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Epidemiological Profile of Buruli Ulcer and its Management at the Buruli Ulcer Detection and Treatment Center (CDTUB) in Pobè, Republic of BENIN

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ABSTRACT

Background: *The first clinical description of a new necrotizing skin disease appeared in 1948 with the work ofPeter MacCallum. In fact, the latter described the causative agent for the first time by discoveringacid-fast bacilli (AFB) in a biopsy taken from a leg ulcer in a child from Bairnsdale (Australia) in 1940. He would also publish eight years later his detailed report on six patients with ulcers on their arms or legs due to this new mycobacterial infection. However, the bacteria responsible forthis necrotizing skin disease was not named in the original publication; the name Mycobacteriumulcerans would not be proposed until 1950 by Frank Johannes Fenner [1].*

However, this disease was already known in Africa before 1948: suspected cases of Mycobacterium ulcerans infections were reported as early as the middle of the 19th century. The detailed description of the infection mentioned by the explorer James Augustus Grant in his book"A walk across Africa" in 1864 is currently considered the first reported case of Buruli ulcer. Sir Robert Cook had also described extensive ulcers in patients in Uganda as early as 1897, almost certainly caused by M. ulcerans [2]. In addition, between 1923 and 1935, a missionary doctor innortheastern Congo (formerly Zaire), named Kleinschmidt, also observed skin lesions withsunken edges containing numerous acid-fast bacilli [3]. In 1960, numerous cases occurred in Buruli County in Uganda (now Nakasongola District), hence the most commonly used name forthis disease, Buruli ulcer [4]. Currently, Buruli ulcer is reported in at least 33 countries in tropical, subtropical and temperateregions of Africa, South America and the Western Pacific. Very recently, a first case of Mycobacterium ulcerans infection was also reported in Jordan [5]. It is noteworthy that West andCentral African countries, such as Benin, Cameroon, Côte d'Ivoire, Ghana and the Democratic Republic of Congo, account for most of the reported cases (Figure 1). On the other hand, cases reported in Australia and Japan, countries with moderate non-tropical climates, have raised theinterest of scientists in the biology of the bacteria responsible for the disease: different strains of M. ulcerans in different continents have been identified [6]. Every year, more than 7,000 people are infected with Buruli ulcer, making it the third most common mycobacterial infection [7] the most common in humans after tuberculosis (Mycobacterium tuberculosis) and leprosy (Mycobacterium leprae).

