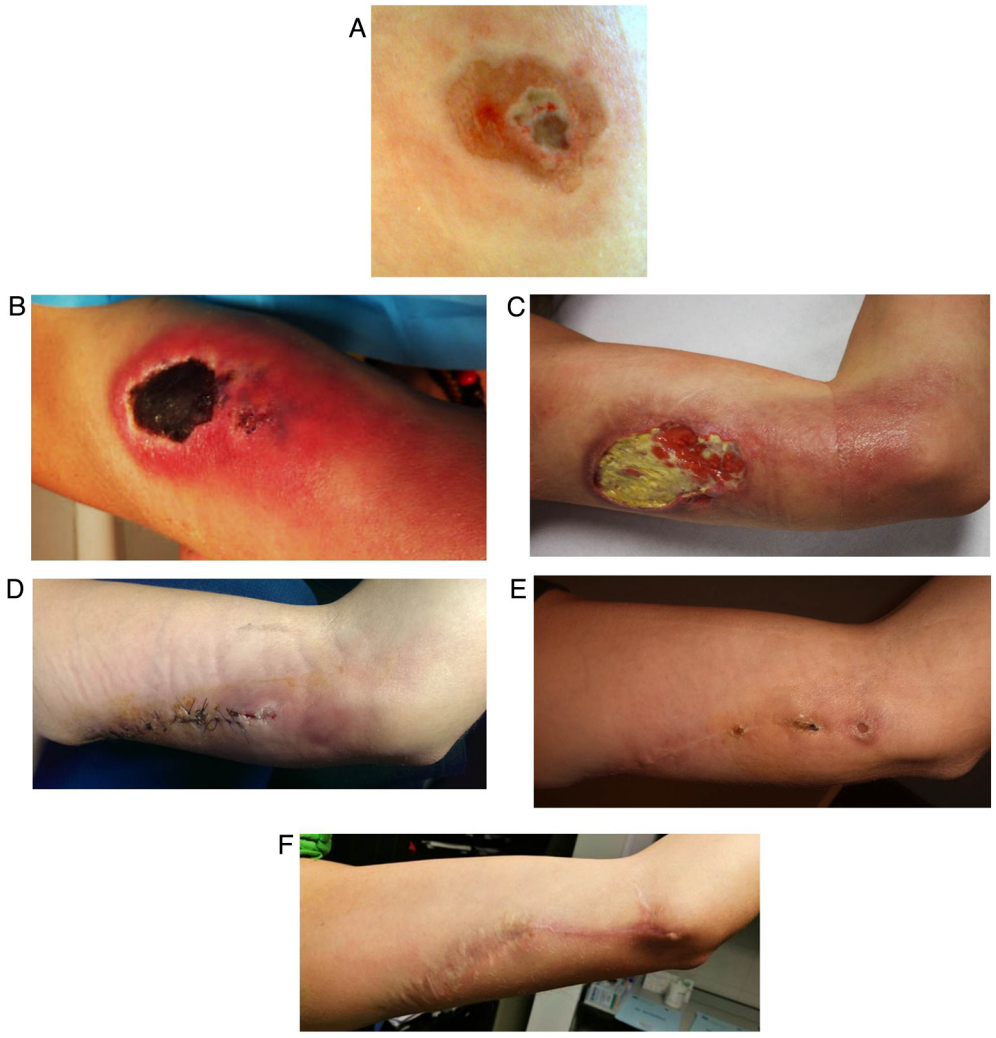

Figure 1. Progression of Buruli ulcer in a Spanish aid worker after her return from Peru. (A) March 2014, on the patient's return to Spain; (B) April 2014, before *Mycobacterium ulcerans* therapy; (C) lack of response with steroid treatment; (D) extensive debridement, 8 weeks after first being seen; (E) November 2015, after 9 months of antimicrobial drug therapy; (F) May 2017, 8 months after the end of treatment.

rifampicin and clarithromycin were completed with good adherence.

In September 2015, due to the persistence of pain and local inflammation (Figure 1E), a new surgical intervention was performed. The anatomopathological study revealed the presence of necrotizing granulomas with extensive caseous necrosis surrounded by an inflammatory infiltrate rich in histiocytes and some multinucleated cells, interpreted as potentially persistent BU. An additional culture of a biopsy specimen was negative, but IS*2404* PCR was positive. Antibiotic treatment with rifampicin, clarithromycin, and levofloxacin (500 mg/day) was restarted for 12 months, resulting in the disappearance of all skin lesions. As of follow-up consultations in May 2017, the patient was asymptomatic (Figure 1F).

