# Buruli ulcer

## Úlcera de Buruli

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**Abstract:** Buruli ulcer, an infectious disease caused by Mycobacterium ulcerans, is the third most prevalent mycobacteriosis, after tuberculosis and leprosy. This atypical mycobacteriosis has been reported in over 30 countries, mainly those with tropical and subtropical climates, but its epidemiology remains unclear. The first autochthonous cases of infection in Brazil have recently been described, making this diagnosis important for Brazilian dermatologists. Clinical manifestations vary from nodules, areas of edema, and plaques, but the most typical presentation is a large ulcer, usually in the limbs. Despite considerable knowledge about its clinical manifestations in some endemic countries, in other areas the diagnosis may be overlooked. Therefore, physicians should be educated about Buruli ulcer, since early diagnosis and treatment, including measures to prevent disability, are essential for a good outcome.
Keywords: Buruli ulcer; *Mycobacterium infections*, atypical; *Mycobacterium ulcerans*; World Health Organization

**Resumo:** A úlcera de Buruli, uma doença infecciosa causada pela *Mycobacterium ulcerans* (*M. ulcerans*), é a terceira micobacteriose em ocorrência, após a hanseníase e a tuberculose. Essa micobacteriose atípica tem sido relatada em mais de 30 países, principalmente, nos que têm climas tropicais e subtropicais, mas a sua epidemiologia permanece obscura. Recentemente, os primeiros casos autóctones do Brasil foram relatados, fazendo com que dermatologistas brasileiros estejam atentos a esse diagnóstico. O quadro clínico varia: nódulos, áreas de edema, placas, mas a manifestação mais típica é uma grande úlcera, que ocorre, em geral, nas pernas ou nos braços. Apesar do amplo conhecimento quanto ao seu quadro clínico em países endêmicos, nas outras áreas, esse diagnóstico pode passar despercebido. Assim, médicos devem ser orientados quanto à úlcera de Buruli, pois o diagnóstico precoce, o tratamento específico e a introdução de cuidados na prevenção de incapacidades são essenciais para uma boa evolução.
Palavras-chave: Infecções atípicas por *mycobacterium*; *Mycobacterium ulcerans*; Organização Mundial de Saúde; Úlcera de Buruli

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## INTRODUCTION

Buruli ulcer (BU), an infectious disease caused by *Mycobacterium ulcerans,* [1] is one of the most neglected tropical diseases. It is the third mycobacteriosis in prevalence, after leprosy and tuberculosis. *M. Ulcerans* is capable of producing mycolactone, an immunomodulatory macrolide toxin that causes tissue necrosis [2,3] and destroys the skin and soft tissues with the formation of large ulcers, often in the arms or legs. In general, the clinical presentation of ulcer is associated with a delay to seek medical treatment [4] and with lack of adequate treatment. Patients not treated early often suffer from functional disabilities in the long run, with joint movement impairment, which restricts their ability to execute and participate in daily activities.[5,6]

Early diagnosis and a specific treatment for BU associated with interventions that prevent disabilities are crucial for satisfactory treatment results. [7,8] When necessary, surgery associated with antimicrobial therapy is the recommended treatment. [7] When extensive lesions and complications exist, the patient may need long periods of hospitalization, with severe socioeconomic and psychosocial implications.[9,10]

Buruli ulcer has been reported in more than 30 countries, especially in those with tropical and subtropical climate, but it can also occur in a few countries where the disease has not yet been recognized. The number of reports of affected patients grew considerably over the last few years. [7] Despite the several cases described, the epidemiology of BU remains unclear, even in endemic countries. [8] Limited knowledge of the disease, its focal distribution, and the fact that it affects mainly poor rural communities contribute to the low notification of cases. [2]

## HISTORY

In 1897, Sir Albert Cook, a British physician who worked at the Mengo Hospital in Kampala, Uganda, described skin ulcers that were clinically consistent with Buruli ulcer (BU), [7] but the first detailed description of the disease caused by *Mycobacterium ulcerans* is attributed to MacCallum et al. in Australia in 1948. [11] In 1950, in the Belgium Congo (current Democratic Republic of Congo), the first African case was reported and, in that same year, Fenner identified the bacillus and named it *Mycobacterium ulcerans*. [1] Since 1959, several authors have described many patients with this disease in tropical and subtropical regions of Central and West Africa. [7,12] In the Americas, it is a rare disease and very few cases have been reported. This mycobacteriosis received several names according to the place where it occurred or was observed. For instance, it was called Bairnsdale ulcer in Australia, Buruli ulcer in Uganda, and Tora and Mexican ulcer in Mexico. [12] The first Brazilian case was reported by do Santos et al. only in 2007. [13]

In 1998, after an international conference organized by the World Health Organization about the control of and research in Buruli Ulcer, held in Yamoussoukro, Côte d'Ivoire (Ivory Coast), the Global Buruli Ulcer Initiative was implemented. The initiative remains active until today, including several research projects in different African countries, where Buruli ulcer is an endemic disease.

## EPIDEMIOLOGY

Buruli ulcer frequently affects individuals who live close to water bodies – slow flowing rivers, ponds, swamps, and lakes; however, cases of the disease have been reported after flooding. Activities that are developed close to the water, such as farming, constitute risk factors. Protective clothing appears to reduce the risk of contracting the disease. [7,8] In Benin, an inverse relationship between the prevalence of the disease and how far the patient lived from a river was found. The prevalence gradually increased from 0.6 to 32.6/1000 when the distance to a river shortened by 10 km. [14]

All ages and genders are affected, but most patients are children younger than 15 years, [14,15,16] with peak age of onset between 10 and 14 years. In adults, it is between 75 and 79 years. [14] The high rate of detection in elderly patients may be associated with the reactivation of a latent disease. [17] A study showed that even though there are no gender differences among children and adults, men older than 59 years had a higher chance of developing BU than women. [17] This difference may be related to professional activities and differences in access to health services. [14]

Buruli ulcer has been reported in 30 countries from Africa, the Americas, Asia and Western Pacific, especially in tropical and subtropical regions (Figure 1). The disease is a public health problem in Uganda, Nigeria, Gabon, Ghana, Cameroon, Liberia, Côte-d'Ivoire, Malasia, Papua New Guinea, Togo, French Guiana, and Republic of Benin. [7, 12] In Côte-d'Ivoire, approximately 15,000 cases have been registered since 1978. In Benin, nearly 15% of the population is affected. In 1999, 6,000 new cases were reported in Ghana. [18] A few cases reported in North America and Europe were associated with international travelers. [19] Some cases have been reported in China, but the extension of the disease is unknown. In the Americas, the disease appears to be more common in the French Guiana (although fewer than 200 cases have been reported in 35 years) than in Suriname, Mexico or

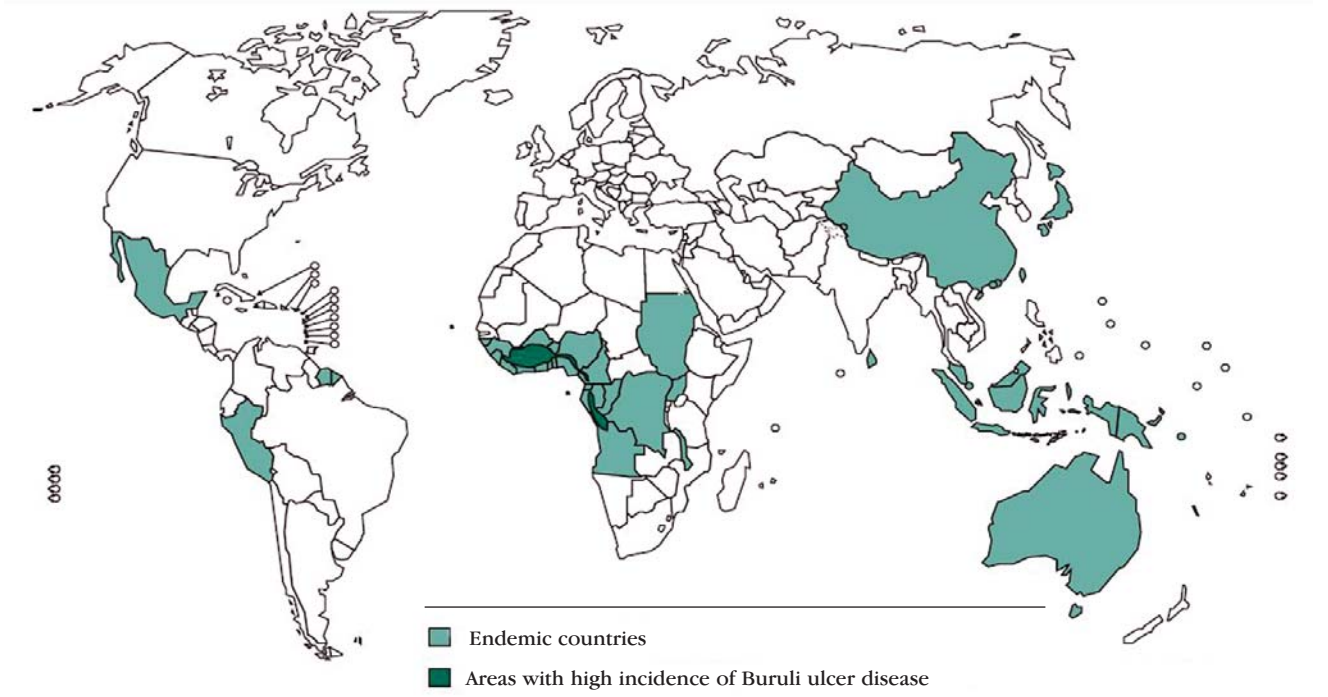


**Figure 1**: Geographical distribution of Buruli Ulcer
Modified Source: van der Werf et al.[8]

Peru, where very few cases have been confirmed – fewer than 10 cases per country over the last 50 years. These numbers may be only an indication of the presence of the disease, but do not reveal the extent of the problem. [8]

The first Brazilian case was reported in 2007. A 65-year-old patient was seen at a health center in Brasilia showing two years of clinical evolution of BU associated with osteomyelitis. The patient lived in a rural area close to a water body, where the climate is hot and humid. Initially, leishmaniasis, a common disease where the patient lives, was wrongly diagnosed. After a positive culture for *M. ulcerans* from skin and bone tissue samples, the diagnosis of BU was confirmed. [13] Despite being the first Brazilian case of BU, similarities between the climate, vegetation, and habits in Brazil and endemic countries suggest that Brazil may be a focus of the disease. Health professionals' lack of knowledge about the disease makes the identification and epidemiological monitoring of BU difficult in Brazil.