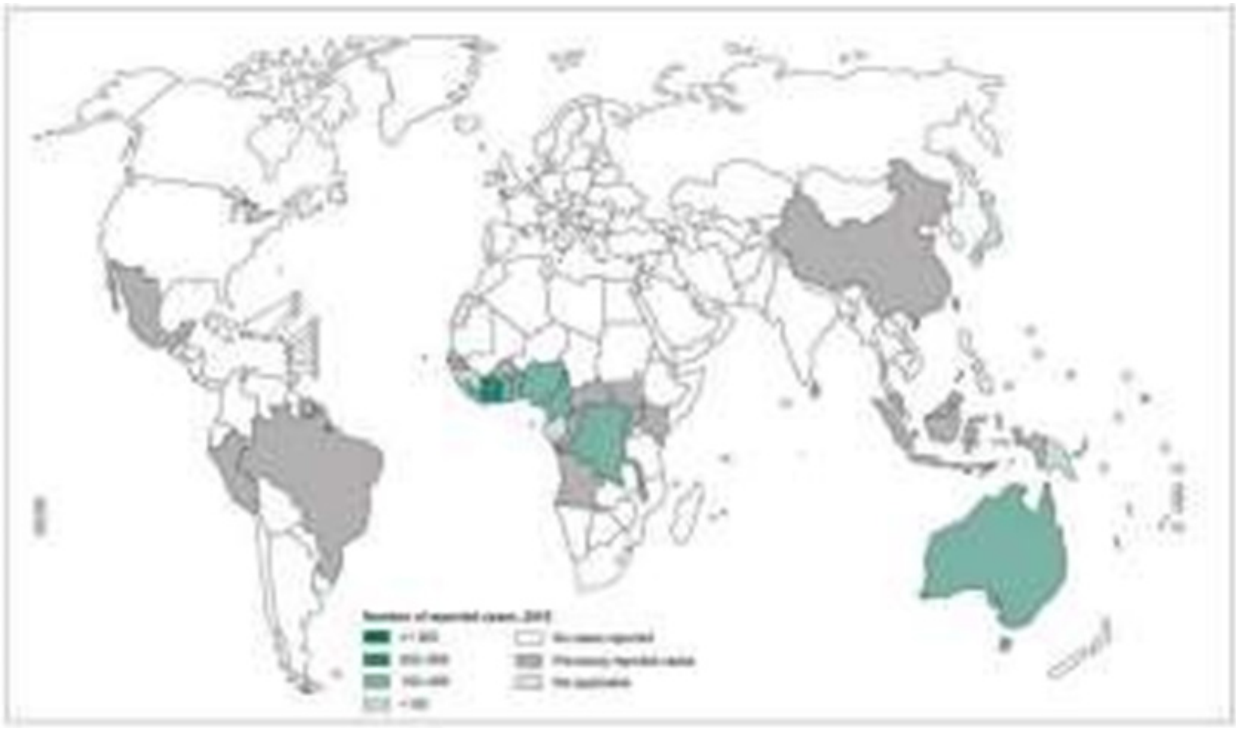

Figure 1: Global distribution of Buruli ulcer in 2015. Figure reproduced from ref. 1 withpermission. Copyright 2016 – *WHO.*

Keywords

Clinical epidemiological profile, Burili ulcer, Therapeutics, Mycobacterium ulcerans, Treatment, Pobe.

Summary

The aim of this study was to describe the epidemiological, clinical, therapeutic and evolutionary profile of Buruli ulcer (BU) at the CDTUB in Pobè, Benin. This was a retrospective descriptive study of the records of patients treated for BU between June 2017 and December 2020. During the study period, 238 patients (56.3% male) were treated at the CDTUB for BU. The median age of the patients was 14 years. The proportion of children (age < 15 years) was 56.3%. On admission, 170 patients were in the ulcerative stage and 68 patients in the preulcerative stage. The main locations were the lower limbs (50.4%), upper limbs (32.6%) and trunk (13.3%). Lower limb localizations were more frequent in patients aged over 15 years ($p < 0.001$). In contrast, upper limb ($p = 0.002$) and trunk ($p = 0.03$) localizations were more frequently observed in patients aged under 15 years.

All patients had received medical treatment with the combination of rifampicin and streptomycin for eight weeks. This treatment was supplemented by surgical treatment in 30 patients. The course was marked by complications in 14 patients, limb amputation in 6 patients and sequelae in ten patients. This study allowed us to confirm that UB is the prerogative of young subjects and is preferentially located in exposed areas. Apart from these classic characteristics, certain original aspects, in particular the age-dependent localization, are related to the pathogenesis of this condition.

Patients in this study, with a median age of 14 years, predominantly presented with single lesions (9.6%), large lesions exceeding 15 cm (36%), and ulcerative lesions (6.6%), most commonly affecting the lower limbs (60%). This reflects an atypical presentation of Buruli ulcer (UB), with osteomyelitis caused exclusively by Mycobacterium ulcerans. The sex ratio varied with age, with boys representing 57% of cases under 15 years and women comprising 33% of adult cases. Clinical presentation also differed by sex, as 9% of male patients had osteomyelitis compared to 4% of females. At the end of treatment, 22% of patients experienced permanent functional sequelae, with severe cases—characterized by extensive edema, osteomyelitis, large lesions, or multiple lesions—significantly associated with functional impairments (Odds Ratio: 7.64, 95% CI [8]). Understanding the physiopathology of M. ulcerans infection is crucial for developing new therapeutic approaches and vaccines.

Introduction

The ulcer from Buruli (UB), infection has *Mycobacterium ulcerans,* third mycobacteria where is it world, is experiencing an emergence rapid since 1980, essential in sub - Saharan African countries. So far, THE epidemiological knowledge on the UB were founded on series of e case clinics No confirmed by the laboratory. Buruli ulcer (BU) is a necrotizing infection of subcutaneous fatty tissue caused by Mycobacterium ulcerans that occurs in more than 30 countries in Africa, Latin America, Oceania and Asia [9]. In some African countries, BU has become the second most common disease caused by mycobacteria after tuberculosis [10]. We aimed to describe the epidemiological, clinical, therapeutic and evolutionary profile of BU cases followed at the CDTUB.

