Buruli Ulcer: A Review

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Abstract: Buruli ulcer (BU) is a severe necrotizing infection of skin and soft tissues caused by the fresh-water mycobacterium, *Mycobacterium ulcerans*. While it is rarely fatal, it can profoundly debilitate those it affects, resulting in extreme individual morbidity, as well as a profound financial, emotional and social burden for the families and communities affected. This review of Buruli ulcer details the history, pathogenesis, diagnosis and management of this neglected tropical disease.

Keywords: Buruli ulcer, Bairnsdale ulcer, Daintree ulcer, *Mycobacterium ulcerans*, tropical diseases, infectious diseases.

INTRODUCTION

Buruli ulcer (BU) is a devastating, necrotizing infection of skin and soft tissues, which rarely kills but can profoundly debilitate its victims. Buruli ulcer is caused by a fresh-water mycobacterium, *Mycobacterium ulcerans*, found in humid and tropical climates. It is the third most common mycobacterial infection, behind tuberculosis and leprosy. It occurs predominantly in the West African countries of Côte d'Ivoire, Ghana, and Bénin, where access to healthcare is limited. However, pockets of endemic infection have been reported in several other countries worldwide, including Papua New Guinea, South America, Mexico, Japan and Australia. In those scattered places where it occurs, its prevalence is high and its morbidity dramatic, leading the World Health Organization (WHO) to recognizes BU as one of the most important emerging infections globally.

History

In 1897, Sir Albert Cook, a medical missionary in British-ruled Uganda, described several patients who had leg ulcers that were probably Buruli ulcer. In the 1930s and 1940s, an outbreak of deep skin ulcers occurred in the vicinity of Bairnsdale, in southeastern Victoria, Australia. In Australia, BU is also known as "Bairnsdale ulcer" or "Daintree ulcer". In 1948, Peter MacCallum, an Australian physician, isolated the pathogen and named it *Mycobacterium ulcerans*. Later in the 1960s, the disease acquired the common name Buruli ulcer from a focal outbreak in the Buruli region, a marshy region in southern Uganda [1, 2]. In 1998, the WHO recognized Buruli ulcer as an emerging infection and established the Global Buruli Ulcer Initiative (GBUI) [3]. Since then, the World Health Assembly and Global Network on Neglected Tropical Diseases has continued to focus attention on controlling Buruli ulcer.

Epidemiology

Worldwide, BU is the third most common mycobacterial disease after tuberculosis and leprosy. There are approximately 5000 new cases per year in over 30 countries [4]. In West Africa, approximately 70% of the cases occur in children under the age of 15. While the median age in Australia is much older, 66 years [5].

The causative bacterium, *M. ulcerans*, occurs naturally in moist tropical soils and as a saprophyte on decaying vegetation in tropical wetlands, including slow-moving streams, stagnant ponds, swamps, and marshes. The disease has a distinct geographic distribution with most cases occurring in rural tropical wetlands, particularly in equatorial West Africa (Figure **1**). In Australia, BU continues to be endemic to areas of Victoria and Queensland. While in Japan**,** BU is uniquely caused by the *M. ulcerans* subspecies *shinshuense.* In some regions, especially among the West African nations of Benin, Togo, Ghana, and Côte d'Ivoire, the incidence has increased dramatically over the past few decades [2]. This is due not only in part to increased awareness, but also to environmental