The second case, probably originary from the Brazilian territory, was published by McGann et al. in November of 2009. It refers to an English tourist who contracted the disease after a visit to the Brazilian Pantanal region, [20] which shows that the disease may be more of a reality than imagined. Cases from non-endemic countries are very rare. In the literature, only 21 such cases have been reported so far, [8,21,22] including the last Brazilian case. One explanation for them could be immigration from an endemic country for BU to non-endemic areas. The disease could be contracted in the country of origin or by a traveler from a non-endemic country. Infection by *Mycobacterium ulcerans* is among the main ulcers diagnosed in travelers to West Africa, Central America and other Western countries, together with leishmaniasis, diphtheria, and profound mycoses. [19,21] Despite advanced knowledge about the clinical symptoms of BU in endemic countries, in other areas this diagnosis may be overlooked. Therefore, physicians should be aware of its symptoms, since early diagnosis and proper treatment help prevent functional disabilities resulting from the infection. BU is probably endemic in a broader area than often considered.

The exact prevalence of the disease is unclear due to lack of knowledge about the disease among health professionals and the population in general. This leads to significant undernotification. People who are mostly affected by the disease live in remote rural areas, with little access to the health system. There is great variation in the clinical presentation of the disease, which makes confusion of BU with other diseases and tropical ulcers common. In addition, there is no compulsory notification in many countries.

Based on various studies, it is now clear that there is a relationship between BU and water, but the precise form of transmission of *M. ulcerans* has not been established. An environmental factor not yet identified associated with slow flowing water, to

which populations that live close to a water body are exposed, is thought to exist. There are reports of a likely transmission by mosquito or insect bites. [22-26] A research study suggests that in Africa some water insects of the order Hemiptera (Naucoridae and Belostomatidae) may harbor *M. Ulcerans* in their salivary glands and transmist the disease to animals (Figure 2). A study showed that infected Naucoris mosquitoes transmit the pathogen to rats through biting, in addition to being naturally colonized by *M. Ulcerans* in endemic areas. [23,24,25] These insects can fly many kilometers from their starting point and this may explain why patients who do not live close to a water body can get infected, but not as often as those who live closer to ponds and swamps.

More recent data from Australia suggest that salt marsh mosquitoes test positive *for M. Ulcerans* DNA, although transmission by this type of insect has not been recognized. Further research is in progress to establish the exact role of insects and other factors in the transmission of the disease to humans. If this is confirmed, BU will be the only disease caused by mycobateria that is transmitted by insects. [7,24,25,26]

Inoculation also appears to occur through trauma. [27] In this case, skin contamination would occur as a result of direct exposure to still water, gases from lagoons and surface of ponds or contaminated objects. *M. Ulcerans* can enter the human body through several types of trauma, from mild ones such as a hypodermal injection, to severe, like landmine injuries, snake or even human bite. Two cases of human bite have been reported and this exemplifies patient-to-patient transmission.[28,29]

A change in the epidemiology of BU has been attributed to flooding, population growth, mining, extraction of wood from tropical forests, and river damming, but there is no evidence of this causal association. Hypotheses consider that *M. ulcerans* is introduced into new regions by insects, human beings or other animals. Alternatively, the organism can be widely distributed in the environment, in low numbers, but they significantly increase after certain events, such as deforestation and flooding. [16] Recent research studies suggest that the prevalence of BU in Uganda appears to have reduced significantly. In this country, the damming of Lake Vitoria dried the swamps at the margin of the Nile, and this may have contributed to the dramatic reduction of the incidence of the disease. [14]

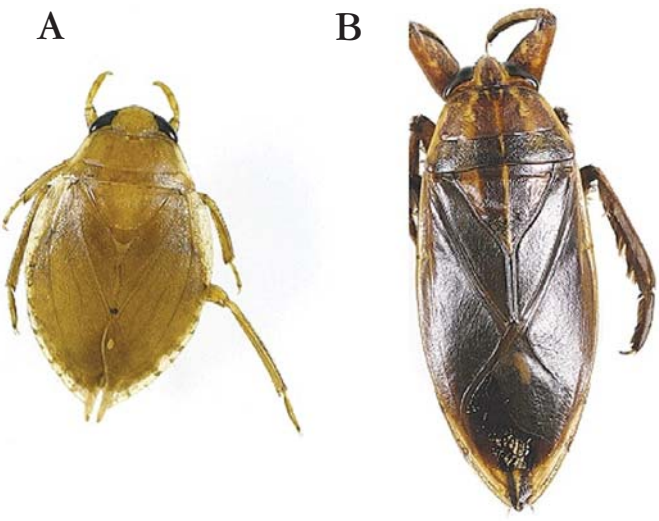


**Figure 2**: Acquatic insects naturally infected with Mycobacterium ulcerans; A. Naucoris fl avicollis (size 1·5 cm); B. Belostoma cordofna (size 10 cm)
*Source: Wansbrough-Jones M et al.*[2]

## IMMUNOLOGY AND ETIOPATHOGENY

*M. ulcerans* is an acid-alcohol resistant bacillus (BARR) predominantly extracellular. Current data suggest that this mycobacterium does not exist freely in the environment, as previously thought. It probably occupies a specific niche within acquatic environments (for instance, small acquatic animals), from where it is transmitted to humans by an unknown mechanism.[7] Although slow growing, *M. ulcerans* can be cultured on media used for mycobacteria (such as Lowenstein-Jensen medium) provided the incubation temperature is kept between 29-33 ° C (lower than that for *M. Tuberculosis*), microaerophilic environment, and pH between 5.4 and 7.4. There is a wide variability of data about the success of isolation from clinical samples. Reference laboratories have reported high rates of success in clinically confirmed cases using improved transportation and decontamination methods. [8]

There is some variation among strains of *M. ulcerans* from different geographical areas of Africa, the Americas, Asia, and Australia (Figure 3), but the relationship between these various strains and virulence in humans has not been established. [8] The development of polymerase chain reaction (PCR) to promptly identify *M. ulcerans* in clinical and environmental samples has improved the diagnosis and comprehension about the epidemiology of BU. The PCR technique identified repeated DNA sequences in the genome of *M. ulcerans*, IS2404. [8,30] A direct relationship between the virulence of *M. ulcerans* and the number of repeated IS2404 sequences appears to exist. [31]

Mycolactone is a heat-stable exotoxin, lypophilic, that belongs to the group of macrolides and causes extensive, chronic, and necrotizing damage to the papillary dermis, subcutaneous fat, and muscles (fasciae and bones are sometimes affected), resulting in derformity and disability. The molecule is

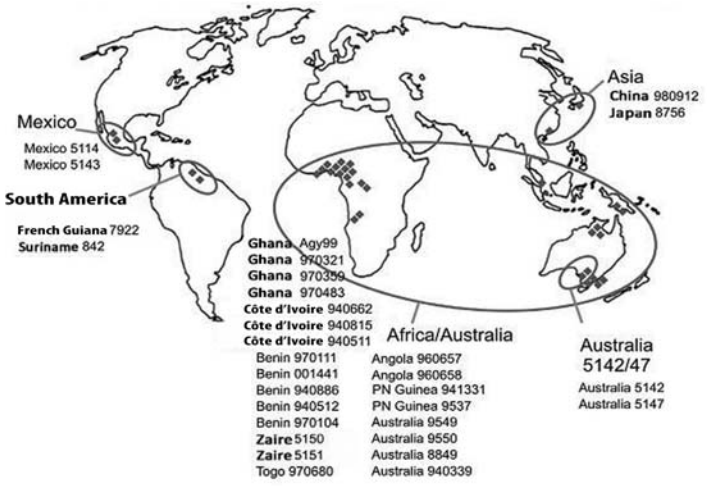


**Figure 3**: Geographical distribution of the different strains of Mycobacterium ulcerans
*Modified source: van der Werf et al.*[8]

active in extremely low concentrations and is not present in laboratory cultures. [16]