The term "neglected tropical disease", which emerged in 2006 at the initiative of the World Health Organization (WHO), represents a diverse group of communicable diseases prevalent in tropical and subtropical environments in 149 countries and affecting more than a billion people. These infections are a consequence of environmental and socio-economic conditions. Indeed, they mainly affect populations living in poverty, without adequate sanitation and in close contact with infectious vectors and animals. Currently, about fifteen diseases are considered neglected tropical diseases [11]. They are the subject of global control plans coordinated by the WHO, with the aim of preventing, controlling, eliminating or eradicating them. These include widespread diseases, such as leprosy, rabies, or dengue fever, but also others that are much less publicized, such as Buruli ulcer. The latter has been classified as a WHO priority because of the limited knowledge about it and the social and economic burden it represents for affected developing regions. A global initiative to mobilize and coordinate international research was launched in 1998.

Infections caused by M. ulcerans are emerging in West Africa. In 2002, at the 5th meeting of the WHO Advisory Group on Buruli Ulcer, all countries reported an increase in the number of cases. This is certainly due to better screening given the implementation of national programs, but it is also linked to a real increase in incidence (Table 1).

Table 1: Incidence and prevalence of Buruli ulcer in five West African countries.

Impact	Cumulative cases	New case in 2011
Benign	4374 from 1988 to 2001	478 and 38 relapses
Ivory Coast	12033 from 1978 to 2001	562
Ghana	3388 from 1993 to 2001	621
Guinea	442 from 1995 to 2001	221
French Guiana	193 in 2001	17

Except for Europe and North America, all continents are affected. Strains from different continents have slight genetic [12] and phenotypic variations. Hayman [13] proposes the following explanation: originating from an ancient common ancestor, the strains would have been separated by continental drift and would have evolved each on its own in different environments.

In any case, it is in wetlands that infections caused by M. ulcerans are found. But in wetlands the incidence of the disease is highly variable: zero in some places, reaching 22% of the population in other places [14]. Other factors must be involved. For Barker [15] it is the plants that are the reservoir of microbes. For Hayman [16] it is deforestation that is the cause of M. ulcerans infections. Australian authors [17] have suggested that M. ulcerans could be transmitted by aerosols produced by watering a golf course with water from a swamp. Recently, Marsollier [18] demonstrated that M. ulcerans colonizes aquatic plants on which it forms biofilms, that aquatic snails are capable of grazing on these grasses and becoming contaminated, they are then carriers of the germ. Attacked and devoured by carnivorous insects present in the swamps (Naucoris, Nepes, Belostomides), these snails infect insects in which M. ulcerans develops in the salivary glands. Very aggressive, these insects bite humans who frequent these swamps and transmit the infection to them at the point of bite where the micro-organism will multiply. In endemic areas, children are most often affected. The lesions can develop all over their body. In adults, ulcers mainly affect the limbs.

Frame – Materials

The CDTUB (Buruli Ulcer Detection and Treatment Center) in Pobè, funded by the Raoul Follereau France association, was founded in 2003 and employs approximately fifty people, including three doctors and a surgeon. It is located in a rural area in eastern Benin, near the Nigerian border. The hospitalization capacity is 58 beds. The operating theater operates twice a week. The CDTUB has an analysis laboratory allowing the main biological examinations to be carried out. X-rays are carried out nearby at the Pobè zone hospital. The CDTUB coordinates BU care in hospital and outpatient care in Pobè and in the 15 advanced care posts in the heart of endemic villages within a radius of approximately 30 kilometers. The organization of care provides for three weekly medical rounds in endemic villages. The annual number of patients treated is around 200. All patients are reviewed after treatment to detect relapses and assess functional after-effects.