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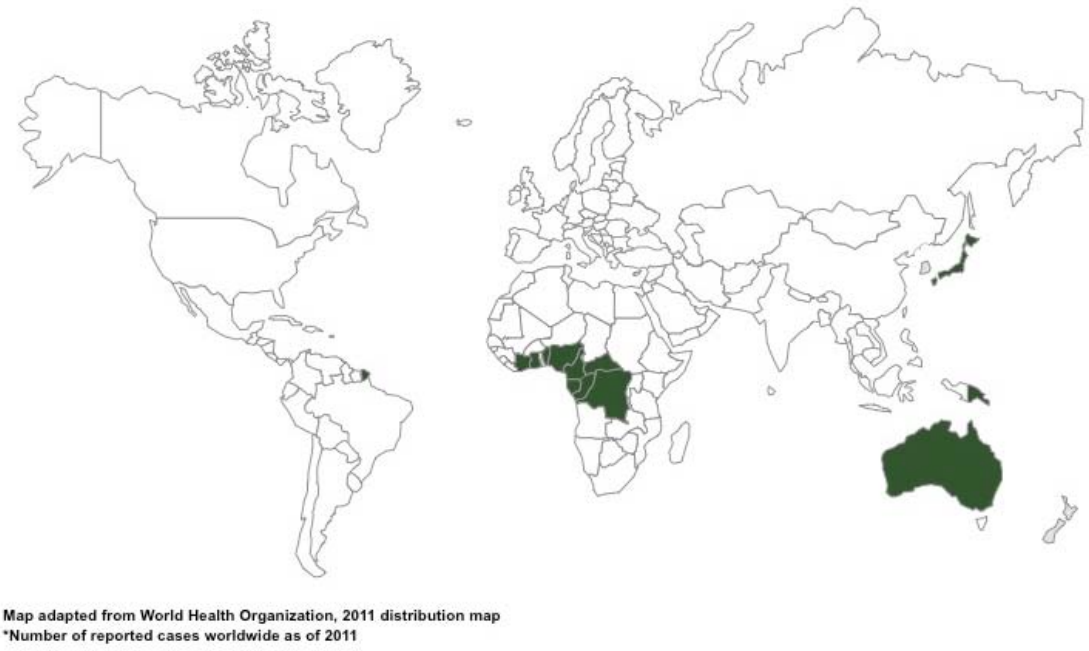

Figure 1: Buruli ulcer. Worldwide distribution (adapted from WHO) [28].

degradations associated with deforestation and altered flooding patterns [2].

Microbiology

Mycobacterium ulcerans, the causative agent of Buruli ulcer, is characterized by slow growth with a 5 to 8 week incubation period, but can take as long as 6 months before it is isolated from its host. This bacterium is one of the mycobacterial pathogens found in the *M. marinum* complex. This group includes species found in aquatic vertebrate and includes *M. marinum*, *M. psudoschottsii*, and *M. liflandii* in addition to *M. ulcerans*. *M. ulcerans* is thought to have evolved from *M. marinum* though accumulation of insertion sequences and mutations. These mutations have decreased its ability to grow in low oxygen environments and direct sunlight [2].

Like *M. marinum,* an important characteristic of *M. ulcerans* is the low temperature that is required for optimal growth. A narrow temperature range between 30-33 °C is where the bacterium is found to have the most optimal growth. This in turn, confines the infection to the skin and limits its ability to spread throughout the body to various organs [2].

Pathogenesis

The disease starts as a painless nodule in the skin and subcutaneous fat, characterized histologically by necrosis and a lack of inflammation. *M. ulcerans* produces an exotoxin, a polyketide macrolide called mycolactone. The mycolactone diffuses into and destroys surrounding tissues. Over time, this diffusible toxin ravages subcutaneous fat causing subsequent destruction of overlying tissues.

Unique to *M. ulcerans*, however, is that it produces the exotoxins, mycolactone A and B (African strains) and mycolactone C (Australian strains). These toxins suppress the immune system by inducing apoptosis in immune cells [6, 7]. A unique property of mycolactone is that it changes its conformation when exposed to visible light, causing significantly reduced cytotoxicity [8]. Mycolactone has also recently been shown to induce local anesthesia through the type 2 angiotensin II receptor leading to potassium-dependent hyperpolarization of neurons [9]. In West Africa, the disease is more virulent than it is in Australia or the Americas. The geographic differences in the severity of the disease may be due to regional variations in the virulence of the mycolactones produced by the bacteria.

Transmission

The precise mechanism of transmission of the disease has not been fully established. The presumed typical sequence of events is as follows: *M. ulcerans* is inoculated into the skin, usually on an extremity after minor or possibly unnoticed trauma, such as an abrasion on decaying wood. Several studies have discovered potential animal reservoirs for *M. ulcerans*,

such as the amoeba[8]or aquatic insects,[9]and vectors, such as the creeping water bug (*Naucoris cimicoides*) [10-12]. One study hypothesized that mosquitoes are potential vectors after *M. ulcerans* DNA was detected in roughly 0.2% of mosquitoes in southeastern Australia [13]. Similar bacteria such as *M. abscessus*, *M. fortuitum*, and *M. marinum* are prevalent in fish who serve as vectors [14]. Of note, person-to-person transmission has not been reported. An important consideration regarding Buruli ulcer is the local view of the disease and perceived causes of transmission. In Ghana, 53.3% of people with pre-ulcers said they believed they were infected while swimming in ponds and rivers, while 64.5% of people with ulcers attributed the disease to witchcraft [15].