Mycolactone molecules, when injected in laboratory animals, are capable of producing massive necrosis similar to what is observed when these animals are inoculated with *M. ulcerans*. With the full genome sequencing of *M. ulcerans*, it became evident that the genetic information for mycolactone synthesis is synthesized from a 174 kb plasmid known as pMUM001. This makes *M. ulcerans* the only mycobacterium whose virulence is mediated by a plasmid. *Mycobacterium marinum* and *M. ulcerans* are phylogenetically close, sharing from 98 to 99.8% of their genetic material. [32] The molecular finding that most clearly separates these two species is the production of mycolactone by *M. ulcerans*· [8,16,31,33] Another mycobacterium, *M. Lifandii*, isolated from frogs in West Africa, is a pathogen associated with *M. ulcerans* and *M. Marinuns*. *M. Liflandii* also presents the IS2404 sequence and all the genes that codify mycolactone, except the one that codifies monooxygenase p450. [2] *M. Liflandii* can produce ulcers similar to BU in frogs. [8]

*M. ulcerans* strains isolated in particular regions show remarkable similarity, but important differences in the production of mycolactone according to geographical areas have been identified. This may reflect regional differences in the clinical presentation and virulence of *M. ulcerans*. This macrolide has been shown to be the main virulence factor and presents cytotoxic, analgesic, and immunosupressive activity in the infection caused by *M. ulcerans*. [33]

The various strains of *M. ulcerans* produce a macrolide family with identical biological activity, but with different potency. [34] The first type of macrolide identified was mycolactone A/B, followed by a family of variants of this toxin (mycolactones C, D, and E). Studies compared strains of *M. ulcerans* from different continents and verified that mycolactone A/B was the most powerful macrolide. [35] African strains and one from Malasia produced more mycolactone variants (at least four variants in addition to types A/B). This could contribute to greater cytopathogenicity of this particular strain. [36] Therefore, a region with more extensive and disseminated BU cases is found with higher frequency in Africa, where mycolactones A/B are more abundant. This is different from Australia, where mycolactone C appears more often than mycolactones A and B.

*In vitro* studies have shown that the immunosupressive activity of mycolactone occurs through the inhibition of the production of Th1-interleukin (IL) 12 cytokines, interferon (IFN)-alpha, and suppression of the production of tumoral necrosis factor (TNF) by monocytes. These cytokines are important in the control of mycobateria infection. [37] Studies on tuberculosis and leprosy have shown that Th2 cytokines (IL-4, IL-13) and antiinflammatory cytokines, such as IL-10 and tumoral growth factor (TGF), have a negative effect on the control of mycobacteria proliferation. [38,39] Kiszewski et al. showed that in ulcers with granulomas there is a significantly higher expression of IFNγ and lower bacillary load. However, the cytokine profile found in BU without granulomas was similar to the one found in active progressive tuberculosis, in which there is reduction of Th1 cell funtion and increase of Th2 activity, associated with a high production of IL 10 and TGFβ. [34,38] This could indicate that the presence of granuloma signals better immunological protection. [34] Hence, it appears that there is a mixed model of pro- and antiinflammatory cytokines in areas of ulcerative lesions of BU, which vary based on the evolution of the disease. Recent ulcerated lesions have a predominant immunosuppressive cytokine profile, with high bacillary load, whereas old ulcers have a combination of cytokines with predominance of IFNγ and low bacillary load and typical granuloma. In addition, disseminated disease and osteomyelitis have been associated with defects in the formation of granulomas. [40,41]

Many healthy individuals in endemic areas for Buruli ulcer show a specific humoral immune response to *M. ulcerans*, suggesting that the disease thrives only in a limited group of individuals who have been infected by *M. ulcerans*. The immune response mediated by Th1 lymphocytes is protective against BU; however, Th2, which is targeted to the production of antibodies, does not control the disease. Humoral immune response with production of IgM antibodies appears to be more frequent in patients with active ulcers than in their relatives. Co-infection by HIV has

not been reported as a risk factor, but more severe clinical forms of BU have been described in seropositive patients. [42] A few individuals could escape the disease due to an individual protection centered in a cellular immune response.

**HISTOPATHOLOGY**

It is possible to divide BU into four histopathological stages of evolution: [43]

• Non-ulcerated necrotic stage: the epidermis is intact, but it is often hyperplastic. The upper dermis is usually preserved, but it can show various degrees of collagen degeneration, edema, and discreet infiltration of inflammatory cells. Vasculitis, with occlusion of vessels by thrombus, can also be observed. In this stage and in the initial ulcerative phases, coagulation necrosis in the lower portion of the dermis, subcutaneous tissue, and fascia is easily identified. In these areas, predominantly extracellular BAAR form clusters that are easily detected in the center of the lesion in the deep layers of the dermis and in the adipose paniculus. Calcification can also be detected. Another finding is the presence of denucleated adipose cells, called "ghost cells".

• Ulcerated necrotic stage: there is loss of epidermis and reepitalization attempt in the borders of the ulcer. The adjacent epidermis is often hyperplastic (pseudoepitheliomatous hyperplasia). The base of the ulcer exposes the dermis with necrotic material and fibrosis. Necrosis of the subcutaneous tissue and dermal collagen, with formation of "ghost" cells, is accompanied by edema, minimal inflammation, and presence of numerous, predominantly extracellular BAAR in clusters. Necrosis by coagulation affects the subcutaneous cellular tissue and fascia similarly to the non-ulcerative phase. Vasculitis and extensive areas of calcification in the lower portion of the dermis are frequently observed (Figures 4 A, B, C, and D).

• Initial healing stage (of organization and granulomatous): it is characterized by the start of a granulomatous response in the dermis and subcutaneous cellular tissue. Epithelioid cells, Langhans giant cells and lymphocytes are present in this phase. Eventually, these cells rearrange to form tuberculoid granulomas.

Foamy macrophages, lymphocytes, and plasmatic cells are sometimes seen in the margins of necrotic tissue. The appearance of granulous tissue indicates the beginning of the ulcer healing process. In this stage, BAAR are rarely found in the histopathological examination. [39]

• Late healing stage - fibrosis and atrophic epidermis.

Histopathological findings are considered unspecific; however, considering the different findings altogether, it is estimated that the sensitivity of the anatomopathological exam is around 90%. The sensitivity of the Ziehl-Neelsen staining procedure is between 40 to 80%. [42] In conclusion, the most characteristic findings of a histopathological study of BU lesions are necrosis of the subcutaneous tissue and of the dermal collagen accompanied by minimal inflammation (despite the extensive necrosis observed), and predominantly extracellular acid-alcohol resistant bacilli (BAAR). Neutrophils can be found in the necrotic material. This mild inflammation could be explained by the action of exotoxin, which causes necrosis of adipose cells and of the inflammatory infiltrate. [44]

**CLINICAL PROFILE**

Non-tuberculoid mycobacteria are present in the environment and are in permanent contact with humans and animals. Therefore, the colonization of clinically healthy individuals by these bacteria is very common. [14] Contact with *M. ulcerans* may result in the colonization of healthy tissue without infection. Evolution to the clinical manifestation of the disease depends primarily upon the host's defenses. Another hypothesis is the self-resolution of the primary infection, being thus never noticed. The disease may cause subclinical symptoms or even develop an asymptomatic profile, remaining latent, as shown in figure 5. Later, the patient may have the latent focus activated and manifest the clinical symptoms and signs of the disease. Sometimes, only a superficial trauma is necessary for the reactivation of the infection focus, with a shorter incubation period (about 15 days) than the typical one, between 2 and 3 months. [14]Australian authors have reported the case of a patient, most likely carrying the latent disease, who after immunosuppressive therapy with corticosteroids developed disseminated BU. [45]

The disease caused by *M. ulcerans* has a spectrum of clinical forms mostly associated with the time between the onset of the disease and when the patient seeks a health center for diagnosis. The average incubation period is from two to three months. [14] After primary infection, the disease can remain localized or disseminate. Many factors contribute to the evolution of the disease, such as the immunological status of the host, the size and depth of the inoculation site, and the virulence of the strain. [14,22] The pre-ulcerative form of the disease often does not make the patient seek medical assistance and this period may vary from a few weeks to months.

Clinically, BU can be divided into pre-ulcerative stage (papule, nodule, plaque, and diffuse edema) and ulcerative stage, which may be represented by small ulcers (smaller than 5 cm) and large ulcers (lar-