Patients and Method

Clinical Manifestation

Mycobacterium ulcerans infection, the first signs of which appear after an incubation period of several weeks following inoculation, can be characterized by two distinct stages of the disease: pre-ulcerating lesions and ulcerating lesions. At an early stage, the disease begins with papules, resembling insect bites, or with subcutaneous nodules (Figure 2a) [19]. These skin lesions, which cause some itching, gradually develop into sores with irregular edges and more than 3 centimeters in diameter called plaques (Figure 2b). Diffuse swellings that can extend to an entire limb have also been observed in a few patients (Figure 2c). Since these various pre-ulcerative lesions are all painless, they generally lead infected people to neglect these symptoms. The disease then progresses to massive skin ulceration, the characteristic appearance of Buruli ulcer (Figure 2d). Discoloration of the skin may be observed around these ulcerative lesions, but again they remain painless. However, these open lesions can become painful when a bacterial superinfection or severe edema occurs. A few deaths attributable to septicemia, tetanus or hemorrhage have been reported. An increasing number of bone infections, called osteomyelitis, which complicate the management of cases has also been reported [20]. Furthermore, it should be noted that several spontaneous cures have been observed in patients after a long ulcerative phase, leading to major disabilities due to retractions and bone destruction [21].

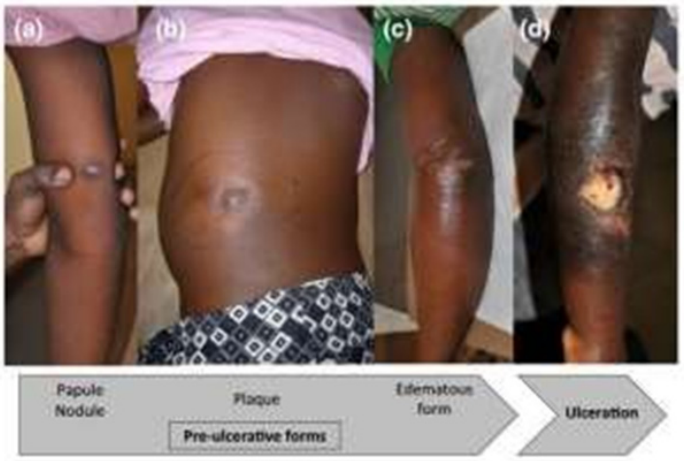

Figure 2: Clinical presentations of different forms of Buruli ulcer progression. Clinical manifestations of Buruli ulcer. Figure reproduced from ref. 12 with permission. Copyright 2017 – *WILEY.*

Generally speaking, all subjects, men and women equally and regardless of their age, can be infected by the bacteria. However, it should be noted that in Africa, children under the age of fifteen are the most affected by the infection. The lesions are located mainly on the lower limbs (50.4%), often on the upper limbs (32.6%) and sometimes on other parts of the body (13.3%) such as the trunk, face or genitals [22]. Indeed, the growth of strains of the bacteria under laboratory conditions is characterized by a remarkably narrow temperature range of 28–34 °C with optimal growth of most strains occurring between 30–33 °C, thus playing an important role in the pathogenesis of Buruli ulcer [23]. Furthermore, unlike leprosy and tuberculosis, which are characterized by person-to-person transmission, direct human-to-human transmission of *M. ulcerans* is extremely rare. However, as with other environmental mycobacteria, it is very likely that *M. ulcerans* requires inoculation into the dermis in order for it to multiply there [24]. Given the predominance of the number of cases of Buruli ulcer reported near rivers, natural or artificial lakes, or marshy areas, the existence of an aquatic reservoir has been suggested [25]. Furthermore, it should be noted that in many regions *M. ulcerans infections* have only appeared after significant ecological disturbances, such as deforestation, dam construction and agriculture [26]. *Mycobacterium* DNA fragments *ulcerans* have been identified in various aquatic insects, fish, animals, or even directly in environmental water sources [27]. However, the exact mode of transmission to humans was unknown, but it has now been completely elucidated. However, experimental tests show that the bacteria can be transmitted to mice by a bite from a contaminated aquatic insect [28].

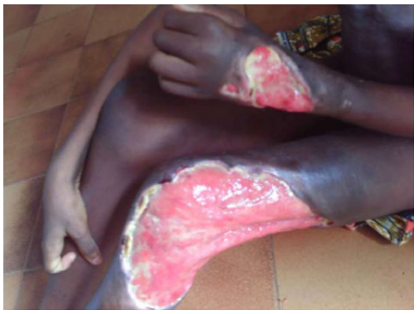

Figure 3: Buruli ulcer of the lower third of the thigh and upper third of the left leg, and left wrist / Buruli ulcer at the union of lower third of the left thigh and upper third of the left leg, and left wrist.