Clinical Features

The disease presents as a painless, occasionally itchy, firm swelling. This lesion evolves into a mobile, non-tender, dermal or subcutaneous nodule, often lacking corresponding constitutional symptoms (Figure **2**). Although typical Buruli ulcers are not associated with pain, 50% of *M. ulcerans* ssp. *shinshuense* infections in Japan are notably tender [4, 16]. Commonly affected sites are exposed body parts such as the face and extremities, especially the lower limbs [4, 17]. The course of the lesion is variable. The nodule may resolve spontaneously, expand into a large painless edematous plaque, or ulcerate (Figure **3**). Recent data suggest in some regions, up to 50% of lesions remain in the edematous form. When ulcers do form, they are painless and patients rarely have fever, pain, or lymphadenopathy. Ulcers that form tend to be quite large. A Ghanaian study of 336 cases found that approximately 60% of lesions were larger than 15 cm in diameter.[19] The ulcers have a central necrotic slough that is usually white in color. Undermined borders may disguise the true subcutaneous extent of the lesion.

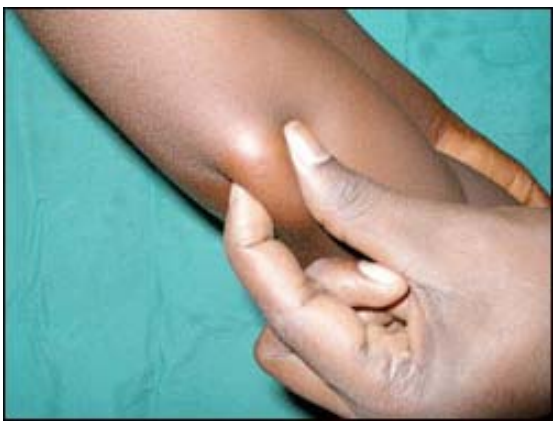

Figure 2: Buruli ulcer. A nontender deep nodule (courtesy of Jim Niquette).

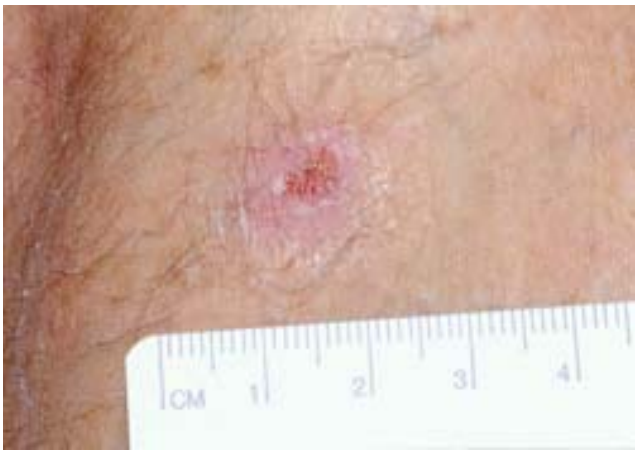

Figure 3: Buruli ulcer. A small ulcerated nodule (courtesy of Jim Niquette).

Rarely, bacteria may spread through the blood to cause metastatic cutaneous ulcers and bony lesions. Bones may also become infected via direct extension from a primary cutaneous lesion. Even with disseminated disease, patients lack systemic symptoms. Ulcers that cross a joint may cause severe and crippling contractures of limbs as they heal with extensive scarring, while destructive facial lesions may cause blindness and disfigurement. The ulcers can also cause significant morbidity when located on the genitals or breasts.

The WHO has characterized lesions into three categories depending on size. Category I represents a solitary lesion less than 5 cm in diameter. Category II represents a solitary lesion between 5 and 15 cm (Figure **4**). Category III is a solitary lesion of greater than 15 cm in diameter (Figure **5**). These categories determine the recommended treatment [20].