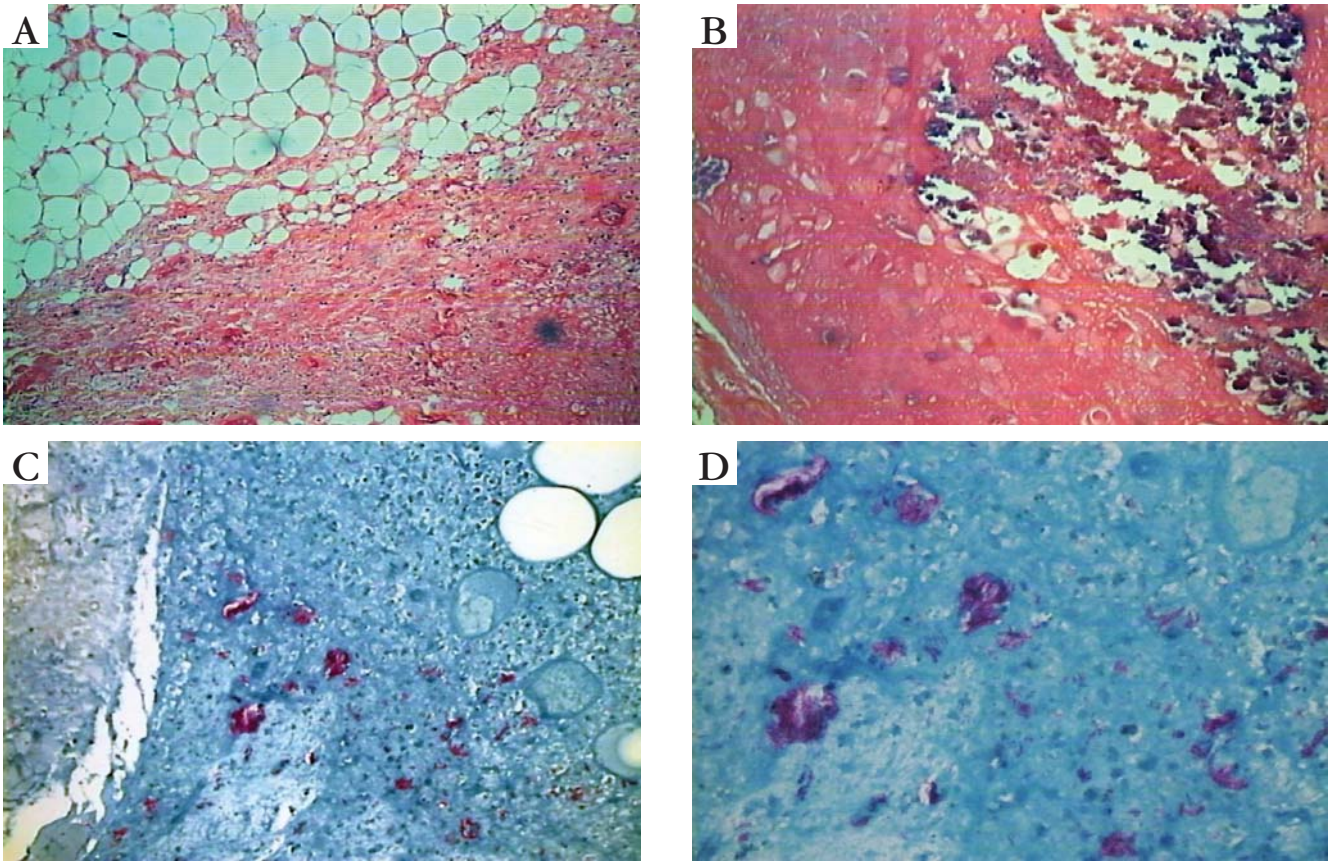


**Figure 4: A.** HE 100X. Denucleated adipose cells - "ghost cells' - accompanied by discreet mononucleate inflammatory infiltrate; **B.** HE 100X. Presence of coagulation necrosis with calcification areas; **C.** ZN 200X. BAAR grouped in extracellular environment forming clusters; **D.** ZN 400X. Grouped BAAR

ger than 5 cm). It can also be classified into localized disease (papule, nodule, and ulcer) and disseminated disease (plaques, diffuse edema, and metastasis). [14,43]

The initial stage of Buruli ulcer often starts with a painless and mobile skin nodule, with less than 5 cm of diameter, that ulcerates usually after a few weeks. It forms an ulcer with poorly demarcated borders, making it appear smaller than it really is (Figure 6A). In the Australian population, [16] it is more common for patients to notice a small pustule or papule, often attributed to insect bites, without the nodular phase. This papule often has less than 1 cm of diameter and is accompanied by erythema in the adjacent skin. This form of the disease has not been reported in Africa. [14] The ulcer develops due to perforation of the necrosis above the epidermis There is no pain or, if present, it is reported as very mild. Borders are characteristically poorly demarcated, undermined, and there is edema in the adjacent skin. The ulcer may remain small, with 1 to 2 cm of diameter, and it is more susceptible to self-resolution (Figure 6B). However, ulcers frequently become larger and destroy the skin around them. They may affect bone tissue and develop into disseminated disease (Figure 6C). Their borders are hyperpigmented and their background, necrotic. The disease can also present with a large area of marked induration, diffuse edema in the legs and arms or a well-demarcated plaque (Figure 7A). [7] Plaques are raised, hard, painless and with a certain degree of depigmentation or spotted erythema. They can have more than 2 cm of diameter, possibly reaching 15 cm. They can develop into large ulcers with irregular borders. When there is only one edema, the profile is more diffuse, without a Godet sign, and with poorly demarcated borders. Edematous lesions can affect an entire limb and a large portion of the trunk. After a few days or weeks exulcerations are formed in these areas. [7]

Lesions can develop in new areas, distant from the original lesion, which characterizes a metastatic form of the disease. This evolution of the clinical condition can be explained by the lymphatic or blood system, especially in relation to the disseminated form of the disease. [14]

Strains of *M. ulcerans* isolated from different clinical forms of the disease in a particular geographi-

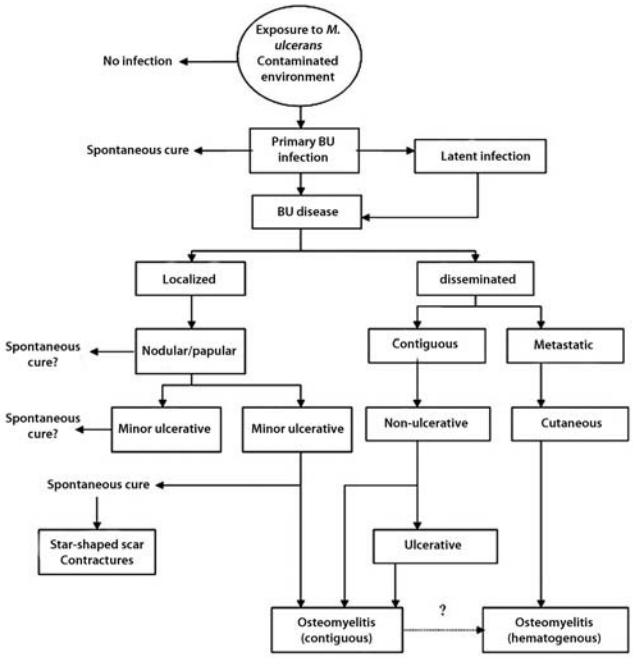


**Figure 5**: Buruli ulcer. Clinical Profile. Flowcharts of the clinical presentations of the disease
*Modified source: Portaels F et al.*[14]

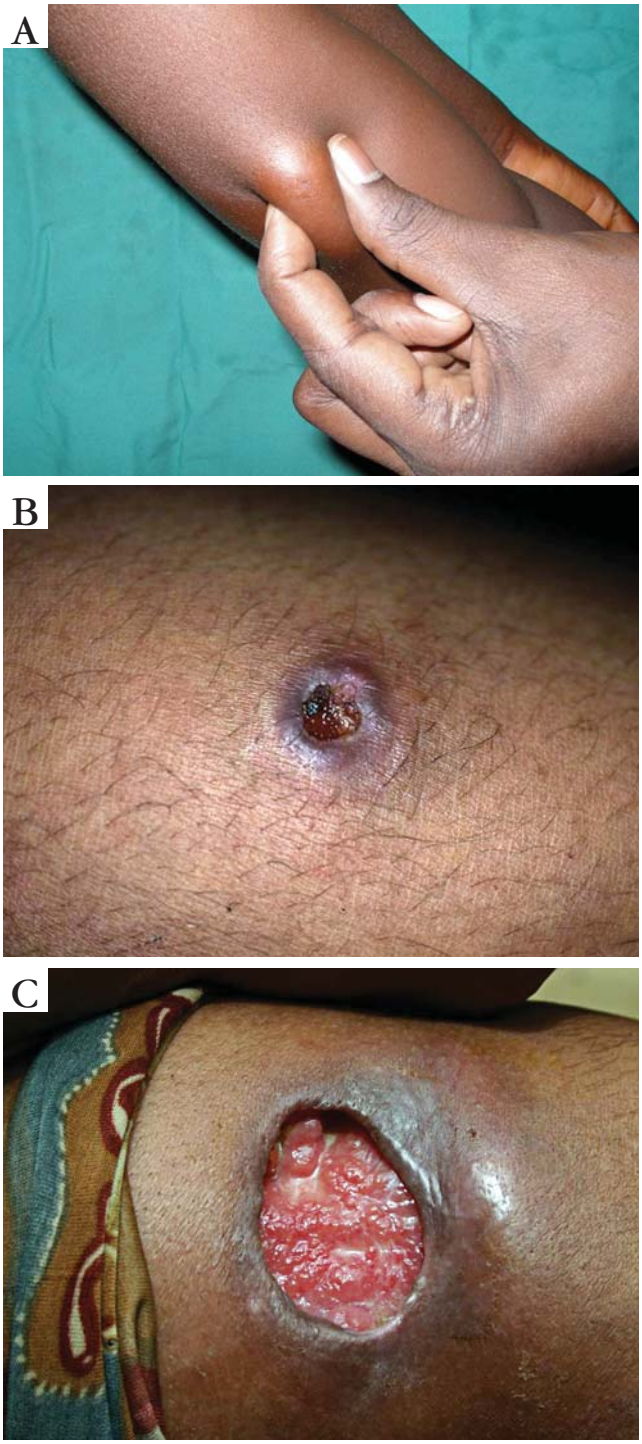


*Photo courtesy of: P. Couppié/French Guiana, H. Guerra/Peru*

**Figure 6: A**. Initial nodular lesion of Buruli Ulcer; **B.** Small ulcerated lesions; **C.** (old10) Large ulcerated lesion with undermined borders

cal region appear identical, suggesting that host factors may play an important role in the establishment of the various clinical presentations. [7, 16] Due to the local immunossupressive properties of mycolactone or, perhaps as a consequence of other unknown mechanisms, the disease evolves without pain, fever or systemic inflammatory response. [15] This may partly explain why affected individuals do not seek immediate treatment. Nevertheless, without treatment, large ulcers develop. The base of the initial ulcer often contains a whitish, frothy-like secretion; sometimes it can form crusts. The skin surrounding the lesion becomes increasingly pigmented. [7] When ulcers are large, they may affect an entire extremety or a large portion of the trunk (Figure 7B).