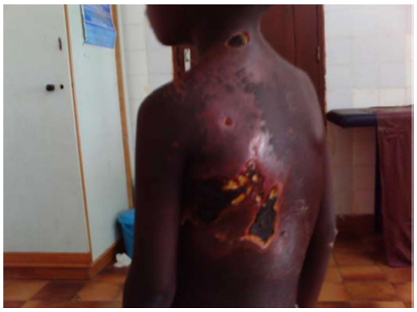

Figure 4: Plaque-like form of Buruli ulcer at the start of ulceration / Plaque form of Buruli ulcer with ulceration.

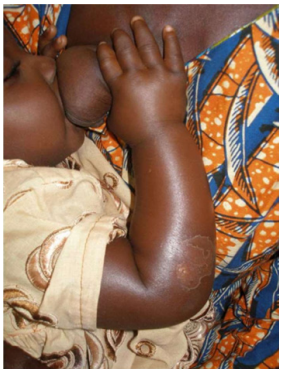

Figure 5: Oedematous form of Buruli ulcer of the upper limb right / Edematous form of Buruli ulcer of the right upper limb.

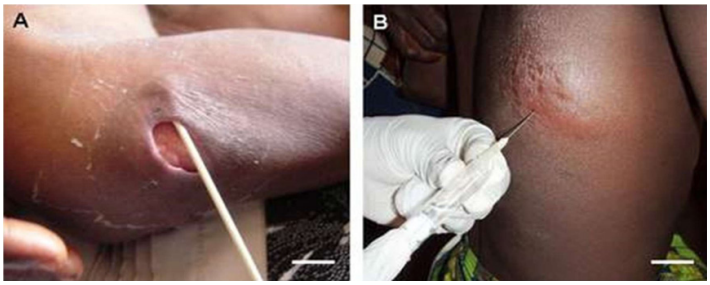

Figure 6 A and B: Fine needle aspiration of a nodule or plaque (HAS) Levy by eco village - system on a shape ulcerated. TEA levy must be accomplished in deprodersour THE b or d s taken off. Hey East advice of realize several samples to differents right ts. (B) Levy to the needle fine. This method of levy little in goes if ve and little painful is t recommanded for THE lesions closed. *Photos and comments: Dr A. Chauty.*

Tableau 1 Localisation des lésions d'UB / *Sites of BU lesions*

Localisation	Nombre	Fréquence (%)
Membres supérieurs	***44***	***32,6***
Bras	13	9,6
Avant-bras	17	12,6
Main	5	3,7
Membre entier	9	6,7
Membres inférieurs	***68***	***50,4***
Jambe	20	14,8
Cuisse	16	11,9
Pied	15	11,1
Membre entier	17	12,6
Tronc	***18***	***13,3***
Fesses	***4***	***3,0***
Visage	***1***	***0,7***

Table 2: Distribution of patients by age group and by location / Distribution of patients according to age and location.

Location of lesions	Age of patients **< 15 years≥ 15 year Old**		RR	95% CI	*p*
Lower limbs					**< 0.0001**
Yes	24 (35.5)	39 (75.0)	2.09	1.47-2.99	
No	43 (64.2)	13 (25.0)			
Upper limbs					**0.002**
Yes	31 (46.3)	11 (21.2)	2.19	1.22-3.93	
No	36 (53.7)	41 (78.8)			
Trunk					**0.03**
Yes	14 (20.9)	4 (7.7)	2.72	1.01-7.76	
No	53 (79.1)	48 (92.3)			