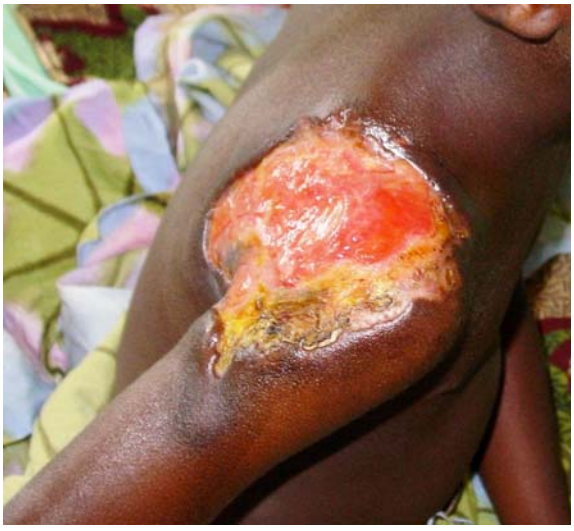

Figure 4: Buruli ulcer. A child with moderate but deep ulceration of the shoulder (courtesy of Jim Niquette).

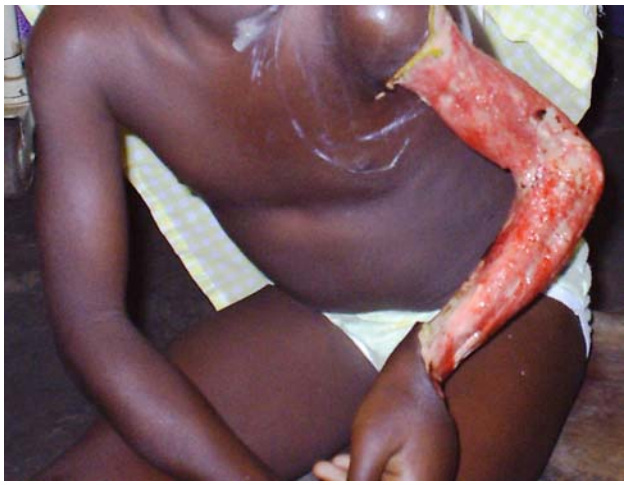

Figure 5: Buruli ulcer. A young child with extensive and disfiguring ulceration of the entire arm (courtesy of Jim Niquette).

In order to cause these edematous and ulcerative lesions in its host, *M. ulcerans* produces a local immunosuppressive state. This allows the bacteria to blunt the host immune response and survive longer. It does so by inhibiting the inflammatory cytokines IFN-gamma, IL-10, TNF-alpha, and TFG-beta during the active phase of infection [18, 19]. Only when the local immunosuppressive state induced by *M. ulcerans* fades, is the host able to produce an inflammatory response with organization of lymphoid structures and up regulation of phagocytic activity [20].

There is evidence that not all people infected with *M. ulcerans* develop the disease. Th1 CD4 T cells and their derived cytokines are crucial for protection against mycobacterium infections. Co-infections that lower CD4 counts or decrease the Th-1 response can lead to a more aggressive course of Buruli ulcer. Reports from Benin and Cameroon have proven that those with HIV are more prone to Buruli ulcer which may be due to decreased CD4 count and immunosuppression [21, 22]. Schistosomiasis co-infection may also worsen the course as it decreases the Th-1 response, diminishing the body's natural immune response against the mycobacterium [14].

Another interesting immunologic aspect to this disease is noted. A transient paradoxical worsening of the ulcer can occurafter initial clinical improvement with appropriate therapy. The reaction occurs because once the antibiotics decrease the exotoxin mycolactone, which is known to cause immune suppression of the host, there is a restoration and hyperactivity of local and systemic immune response [20]. This can occur up to 8 weeks after the start of treatment because the immunosuppressant effect of the mycolactone is still present for some time despite therapy [23].

Diagnosis

The prompt diagnosis of Buruli ulcer remains a challenge in endemic regions, many of which are not equipped with specialized lab equipment necessary for a definitive diagnosis. The differential diagnosis depends on the stage of disease. The ulcerative stage differential includes cancrum oris (noma), tropical ulcer, yaws, leshmaniasis, squamous cell carcinoma, pyoderma gangrenosum and vascular ulcers.

A thorough history and physical examination are the most important steps in establishing the diagnosis of Buruli ulcer. Current or previous residence in, or travel to, an endemic area are important considerations.