One of the consequences of an extensive ulcer is the fact that it may affect bone tissue, increasing the risk of osteomyelitis, [16, 46] and later lead to deformities and even amputations. Involvement of other organs is extremely rare. [22] Metastatic osteomyelitis has also been reported and it can affect approximately 10% of the patients with BU. It is directly proportional to the number of skin lesions. [16]

Involution of lesions may result in atrophic sequelae or symmetrical scars, sometimes hypotrophic or keloid-like, with contractures and impairment of the function of limbs when localized near or over joints. Scars may restrict limb movement, limiting the patient's ability to perform daily activities and making participation in work activities difficult (Figure 8). [47,48] The cosmetic aspect of a scar may also cause social problems, withdrawing the patient from routine activities. Permanent disabilities affect around one fourth of the patients. [7] The disease may affect any part of the

*Photo courtesy: H. Assé/ Côte d'Ivoire*

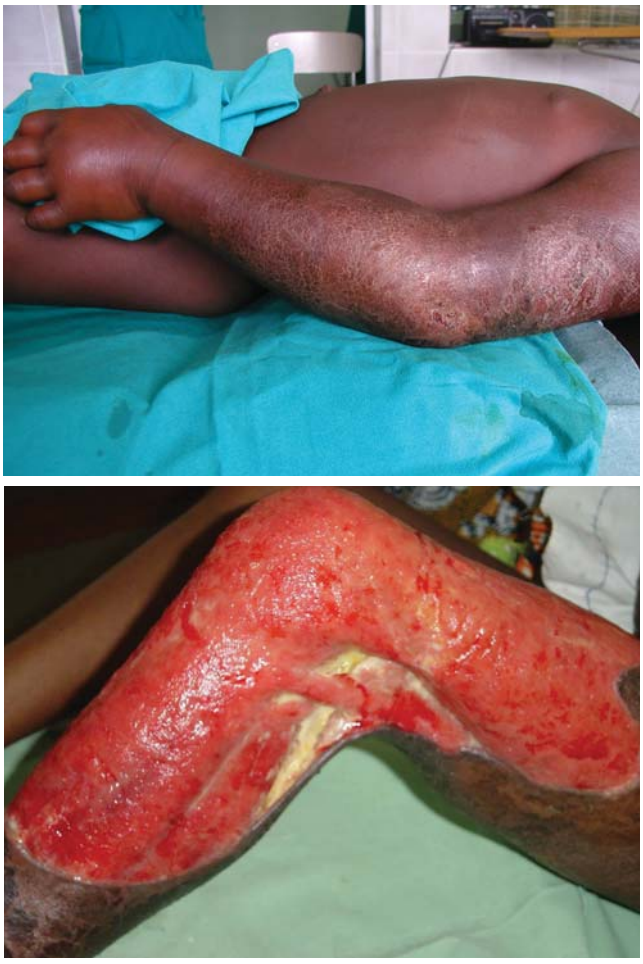

**Figure 7: A**. Edematous area affecting the entire upper limb; **B.** Large ulcerated lesion affecting the entire lower limb

body, but in about 90% of the cases lesions develop in the limbs, with nearly 60% of all lesions occuring in lower limbs. [7,8]

Differently from tuberculosis (TB), there is no evidence that HIV infection predisposes individuals to Buruli ulcer. [12] But the disseminated form of the disease associated with HIV has been previously reported. [49, 50] There is little seasonal variation in the incidence of the disease, [8] but studies have shown that in Australia, Papua New Guinea, Cameroon, Uganda, Ghana, and Côte D'Ivoire there is a slight higher incidence of BU in relatively dry periods. [51]

Factors associated with poor nutrition, such as hypoproteinemia or anemia, have been associated with the development of symptoms and the susceptibility of the host to BU. [16,51] This could be explained by a defficiency in micronutrients such as zinc, which lowers the defenses against bacterial toxins and reduces the function of T cells and immunity mediated by cells. In addition, lack of knowledge about the disease, poor hygiene, infrastructure problems such as poor living conditions or lack of sewage and medical resources, little access to health services, risky cultural practices, poor nutrition, and general poverty increase the risk of contact with and infection by aggressive pathogens. [51]

## DIFFERENTIAL DIAGNOSES

Differential diagnosis depends upon the stage of presentation of the disease and relevant conditions in the area where the patient lives. [8] In a few endemic countries, particularly in West Africa, Buruli ulcer may be confused with onchocercoma (onchocercosis nodules), noma (*cancro oris*), bouba, nodular form of leishmaniasis, spinocellular carcinoma, Kaposi sarcoma, cutaneous tuberculosis, pyoderma gangrenosum, leprosy, and syphilis. [8,14]

Plaque and edematous BU can simulate profound mycosis, erysipelas, bruise, lupus vulgaris, eczema, disseminated sarcoidosis with plaques, necrobiosis, lipoma, epidermal cyst, lymphadenitis or lymphadenopathy, and hydradenitis. The differential diagnosis of the edematous form of the disease is osteomyelitis due to other causes, pyomyositis, elephantiasis, renal or cardiac insufficiency edema, anemia, malnutrition and tumor lymphatic compression. [14]

Ulcerative lesions may be confused with tropical ulcer (lower limbs). [8] However, tropical ulcers are often painful and found only in the lower part of the legs. Leishmaniasis is an important differential diagnosis in South America, and spinocellular carcinoma can also present as ulcers. The undermined border of the ulcer orients to the diagnosis of pyoderma gangrenosum. There is still the possibility of diagnostic confusion with necrotizing fasciitis, sporotricosis, anthrax, and other tropical phagedenic and stasis ulcers. [14]

In the healing form, differential diagnosis should be made with scars resulting from third degree burns and healing lesions of chronic osteomyelitis. [14]

## DIAGNOSIS

Buruli ulcer is frequently diagnosed and treated, based on clinical information, by experienced health professionals in endemic areas. The clinical criteria for higher suspicion of BU are [14,16,52] 1) presence of chronic lesion – weeks or months; 2) absence of fever or regional lymphoadenopathy; 3) nodular lesion, hard plaque or edema; 4) one or more chronic ulcers with poorly demarcated, undermined borders or depressed scars; 5) edema over a painful joint, suggesting bone involvement; 6) patient younger than 15 years; 7) patient who lives at or visited an endemic area.

Laboratory diagnoses are not commonly used to make decisions about treatment due to logistic and operational difficulties. [16] Even though most health professionals in endemic regions are able to arrive at

Photo courtesy of: WHO and S. Etuaful/Ghana

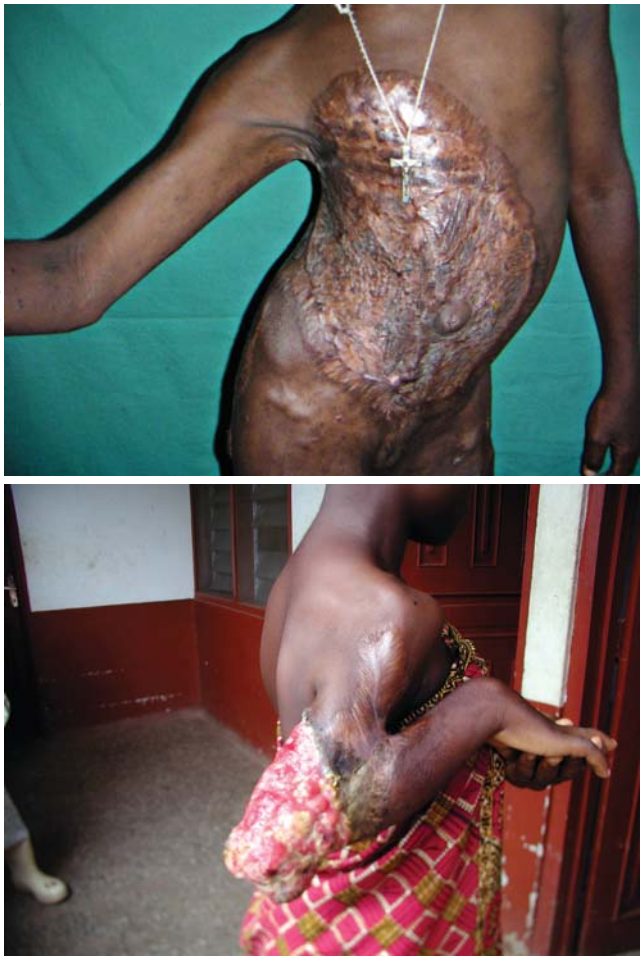

**Figura 8: A**. Extensive area of scar secondary to Buruli ulcer; **B.** Extensive scar in the upper limb degenerating to epidermoid carcinoma

a precise clinical diagnosis, microbiological confirmation is important, whenever possible.[14] This measure helps to elucidate the real prevalence and incidence of BU, confirm new foci of the disease, and verify relapse or reinfection after treatment. Laboratory diagnosis can also be used to confirm the clinical diagnosis retrospectively on swabs and tissues removed during treatment, but this is rarely done. Sample collection depends upon the clinical form of the disease and the objective of the test being performed. [8,14] Two samples are often collected per lesion. [43,53] Multiple swabs are taken from the border of lesions, since the center is often negative for *M. ulcerans*. In patients undergoing surgery, samples should be collected from the tissue removed for bacteriological and histopathological analysis. [14,43] To confirm osteomyelitis, curettage of the bone tissue should be performed. Despite improvements in material collection techniques, access to competent laboratories to read these exams is complicated.