The presence of IS*2404* was tested using a nested PCR protocol (Stienstra et al., 2003). The strain of *Mycobacterium* sp. was sequenced by Illumina MiSeq. The resulting DNA sequence reads were mapped to the 5.6-Mbp *M. ulcerans* reference genome Agy99 to identify all sites of nucleotide differences (polymorphisms). A high-resolution phylogeny was inferred based on shared nucleotide sequences among a collection of *M. ulcerans* and *M. marinum* isolates. The strain identified corresponded to a strain of *M. ulcerans* that shares 99.96% DNA identity to an *M. ulcerans* isolate from French Guiana (MY, Figure 2). In addition, like all *M. ulcerans* strains, the strain of the patient contained the pMUM plasmid and was predicted to produce the immunosuppressive toxin mycolactone.

MLVA was performed using six previously described variable number tandem repeat (VNTR) markers: VNTR18 and VNTR19 (Ablordey et al., 2005), mycobacterial interspersed repetitive units MIRU5 and MIRU33 (Stragier et al., 2005), ST1 (Hilty et al., 2006), and microsatellite SSR (Ablordey et al., 2007). To determine the geographic linkage from our *M. ulcerans* isolate, the MLVA results were interpreted according to previously published data (Reynaud et al., 2015). The pattern obtained was JLAAC (VNTR-18: J, VNTR-19: L, MIRU5: A, MIRU33: A, MST1: C) and 34 repeats for the SSR microsatellite. This pattern corresponds to genotype I described by Reynaud et al., with the exception of the SSR marker, which had only 10 repeats in their strains.

Discussion

BU is the third leading cause of mycobacterial infection in the world after tuberculosis and leprosy and the least well understood. This disease is rare in South America, although it is present in several countries. In Peru, only 14 cases of BU have been described since 1969 (Caro and Llerena, 2006; Guerra et al., 2008; Moyano et al., 2008; Ward, 1970), all in autochthonous patients from the north of the country. Two cases of BU in European travellers upon