Laboratory confirmation of infection can be obtained by two or more of the following laboratory tests:

1. Acid-fast bacilli (AFB) identified on microscopic smear stained by Ziehl-Neelsen technique

2. Mycobacterial culture

3. Polymerase chain reaction (PCR) of exudate or fresh tissue

4. Histopathologic evaluation with visualization of mycobacterium with Ziehl-Neelsen stain.

The sensitivity and specificity of these tests vary widely and are impacted by collection and storage conditions. The WHO provides guidance on sampling techniques for diagnosing *M. ulcerans* infections [24]. A fine needle aspiration is preferred for non-ulcerated lesions while a punch biopsy is indicated for ulcerated ones.

Direct microscopy has the advantage of requiring minimal specialized equipment. Testing can be done on tissue from swabs, fine-needle aspiration and biopsy samples. Microscopy can be performed locally, on site and the result is available immediately. It has a reported sensitivity of 42-57%. While it will detect the presence of mycobacterium, is not specific for *M. ulcerans.*

Tissue culture for *Mycobacterium ulcerans* has the lowest sensitivity of testing methods, from 20-60%. Specimens can be obtained from swabs, fine-needle, aspiration and biopsy samples. Cultures must be

performed in specialized laboratories and the results are quite slow, taking more than 8 weeks due to the slow-growing nature of *M. ulcerans*.

PCR for the IS*2404* repeat sequence has the highest sensitivity for detecting *M. ulcerans* at 85-98% and results are determined quickly, in less than 48 hours [25]. This sequence is unique and prevalent within *M. ulcerans* DNA. The sensitivity of PCR is impacted by tissue collection methods, transport media, and sample storage. While PCR is highly sensitive in detecting active infection, it is not as useful for determining response to treatment in patients on therapy [26].

Histopathologic tissue specimens should be stained with hematoxylin-eosin stain as well as Ziehl-Neelsen to help detect mycobacterium. The sensitivity for histology is around 82-90%. A 4mm punch biopsy provides an adequate sample for both histology and microbiologic testing with minimal wound care [25]. Of note, samples should be taken from the middle of the necrotic tissue where the mycobacterium is at greatest density.

Histopathology

The hematoxylin-eosin stain shows an ulcer with coagulative necrosis in both the dermis and adipose tissue, with some extension into the fascial layer in severe cases [27]. While a lymphocytic infiltrate is rare, there can also be inflammatory cells in the nerve bundles and perineural spaces as well as apoptosis of nerve bundles which contribute to the typical painlessness of the ulcers [18]. Underneath the ulcer, a secondary leukocytoclastic vasculitis or thrombosis of small and medium sized vessels may be seen. The cutaneous nerves of appendages can be destroyed and the necrosis may extend well beyond the edge of the ulcer. With special Ziehl-Neelsen stain, one can see abundant clumps of extracellular acid-fast bacilli in the center of a nodule or at the base of an ulcer [4]. Healing lesions often show a granulomatous reaction with Langhans-type giant cells, suggesting development of cell-mediated immunity. Healed lesions show extensive scarring with dermal fibrosis and epidermal atrophy.

Prevention

While there is no Buruli ulcer vaccine, BCG vaccination may provide some early protection from infection [28]. Wearing shoes, long pants, and protective clothing in at risk areas are also recommended.

Treatment

In endemic areas of the developing world, people with Buruli ulcer often face significant challenges including sequelae from infection, delayed treatment, and the high cost of treatment [29]. Ghana is currently leading the way with its National Buruli Ulcer Control Programme (NBUCP) that gives free care to Buruli ulcer patients [30].

The WHO provides a comprehensive, online manual detailing the current treatment recommendations for Buruli ulcer [31]. Standard therapy consists of oral rifampicin 10mg/kg per day and intramuscular streptomycin (15mg/kg daily) for eight weeks. Alternately, 8 weeks of oral rifampicin (10 mg/kg daily) and oral clarithromycin (7.5 mg/kg twice daily) can be given [32].

The rifampicin and clarithromycin combination is recommended in pregnant patients and those unable to tolerate streptomycin. Alternatively, a combination of rifampicin (10 mg/kg once daily) and moxifloxacin (400 mg once daily) has been used. The treatment schema is listed in Table **1**.

All patients presenting with BU should be evaluated, counseled and tested (if indicated) for HIV and tuberculosis. Appropriate antiretroviral therapy should be initiated in addition to BU treatment noting potential serious drug interactions. In patients co-infected with HIV and *M. ulcerans*, ulcer severity is often worse, with multifocal lesions and osteomyelitis more common.