Five laboratory methods to confirm the disease are often used:

• **Direct Smear Examination -** An examination done on swabs from ulcers or smears from tissue biopsies that can be promptly conducted at local health facilities where TB microscopy is also done. [7] The exam is conducted through the acid-fast (Ziehl Neelsen) stain procedure. Nevertheless, the sensitivity of this method is low (about 40%) [18] because *M. ulcerans* numbers tend to decrease over time. It is important to emphasize that the positivity of the test varies with the clinical form of the disease. It is more useful in the ulcerative stage, [8] but if the lesion is not ulcerated, a skin biopsy is sufficient for the exam. In the nodular form, positivity may reach 60% and in the edematous form, it may reach up to 80%, both in the direct examination and culture.[16] It is considered by many the easiest and most accessible method to arrive at the diagnosis.

• **Culture of *M. ulcerans* -** A procedure done on swabs from ulcers or tissue biopsies through the Lowenstein-Jensen medium that takes 6–8 weeks or more; sensitivity is approximately 20–60%. [7] It is especially difficult to culture *M. ulcerans* when the sample is taken from bone tissue. [16]

• **Polymerase chain reaction (PCR) -** A test whose results can be obtained within two days on swabs of ulcers or tissue biopsies; sensitivity is around 98%. [2,7] Most studies use the IS2404 sequence. [2,8] The PCR technique has been refined with the addition of uracil-DNA glycosylase and deoxyuridine triphosphate to the mixture instead of deoxythymidine triphosphate, which reduces the risk of false positive results due to contamination. [54] PCR with lyophilized reagents and transportation buffers has also been developed to overcome technical difficulties in the tropics. [54] The positivity of PCR and histopathological tests does not vary with the clinical form of the disease.

• **Histopathology.** A method that requires tissue biopsies; sensitivity is about 90% and is useful for differential diagnoses when the results of other methods are negative. It should be done with a scalpel, thus avoiding the use of *punches.* The incision should cover the border of the lesion and extend into the subcutaneous tissue. It may be up to 90% sensitive; [7] in a study conducted in Ghana, despite having found a sensitivity of about 82%, the authors considered this to be the most sensitive method.[53]

• **Fine needle aspiration:** This technique is used in cases of nodular lesions and allows the collection of material for later research, such as direct examination and culture. [14]

Frequently, some laboratory methods, such as PCR, are limited to reference and research laboratories, often remote from endemic areas. Usually, in routine clinical practice cases are managed without

microbiological confirmation. Four laboratory tests are currently available to confirm the diagnosis of BU: 1 – direct examination of secretion with BAAR investigation and Ziehl Neelsen stain or auramine O; 2 – culture; 3- PCR for the IS2404 sequence, and 4- histopathology.[14] The WHO recommends that at least two laboratory tests be carried out for diagnostic confirmation, making the occurrence of false-positive and false-negative results more difficult. A simple and fast diagnostic test for BU is needed because the initial disease (nodule) can be treated locally and inexpensively. The WHO is reconsidering its early recommendation that two confirming tests were needed to arrive at a conclusive dignosis. [8]

**TREATMENT**

Sometimes localized lesions may spontaneously recede; however, without treatment, most cases of BU result in physical deformities that lead to disability, psychological problems, and stigmas. Early detection of active cases, proper treatment, and complete joint movement of the affected area are essential for the prevention of disabilities.

According to the WHO, the following are the current recommendations for treatment: [7,8]

• A combination of rifampicin and an aminoglycoside (streptomycin/amikacin) for eight weeks as a first-line treatment for all forms of the active disease. Nodules or uncomplicated cases can be treated without hospitalization. Side effects have been reported, but are considered rare and are listed in Chart 1. The recommended doses of rifampicin and streptomycin are: rifampicin, 10 mg/kg of body weight, taken orally and daily for eight weeks; streptomycin, 15 mg/kg of body weight, taken intramuscularly and daily for eight weeks (Chart 2). This drug is contraindicated for pregnant women. Amikacin is a viable alternative if streptomycin cannot be used and it is administered in the dose of 15mg/kg of body weight, instramuscularly, daily, for eight weeks. [52]

• Surgery to remove necrotic tissue and skin grafts to aid in healing make recovery faster and more efficient. Surgery is performed to correct skin defects, contractures and the function of affected joints, and for cosmetic reasons.

• Interventions to minimize or prevent physical, emotional and social disabilities. [47,48] The best prevention is to teach the population and health professionals how to diagnose and later treat the disease. Other actions, associated with antibiotic therapy, are needed to avoid complications and disabilities. Education is the best guarantee that patients and their families will learn about and participate in their self-care from the moment of the diagnosis. The most important measures are:

Dressings that aid in healing, preventing the contracture of soft tissues and joints;

Exercises and positioning of the affected part to avoid contractures;

Control of the edema;

Care with the skin and scar, keeping them hydrated, lubricated, mobile (without adhesions), and protected;

Participation in daily activities;

Good nutrition and diet that aid in healing;

Good hygiene, which helps to avoid infections;

Awareness that when needed, help should be asked of others.

For a long time, the treatment of BU was exclusively surgical and often mutilating. Treatment with drugs was only implemented in 2005 with the associ-

**CHART 1:** Main side effects associated with the drugs recommended to treat Buruli ulcer

| Common side effects | Probable causative drug | Conduct |
|---|---|---|
| Anorexia, nausea, abdominal pain | Rifampicin | Continue treatment with administration of the drug with small meals or at bedtime |
| Jaundice and hepatitis (excluding other causes) | Rifampicin | Suspend treatment |
| Shock, purpura, acute renal failure | Rifampicin | Suspend treatment |
| Hypoacusis (in the absence of ear wax confirmed by otoscopy) | Streptomycin | Suspend treatment |
| Dizziness with vertigo and nystagmus | Streptomycin | Suspend treatment |

*Adapted source: WHO/2004*[48]

**CHART 2:** Dosages of drugs recommended to treat Buruli ulcer

| Patient's body weight (kg) | Rifampicin (300mg/tablet) | | Streptomycin (1g/ 2ml) | |
|---|---|---|---|---|
| | Dose (mg) | No. of tablets | Dose (g) | Volume (ml) |
| 5-10 | 75 | 0,25 | 0,25 | 0,50 |
| 11-20 | 150 | 0,50 | 0,33 | 0,70 |
| 21-30 | 300 | 1,00 | 0,50 | 1,00 |
| 31-39 | 300 | 1,00 | 0,50 | 1,00 |
| 40-54 | 450 | 1,50 | 0,75 | 1,50 |
| >54 | 600 | 2,00 | 1,00 | 2,00 |

*Adapted source: WHO/2004* [48]

ation of rifampicin and streptomycin so that the lesions and edema could be reduced, helping surgical excision. [55] The surgical treatment of BU initially consisted in the radical excision of all necrotic tissue and a portion of normal tissue, followed by skin graft. Studies have shown that antimicrobial drugs (rifampicin, aminoglycosides, macrolides, and quinolones) inhibit the growth of *M. ulcerans in vitro* and *in vivo* and that treatment combinations containing aminoglycosides were more efficient than those without this drug. [8] A previous study conducted by the British Research Council in Uganda showed the beneficial effect of clofazimine. [8] In another study in Ghana, patients with a clinical diagnosis of the nodular form of the disease were randomized to receive rifampicin, 10 mg/kg, and streptomycin, 15 mg/kg for two, four, eight or twelve weeks. After these weeks, all patients underwent surgery and skin biopsies were analysed by PCR, culture, and histopathology. In patients treated for two weeks, viable *M. ulcerans* could still be cultured, whereas in all the patients who were treated for at least four weeks, living bacilli could not be isolated. Clinically, however, most patients responded well to streptomycin and rifampicin; in some cases, lesions receded completely. [55]

Nienhuis et al. conducted a study comparing the use of streptomycin and rifampicin for eight weeks with the regimen of rifampicin for eight weeks and streptomycin for four weeks, associated with the use of oral clarithromycin for four more weeks. The authors showed that 96% of the patients who used streptomycin for eight weeks and 91% of those who used streptomycin for four weeks followed by oral clarithromycin for four weeks had their lesions healed and were cured after a year without relapse after antibiotic treatment. The difference between the two therapeutic regimens was very small, suggesting that they are both efficient. This study also demonstrated that initial and limited lesions of BU can be effectively treated without surgical procedures. [56,57] Previously, studies showed that initial lesions removed with a simple excision had a relapse rate of 16% after a year. Even after evidence about the therapeutic effect of antibiotics in the treatment of BU, [56,57] surgery is still necessary in some cases. The type of surgical treatment will depend on the clinical form of the disease. Papules, nodules, and small ulcers are excised with simple closure. The avoidance of aggressive surgical procedures produces more satisfactory functional results. [58] It has been observed that extensive and unnecessary surgeries can damage healthy tissues and do not prevent relapses. For patients with facial lesions, surgery is not a treatment option. Patients should be referred to an orthopedist when there is bone tissue involvement.