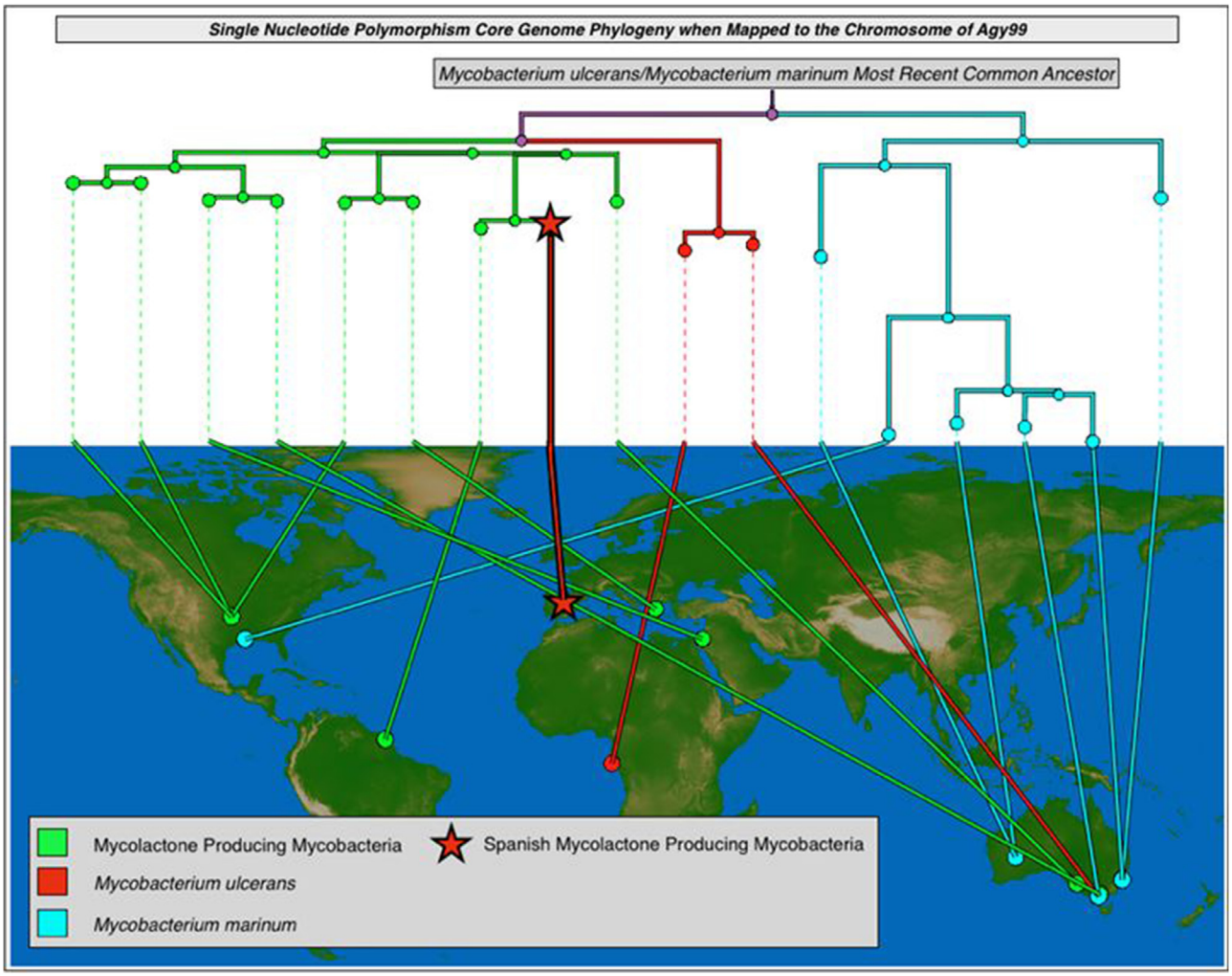

Figure 2. Phylogenetic relationship of this strain with other *Mycobacterium ulcerans* and *Mycobacterium marinum* isolates. The red star indicates the close relationship to the MY strain from French Guiana. Also shown is the connection between the geographic origin of the isolate and the phylogeny.

their return from South American countries have also been published (Mougin et al., 2011; Wadagni et al., 2018). The case patient here became infected in the departments of Loreto and San Martín. Four cases occurring in Loreto have been described previously (Guerra et al., 2008; Moyano et al., 2008).

Before the introduction of antibiotic treatment in 2004, the management of this infection was almost exclusively surgical. Currently, this strategy is reserved for ulcers of larger size (>10 cm), for multiple ulcers, for those affecting the head and neck, or for those associated with osteomyelitis. However, prolonged antibiotic treatment appears to be a good alternative to surgery for large ulcers (Wadagni et al., 2018). In the case presented herein, the lesion was >12 cm in diameter, and after the recommended 8 weeks of antibiotic treatment the lesion had not resolved. For this reason it was decided to prolong the treatment for an additional 7 months. Nevertheless, we must take into consideration the possibility that the evolution described reflects a paradoxical reaction, one of the hallmarks of which is an intense inflammatory reaction revealed by histopathology (O'Brien et al., 2013), which was the case in our patient. However, the other characteristic outcome, i.e. deterioration of the clinical evolution after an initial improvement, was not evident. The patient did not show the expected improvement, but there was no apparent deterioration. Additionally, although extended paradoxical reactions have been described, most of them have occurred less than 10 weeks after antibiotic initiation (O'Brien et al., 2013).

BU initially manifests as a nodule, papule, or painless plaque on the leg, arm, or face and evolves into a painless ulcer with characteristic indeterminate borders. The lack of suspicion for this infection in patients from South America results in delays in its diagnosis.

M. ulcerans is difficult to grow from clinical samples. Culture sensitivity varies from 34% to 79%. The etiological agent could not be isolated from either the patients reported with BU from Peru (Guerra et al., 2008; Moyano et al., 2008) or the two travellers infected in South America (Mougin et al., 2011; Wadagni et al., 2018). Nevertheless, we successfully cultured *M. ulcerans* from biopsy #2. The most sensitive diagnostic methods are molecular. IS*2404* PCR, the most widely used diagnostic test in endemic countries, has 79–85% sensitivity and variable specificity of between 65% and 100%. Histological analysis is also frequently used, but sensitivity is lower at 70% in cases with a clinical suspicion (Eddyani et al., 2018).

Comparison of the genomes of *M. ulcerans* and *M. marinum* suggests that the first emerged as an evolution of the second after the acquisition of genes for the production of mycolactone, an immunosuppressive molecule and the main determinant of pathogenicity in *M. ulcerans*. Although the 16SrRNA gene

sequences are nearly identical, the two species are surprisingly different phenotypically (Stinear and Johnson, 2007).

In conclusion, the differential diagnosis of infection by *M. ulcerans* should be included for patients from tropical areas presenting with ulcers, especially if such ulcers are painless, exhibit rapid growth, and are located on the extremities. A lack of familiarity with the disease results in delays in diagnosis and subsequent treatment, leading to severe deformities and disabilities.

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