A paradoxical worsening of disease can occur with initiation of antibiotic therapy, a reaction similar to a reversal reaction seen in leprosy. Approximately 20% of patients develop this reaction, typically occurring a median of 39 days into treatment (range 20-73 days) [33]. Reactions are seen at the borders of the existing ulcer, within the region of the ulcer and distally. Generally, local wound care is all that is required with evaluation for osteomyelitis if suspected. In severe cases, however, systemic corticosteroids may be used to blunt the unwanted inflammatory response [33].

Other treatments for BU, should be used in conjunction with standard therapy. Because *M. ulcerans* grows best at cooler temperatures, local heat application, with a circulating hotwater jacket for instance, may be used to arrest expansion of the

Table 1: Treatment for Buruli ulcer

Category	Manifestations	Treatment schema	Primary aim	Secondary aim	Antibiotics
I	Small early lesions (e.g. nodules/papules/plaques, ulcers <5 cm)	*Papules and nodules:* Antibiotics 24 hours prior to emergent incision and suturing, then for 4 weeks total *All other lesions:* antibiotics 8 weeks	Cure without surgery, except to remove dead tissue	Reduce/prevent recurrences	Rifampicin, 10mg/kg PO daily for 8 weeks and Streptomycin, 15mg/kg IM daily for 8 weeks or (for pregnant patients and those unable to take streptomycin) Rifampicin, 10 mg/kg PO daily for 8 weeks and clarithromycin, 7.5 mg/kg PO twice daily for 8 weeks
II	Non-ulcerative and ulcerative plaques, edematous forms, ulcers >5 cm, lesions of head and neck	Antibiotics for at least 4 weeks then surgery (if needed), then 4 more weeks of antibiotics	Reduce the extent of surgical excision	Reduce/prevent recurrences	
III	Disseminated/mixed disease (e.g. osteitis, osteomyelitis, joint involvement)	Antibiotics for at least 1 week before surgery, then 7 more weeks of antibiotics	Reduce infection and dissemination	Reduce/prevent recurrences and reduce the extent of surgical excision	

Modified from World Health Organization. Provisional guidance on the role of specific antibiotics in the management of Mycobacterium ulcerans disease (Buruli ulcer). World Health Organization. Available at http://www.who.int/buruli/information/antibiotics/en/. Accessed Sept 2, 2014.

ulcers. Where available, ozone and hyperbaric oxygen have also been used successfully in the management of Buruli ulcer [34]. In parts of Africa, however, traditional therapies are often used first, before the case comes to the attention of conventional health officers, thus delaying treatment.

Along with antibiotic therapy, surgical management of Buruli ulcer is recommended to remove necrotic tissue, cover skin defects and correct deformities. Excision of an early lesion while it is still in the nodular stage can be curative; but for ulcers, excision followed by skin grafting and physical therapy is often necessary. In rural West Africa, patients often present late for formal medical care when their ulcers may be deep and extensive. Occasionally amputation of a limb is necessary to treat these advanced lesions. Hospitalization for Buruli ulcer averages about 100 days and rehabilitative physical therapy is often unavailable.

CONCLUSION

Buruli ulcer is a severe, necrotizing disease, caused by *Mycobactium ulcerans*. It primarily affects the impoverished areas of West Africa; and the impact of this disease on the endemic communities where it occurs is profound. The dermatological community can help by actively supporting international programs aimed at preventing or treating Buruli ulcer. Recognition, understanding and action are required to control this neglected and devastating disease.

REFERENCES

[1] Clancey J. Mycobacterial skin ulcers in Uganda. Lancet 1961; 2: 951-4. http://dx.doi.org/10.1016/S0140-6736(61)90793-0

[2] Merritt RW, Walker DE, Small PLC, *et al*. Ecology and Transmission of Buruli Ulcer Disease: A Systematic Review. PLoS Negl Trop Dis 2010; 4(12): e911. http://dx.doi.org/10.1371/journal.pntd.0000911

[3] WHO, Buruli Ulcer: Progress Report 2004-2008. Wkly Epidemiol Rec 2008; 83: 145-154.