The WHO has created a didactic division [52] into treatment categories according to the size of the lesion and other complications (Chart 3). The three treatment categories seek to help manage the disease and do not cover all of its clinical forms. A careful and adaptive analysis of the clinical condition should be conducted to control all forms of the disease. Moreover, findings from Nienhuis et al. should be considered so that unnecessary surgical procedures can be avoided.

Complete mobility of the affected area when diagnosis is accomplished, during, and after treatment may prevent contractures due to the healing process. Patients and their families should learn how to make these movements as part of their daily self-care routine. The health team and the community health agent must instruct patients and their families and practice the movements with them. Most physiotherapy and rehabilitation services are offered in reference centers for the treatment of BU in endemic countries. Unfortunately, access to these centers is limited, but they are very important to complement surgical treatment. [7,47] Other topical treatments have been suggested, including thermal treatment, hyperbaric oxygen therapy, medicinal argyle, powder phenytoin, and nitrite ointment. [8]

Recurring infections are a problem, especially in immunocompromised individuals and patients with disseminated disease. They also occur frequently

**CHART 3:** Division in categories to aid in the treatment of the Buruli ulcer patient

| Categoria | Form of disease | Treatment | Primary objective | Secondary objective | Health system level | Diagnosis |
|---|---|---|---|---|---|---|
| I | Recent small lesions (nodules, papules, plaques, and ulcers with less than 5 cm of diameter) | For papules and nodules, if immediate excision is possible, start antibiotic therapy at least 24 hours before the procedure and continue for 4 more weeks Alternatively, treat all lesions in this category with antibiotics for 8 weeks | Cure without surgery, excluding debridement of necrotic tissue | Reduction and prevention of recurrences | Community health centers and district hospitals | Precise clinical diagnosis and laboratory exams |
| II | Non-ulcerative and ulcerative plaques and edematous forms Extensive ulcerative lesions with more than 5 cm of diameter Lesions in the head, neck, and especially face | Treat with antibiotics for at least 4 weeks after surgery (if needed) followed by 4 more weeks of antibiotic therapy | Reduction of the extension of surgical excision | Reduction and prevention of recurrences | District and tertiary hospitals | Precise clinical diagnosis and laboratory exams |
| III | Disseminated, combined forms; for instance, osteomyelitis, osteitis, joint involvement | Treat for at least a week before surgery and continue with therapy for a total of 8 weeks of treatment | Reduction of infection and dissemination by M ulcerans after surgery | Reduction and prevention of recurrences Reduction of the extension of surgical excision | District and tertiary hospitals | Precise clinical diagnosis and laboratory exams |

*Adapted source: WHO/2004* [48]

in patients who develop osteomyolitis caused by *M. ulcerans*. Since patients with lesions that affect the joints are prone to developing contractures, most of these patients benefit from actions to prevent disabilities that include activities or exercises to maintain or improve movements, from the time of diagnosis until after treatment. If joint movement does not improve or worsens, the patient should be referred to reference centers where he can undergo physiotherapy. [2] Recurrence rates after treatment with antibiotics are lower than 2%, compared with the 16-30% rate of exclusive surgical treatment. [7,56] These data motivated a change in the treatment strategy for BU, which was geared towards surgical treatment until 2004.

**MONITORING AND PROGNOSIS**

After conclusion of the antibiotic treatment, the patient should be monitored for at least 10 months so that cure can be confirmed. Monitoring is also important for the diagnosis of possible complications and to notice any recurrences. [52] Execution of the ten tasks listed in chart 4 can prevent or minimize disabilities and should be initiated at diagnosis and continue after the treatment is concluded, when necessary. [59]

The evolution of the disease may vary in severity. In some areas, ulcers heal slowly with fibrosis and retraction. In the limbs, retraction can be extensive and impair their function permanently. Facial lesions can lead to serious cosmetic deformity or even loss of the eyeball. Death due to infection by *M. ulcerans* is rare, although secondary bacterial infection may aggravate extensive areas of ulceration. [16] Often, the general condition of the patient is not affected. [12] Spinocellular carcinomas have been reported to develop in healing areas after BU. [60] Care [59,61] with scars is very important to reduce skin dryness, fissures, trauma during work or leisure, sunburns, and problems with the mobility of soft tissue and joints. [47]

**Chart 4:** Ten tasks for the prevention of disabilities caused by Buruli ulcer:
"Tasks for individuals affected by Buruli ulcer who wish to prevent disability - I can!"

| 10 Tasks | Key point 1 | Key point 2 | When to start | Frequency? | Expected results |
|---|---|---|---|---|---|
| Task 1 Diagnosis &Treatment | Early diagnosis - find other cases of BU as early as possible, before many injuries have occurred | Undergo treatment | Immediately! | Daily for 8 weeks | Germs will be dead, but you will need to take other actions to help your body heal completely |
| Task 2 Hygiene | Wash your body | Wash hands frequently | Now! | Daily | Stay clean<br>Prevent infections<br>The body will heal faster |
| Task 3 Nutrition | Know which foods aid in healing | Eat foods that you are able to | Now! | Daily 2-3 times | Wounds heal |
| Task 4 Wound & skincare | Wash with water<br>Apply oil to keep the skin flexible | Wear clean clothes 1<br>Avoid tight dressings (restrictive) and favor those that encourage movement [2] | As soon as the wound is discovered - even before diagnosis is accomplished | Daily, until it heals | Skin is softer and more flexible |
| Task 5 Movement & Exercise | Try to move the affected area and the other side as well[2] | Engage in routine activities [2] | Start movements and exercises as soon as BU is diagnosed [2] | Many times a day (Every 1-2 hrs.) | Normal movement |
| Task 6 Position | When at rest or sleeping to stretch the wound or scar | Position that allows drainage of the edema | At diagnosis, if there is any limitation of movement or edema | Daily | Avoid contractures<br>Reduce edema |
| Task 7 Edema | Raise the affected limb and encourage movement | Dressings from the end of the limb upward | At diagnosis, if there is edema | Most part of the day and night, until the edema subsides | Reduces the pain and allows complete movement |
| Task 8 Care with the scar | Soap & Oil | Massage & protector 1 against skin extension | As soon as wounds are healed | Daily for 1- 2 years | Mobile, discreet scar<br>Complete movement |
| Task 9 Participation | Practice self-care<br>Involve relatives | Participate in school, work and social activities | Now! | Daily | Lead a normal life |
| Task 10 Extra help | Know when help is needed | Know where to look for help<br>Use telephone or email | When necessary | When necessary | Solve problems<br>Improve functionality |

[1] Apply light pressure with a sponge; [2] It is not necessary to practice exercises for 10 days after skin graft; movement is beneficial, but can cause discomfort. Forced movement that causes intense pain is harmful and should be avoided - Modified from Lehman L & Saunderson P, American Leprosy Missions 08/20/2009

*Adapted source: Lehman L et al.*[59]

**PREVENTION**

Prevention of BU is difficult because there is no clear knowledge about the forms of transmission of the disease or isolation of a specific antigen to develop a vaccine. The Calmette-Guerin bacillus (BCG)

appears to offer some short-term protection against the disease. Even though there is still some debate, BCG vaccine seems to protect against osteomyelitis.[2,7,62]

Due to the absence of efficient tools to control BU, current control strategies aim at reducing the prolongued suffering, disability, and socioeconomic burden associated with the disease.[7,8] In the annual meeting of the WHO for the control and management of BU, held in Geneva, Switzerland, in March of 2005, the following control strategies were suggested:[8]

- Early detection of cases in communities through information, education, and communication;
- Prevention of disabilities;
- Education of health workers in communities;
- Case management (a combination of antibiotics, surgery, and prevention of disabilities/ rehabilitation);
- Laboratory confirmation of cases;
- Standardized storage of data and communication system using forms BU 01 and BU 02 and a *HealthMapper;*
- Strengthening of reference health services;
- Monitoring and evaluation of control activities.

## CONCLUSION

Buruli ulcer, caused by *Mycobacterium ulcerans*,[1] has been reported in more than 30 countries, especially with tropical and subtropical climate,[7] but its epidemiology remains unclear, even in endemic countries. For instance, it is recognized as a public health problem in Uganda, Nigeria, Gabon, Ghana, Togo, French Guiana, Cameroon, Liberia, Côte D'Ivoire, Malasia, Papua New Guinea, and Benin.[7] The limited knowledge about the disease, its focal distribution, and the fact that it affects mainly rural poor communities contribute to the low reporting of cases.

Similarities between the climate, vegetation and habits in Brazil and endemic countries suggest that Brazil may be a likely focus of the disease. Lack of knowledge about the disease by health porfessionals makes the identification and epidemiological monitoring of the disease difficult in Brazil. In 2007, the first case of BU in Brazil was published[13] and, in November of 2009,[20] McGann et al. reporte the occurrence of a second case in the Brazilian territory. This shows that the presence of the disease in national territory is plausible.