[4] Yotsu RR, Nakanaga K, Hoshino Y, Suzuki K, Ishii N. Buruli ulcer and current situation in Japan: a new emerging cutaneous Mycobacterium infection. J Dermatol 2012; 39(7): 587-93. http://dx.doi.org/10.1111/j.1346-8138.2012.01543.x

[5] Veitch MG, Johnson PD, Flood PE, Leslie DE, Street AC, Hayman JA. A large localized outbreak of Mycobacterium ulcerans infection on a temperate southern Australian island. Epidemiol Infect 2007; 119(3): 313-8. http://dx.doi.org/10.1017/S0950268897008273

[6] Silva MT, Portaels F, Pedrosa J. Pathogenetic mechanisms of the intracellular parasite Mycobacterium ulcerands leading to Buruli ulcer. Lancet Infect Dis 2009; 9(11): 699-710. http://dx.doi.org/10.1016/S1473-3099(09)70234-8

[7] George KM, PL, Welty DM, Small PL. Mycobacterium ulcerands toxin, mycolactone, causes apoptosis in guinea pig ulcers and tissue culture cells. Infect Immun 2000; 68(2): 877-83. http://dx.doi.org/10.1128/IAI.68.2.877-883.2000

[8] Xing Y, Hande SM, Kishi Y. Photochemistry of mycolactone a/b, the causative toxin of buruli ulcer. J Am Chem Soc 2012; 134(46): 19234-9. http://dx.doi.org/10.1021/ja309215m

[9] Marion E, Song OR, Christophe T, *et al.* Mycobacterial toxin induces analgesia in buruli ulcer by targeting the Angiotensin pathways. Cell 2014; 157(7): 1565-76. http://dx.doi.org/10.1016/j.cell.2014.04.040

[10] Gryseels S, Amissah D, Durnez L, *et al.* Amoebae as potential environmental hosts for Mycobacterium ulcerans and other mycobacteria, but doubtful actors in Buruli ulcer epidemiology. PLoS neglect Trop Dis 2012; 6(8): e1764.

[11] Ebong SM, Eyangoh S, Marion E, *et al*. Survey of water bugs in Bankim, a new buruli ulcer endemic area in Cameroon. J Top Med 2012; 2012: 8.

[12] Gamboa M, Kimbirauskas RK, Merritt RW, Monaghan MT. A molecular approach to identifying the natural prey of the African creeping water bug Naucoris, a potential reservoir of Mycobacterium ulcerans. J Insect Sci 2012; 12: 2. http://dx.doi.org/10.1673/031.012.0201

[13] Lavender CJ, Fyfe JA, Azuolas J, *et al*. Risk of Buruli ulcer and detection of Mycobacterium ulcerans in mosquitoes in southeastern Australia. PLoS Neglet Trop Dis 2011; 5(9): e1305. http://dx.doi.org/10.1371/journal.pntd.0001305

[14] Stienstra Y, van der Graaf WT, te Meerman GJ, The TH, de Leij LF, van der Werf TS. Susceptibility to development of Mycobacterium ulcerans disease: review of possible risk factors. Trop Med Int Health 2001; 6(7): 554-62. http://dx.doi.org/10.1046/j.1365-3156.2001.00746.x

[15] Ackumey MM, GM, Pappoe M, Kwakye-Maclean C, Weiss MG. Illness meanings and experiences for pre-ulcer and ulcer conditions of Buruli ulcer in the Ga-West and Ga-South Municipalities of Ghana. BMC Public Health 2012; 12: 264. http://dx.doi.org/10.1186/1471-2458-12-264

[16] Onoe H, Yotsu RR, Nakanaga K, Hoshino Y, Ishii N, Takeuchi T. Buruli ulcer accompanied by pain in a Japanese patient. J Dermatol 2012; 39(10): 869-70. http://dx.doi.org/10.1111/j.1346-8138.2011.01430.x

[17] Agbenorku P, Edusei A, Agbenorku M, *et al.* Buruli-ulcer induced disability in ghana: a study at apromase in the ashanti region. Plast Surg Int 2012; 2012: 752749.

[18] Zavattaro E, Boccafoschi F, Borgogna C, *et al*. Apoptosis in Buruli ulcer: a clinicopathological study of 45 cases. Histopathology 2012; 61(2): 224-36. http://dx.doi.org/10.1111/j.1365-2559.2012.04206.x

[19] Pahlevan AA, WD, Andrews C, George KM, Small PL, Foxwell BM. The inhibitory action of Mycobacterium ulcerans soluble factor on monocyte/T cell cytokine production and NF-kappa B function. J Immunol 1999; 163(7): 3928-35.

[20] Martins TG, Fraga AG, Gama JB, *et al.* Corticosteroid-induced immunosuppression ultimately does not compromise the efficacy of antibiotherapy in murine mycobacterium ulcerans infection. PLoS Negl Trop Dis 2012; 6(11): e1925.

[21] Christinet V, RK, Serafini M, *et al.* Impact of HIV on Severity of Buruli Ulcer Disease: Results of a retrospective study in Cameroon. Open Forum Infect Dis 2014; 16.

[22] Johnson RC, Nackers F, Glynn JR, *et al.* Association of HIV infection and Mycobacterium ulcerans disease in Benin. AIDS 2008; 22(7): 901-3. http://dx.doi.org/10.1097/QAD.0b013e3282f7690a

[23] Nienhuis WA, Abass KM, Tuah W, *et al.* Paradoxical responses after start of antimicrobial treatment in Mycobacterium ulcerans infection. Clin Infect Dis 2012; 54(4): 519-26. http://dx.doi.org/10.1093/cid/cir856

[24] World Health Organization. Guidance on sampling techniques for laboratory-confirmation of Mycobacterium ulcerans infection (Buruli ulcer disease). http://www.who.int/buruli/Guidance_sampling_techniques_MU_infection.pdf?ua=1. (accessed October 5, 2014)

[25] Williamson H, Phillips R, Sarfo S, Wansbrough-Jones M, Small P. Genetic diversity of PCR-positive, culture-negative and culture-positive mycobacterium ulcerans isolated from buruli ulcer patients in Ghana. PLoS One 2014; 9(2): e88007. http://dx.doi.org/10.1371/journal.pone.0088007

[26] Leigheb G, Zavattaro E, Molicotti P, *et al.* Clinical considerations on Buruli ulcer employing two molecular tests for the detection of Mycobacterium ulcerans in 100 skin biopsies. Int J Dermatol 2014; 53(2): 213-20. http://dx.doi.org/10.1111/ijd.12249

[27] Ruf MT, Schütte D, Chauffour A, Jarlier V, Ji B, Pluschke G. Chemotherapy-associated changes of histopathological features of Mycobacterium ulcerans lesions in a Buruli ulcer mouse model. Antimicrob Agents Chem 2012; 56(2): 687-96. http://dx.doi.org/10.1128/AAC.05543-11

[28] Fraga AG, Martins TG, Torrado E, *et al.* Cellular immunity confers transient protection in experimental Buruli ulcer following BCG or mycolactone-negative Mycobacterium ulcerans vaccination. PLos One 2012; 7(3): 33406. http://dx.doi.org/10.1371/journal.pone.0033406

[29] Agbenorku P, Donwi IK, Kuadzi P, Saunderson P. Buruli ulcer: treatment challenges at three centres in Ghana. J Tropical Med 2012; 2012: 371915. http://dx.doi.org/10.1155/2012/371915

[30] Barimah KB, Mensah J. Ghana's national health insurance scheme: Insights from members, administrators, and health care providers. J Health Care Poor Underserved 2013; 24(3): 1378-90. http://dx.doi.org/10.1353/hpu.2013.0144

[31] World Health Organizaion. Treatment of Mycobacterium ulcerans disease (Buruli ulcer): guidance for health workers. World Health Organization 2012.

[32] Nienhuis WA, Thompson WA, Awuah PC, *et al*. Antimicrobial treatment for early, limited Mycobacterium ulcerans infection: a randomised controlled trial. Lancet 2010; 375(9715): 664-72. http://dx.doi.org/10.1016/S0140-6736(09)61962-0

[33] O'Brien DP, Robson M, Friedman ND, *et al*. Incidence, clinical spectrum, diagnostic features, treatment and predictors of paradoxical reactions during antibiotic treatment of Mycobacterium ulcerans infections. BMC Infect Dis 2013; 13: 416. http://dx.doi.org/10.1186/1471-2334-13-416

[34] Bertolotti A, Izzo A, Grigolato PG, Iabichella ML. The use of ozone therapy in Buruli ulcer had an excellent outcome. BMJ Case Rep 2013.

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