Despite the great knowledge about the clinical profile of BU in endemic countries, in other areas this diagnosis may be overlooked. Therefore, physicians should be educated about BU, because early diagnosis and proper treatment aid in the prevention of functional disabilities resulting from this infection. ❑

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# QUESTIONS

1) What is the etiologic agent of Buruli ulcer?
   a) Mycobacterium avinum
   b) Mycobacterium chelonei
   c) Mycobacterium ulcerans
   d) Mycobacterium bovis

2) What is the likely cause of change in the epidemiology of Buruli ulcer?
   a) It has been attributed to flooding, population growth, mining, wood extraction from tropical forests, and river damming.
   b) Migration from and to rural and urban areas, leading to the development of new cases in large urban centers.
   c) Change in economic activities leading to the economic growth of most countries previously considered endemic.
   d) Higher notification of cases in countries previously not considered endemic

3) How many cases of the disease have been reported in Brazil?
   a) none
   b) one
   c) two
   d) twenty-two

4) Regarding the form of transmission of the disease, it is incorrect to state the following:
   a) An environmental factor associated with slow-flowing water, to which populations who live by the water are exposed, might play a role.
   b) There are reports of a possible transmission through insect bites or insects, probably acquatic insects of the order Hemiptera (Naucoridae and Belostomatidae). Studies have shown that the Naucoris mosquito is naturally colonized by M. ulcerans in endemic areas.
   c) There are no reports of patient-to-patient transmission.
   d) The agent of BU can enter the human body through several types of trauma, from mild ones, such as a hypodermic injection, to severe, such as landmine injuries.

5) It is correct to state the following about the etiologic agent of BU:
   a) This mycobacterium is a predominantly intracellular BAAR and can live freely in the environment, especially in acquatic environments.
   b) It is slow growing and needs a specific culture medium, different from the one used for other mycobacteria.
   c) There is some variation between the strains of M. ulcerans found in different georaphical areas (Africa, America, Asia, and Australia) that can be observed with the use of the PCR technique.
   d) There appears not to be a direct relationship between virulence and the number of IS2404 copies.

6) Mycolactone is a heat-stable exotoxin, lypophilic, that belongs to the group of macrolides and causes extensive, chronic and necrotizing damage to the papillary dermis, subcutaneous fat, and muscles, resulting in deformity and disability. It is incorrect to state the following about this molecule:
   a) The molecule is active in extremely low concentrations and is not present in laboratory cultures.
   b) Mycolactone molecules, when injected in laboratory animals, are capable of producing massive necrosis similar to that observed in animals innoculated with M. ulcerans.
   c) Mycobacterium marinum and M. ulcerans are philogenetically close and may share from 98 to 99.8% of their genetic material, but the production of mycolactone by M. ulcerans differentiates the two species.
   d) M. liflandii also has the IS2404 sequence and all the genes that codify mycolactone. This is why it is impossible to distinguish it from M. ulcerans.

7) The following is not characteristic of the histopathological evolutionary stages of BU:
   a) Late healing stage - fibrosis and atrophic epidermis.
   b) Initial healing stage - without granulomatous response. Numerous BAAR are still found.
   c) Non-ulcerated necrotic stage - the epidermis is intact, but it is often hyperplastic. The upper dermis is preserved, with varying degrees of colagen degeneration and discreet infiltration of inflammatory cells with the presence of denucleated adipose cells, called “ghost cells”.
   d) Ulcerated necrotic stage: there is loss of the epidermis and attempt of reepitelization in the borders of the ulcer. The epidermis is hyperplastic, there is minimal inflammation, and presence of numerous BAAR.

8) What is the main factor that determines which type of clinical lesion we will find in a patient?
   a) Strain of the mycobacterium causing the disease
   b) Work activity of the patient
   c) Patient's immunity
   d) Evolution time

9) What is the incubation period of the disease?
   a) 3 months
   b) 7 days
   c) 1 year
   d) 3 weeks

10) Buruli ulcer can present with a large area of marked induration, diffuse edema in the arms and legs or a well-demarcated plaque. It is correct to state the following about this clinical phase of the disease:
   a) It is also called disseminated BU.
   b) Plaques are raised, indurated, painless, hyperpigmented and smaller than 2 cm.
   c) It does not progress to an ulcer.
   d) It never affects the entire limb, only part of it.

11) It is incorrect to state the following about the clinical profile of BU:
   a) Strains of M. ulcerans isolated from various clinical forms of BU in a particular geographical region appear identical, suggesting that host factors may play an important role in the determination of the clinical presentations of the disease.
   b) The base of the initial ulcer usually contains a whitish, foamy secretion and sometimes it can form crusts.
   c) One of the consequences of an extensive ulcer is the involvement of bone tissue, with high risk of osteomyolitis and later deformities or even amputations.
   d) Due to the local immunological properties of mycolactone, the disease progresses with intense pain, high fever, and systemic symptoms.

12) It is correct to state the following about the healing phase of the disease:
   a) The cosmetic aspect of the scar seldom causes social problems or withdrawal from daily activities.
   b) Permanent disabilities affect about half of the patients.
   c) The risk of disability increases with the development of osteomyelitis during the course of the disease.
   d) The involution of lesions may result in atrophic sequelae or scars with contractures and impairment of the function of limbs when lesions occur on extensor areas.

13) Many diseases can be possible differential diagnoses of Buruli ulcer. It is correct to state the following:
   a) In West Africa, BU can be mistaken for onchocercoma and nodular leishmaniasis.
   b) The presentation of the disease with plaques and edema is completely different from any profound mycosis. Differential diagnosis with this class of diseases is done when the patient is in the ulcerative phase of BU.
   c) Tropical ulcer is rarely confused with BU because it more commonly affects the upper limbs; however, this diagnosis should always be contemplated.
   d) Leishmaniasis is an important differential diagnosis, but not in South America, where it is a rare occurrence.

14) All the following are clinical criteria for suspicion of BU, except:
   a) Presence of chronic lesion
   b) Regional lymphoadenopathy
   c) Edema on painful joint, suggesting bone involvement
   d) Patient who lives in or visisted an endemic area

15) Laboratory diagnoses are not commonly used to make decisions about treatment due to operational and logistic difficulties. It is incorrect to state the following about the possile techniques employed:
   a) Laboratory diagnosis can also be used to confirm clinical diagnosis retrospectively on swabs and tissues removed during treatment.
   b) Bone tissue curetage should be performed to confirm osteomyelitis.
   c) Histopathology is useful for differential diagnosis when the results of other methods are negative. It is up to 90% sensitive.
   d) Culture of M. ulcerans takes from 6 to 8 weeks and is approximately 100% sensitive.

16) Which of the following corresponds to recommendations of the WHO for the proper treatment of BU?
   a) Surgery should not be performed to remove necrotic tissue if wound healing causes joint impairment and cosmetic burden for the patient.
   b) Due to the high rate of complications per and post-surgery, surgery is not performed to correct defects, contractures, and the function of the affected joints. Physiotherapy is used for these purposes.
   c) Association of rifampicin and streptomycin for eight weeks as a first-line treatment for all forms of the active disease.
   d) The recommended doses of rifampicin and streptomycin are: rifampicin, 15mg/kg of body weight, taken orally, daily, for eight weeks; streptomycin, 10 mg/kg, taken intramuscularly, daily, for eight weeks. This drug is contraindicated for pregnant women.

17) It is incorrect to state the following about alternative treatments of BU:
   a) Many antimicrobial drugs (rifampicin, aminoglycosides, macrolides, and quilones) appear to be effective based on clinical impressions.

b) Studies (6) have shown that macrolides, quilonones, and aminoglicosides inhibit the in vivo growth of M. ulcerans.
c) Treatment combinations with aminoglycosides are more efficient than those without this drug.
d) There is a beneficial effect of clofazimine on M. ulcerans.

18) It is correct to state the following about the prevention of disabilities:
a) After the implementation of the so-called "10 tasks", the benefit of physiotherapy started being questioned.
b) Movement of the affected limb from the time of diagnosis is essential for the prevention of disabilities.
c) The "10 tasks" recommended by the WHO should only be executed by competent professionals in reference centers.
d) The main cause of disabilities is the level of immunological response against the infection and is not related with a delay in treatment.

19) After treatment is completed, the patient should be monitored:
a) For at least 6 months
b) Only if there are functional complications
c) Every two years, after the first year
d) Monthly, for at least 10 months

20) Prevention of BU is complicated because there is no clear knowledge about the forms of transmission of the disease or isolation of a specific antigen for the development of a vaccine. In spite of this, it is incorrect to state the following about attempts to prevent the disease:
a) Patients who received the BCG vaccine are less likely to develop osteomyelitis.
b) The bacillus Calmette-Guerin (BCG), when administered in two doses, appears to offer protection against the disease.
c) In the absence of adequate tools to control BU, current control strategies aim at reducing the prolongued suffering, disability, and socioeconomic burdens associated with the disease.
d) Early detection of cases in communities and implementation of disability control were established by the WHO in 2005.

**Answer key**
Occupational dermatosis.
2010; 85(2):137-47.

| | | | |
|---|---|---|---|
| 1 | c | 11 | a |
| 2 | d | 12 | a |
| 3 | d | 13 | c |
| 4 | d | 14 | c |
| 5 | c | 15 | c |
| 6 | a | 16 | d |
| 7 | d | 17 | c |
| 8 | a | 18 | b |
| 9 | b | 19 | c |
| 10 | b | 20 | b |

**Papers**

Information for all members: The EMC-D questionnaire is now available at the home-page of the Brazilian Annals of Dermatology: www.anaisdedermatologia.org.br. The deadline for completing the questionnaire is 60 days from the date of online publication.