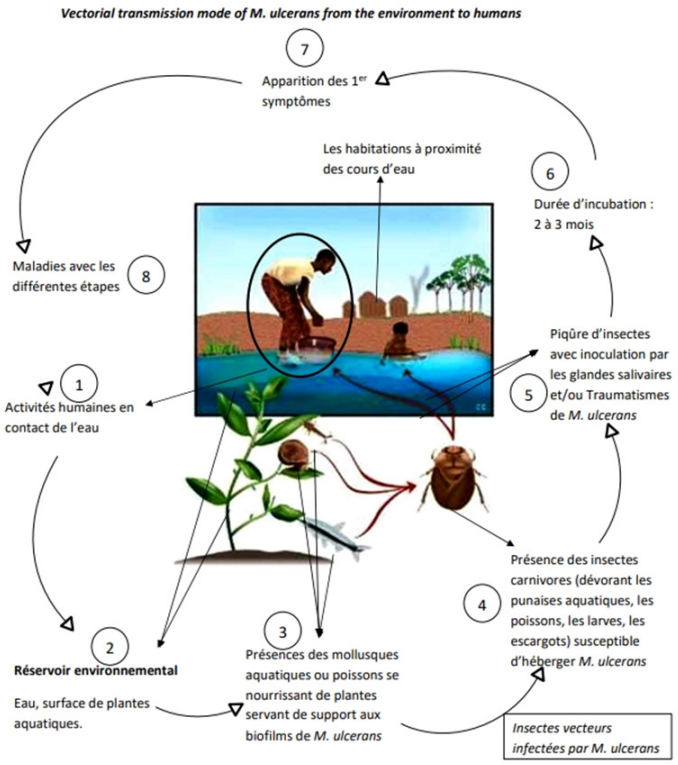

Figure 7: Transmission cycle of *M. ulcerans* from the environment to humans.

Mycobacterium transmission ulcerans from the environment to humans.

1. Human activities in contact with water or habitation near water
2. Environmental reservoir**:** Water, aquatic plants.
3. Presence of aquatic mollusks or fish feeding on plants serving as support for M. ulcerans biofilms
4. Carnivorous insects (devouring aquatic bugs, fish, larvae, snails) likely to harbor M. ulcerans
5. Presence of carnivorous insects (devouring aquatic bugs, fish, larvae, snails) likely to harbor M. ulcerans
6. Incubation 2 to 3 months
7. Appearance of the first symptoms
8. Diseases with different stages

Figure 8 a,b,c: Montrant la proximité accrue entre les habitations et les points d'eau source de vecteurs.

The hypothesis of transmission by mosquitoes is relatively natural: these insects are responsible for many viral and parasitic infectious diseases in the world (such as malaria, dengue fever, yellow fever, filariasis); the bite is frequent, recognized by everyone, and not accidental since it is necessary for their nutrition. On the other hand, it is interesting to note that the transmission of a bacterium by a mosquito has not been described to date. Nevertheless, in Australia, the hypothesis of transmission by mosquitoes is favored. An Australian case-control study observed that patients more frequently remembered being bitten by a mosquito than controls and this bite was localized at the site of ulceration [29]. M. ulcerans DNA was identified in a small but non-zero proportion of mosquitoes captured in an endemic area (4.3 mosquitoes per thousand) [30]. This proportion is geographically correlated with the incidence of UB [31]. However, a laboratory study failed to infect adult mosquitoes with M. ulcerans. Furthermore, while PCRs on mosquito mash are positive after contact with contaminated meals, this is not the case for PCRs on salivary glands or intestine, suggesting simple external contamination of the insect [32].

In Africa, no study has reported the search for M. ulcerans in mosquitoes. The protective effect of the mosquito net does not discriminate between these vectors. A mammalian reservoir is also sought. Overall, it seems clear that the water bug is a vector of the disease in Africa. Vectorial transmission is the most active. The hypothesis of passive transmission from the environment through a skin lesion remains current.

Diagnosis

The unambiguous identification of *M. ulcerans infections* is very difficult in health care centers because of the number of other nontuberculous mycobacterial infections and the general level of technical equipment in endemic regions. Several detection methods exist but are not suitable for all regions where the disease occurs. Indeed, the development of early detection methods that are easy to implement and as minimally invasive as possible, such as skin or serological tests, remains a priority, particularly given the young age of patients and the rurality and isolation of certain endemic areas. However, four different tests have been established to diagnose the disease: direct examination of the sample taken (pus, skin samples, biopsies), culture of *M. ulcerans*, genetic amplification by the PCR (Polymerase Chain Reaction) technique and histopathology. PCR is the test with a sensitivity of around 98%. Direct examination has a sensitivity of only around 40%, culture between 20 and 60%, and histology is around 90%. However, it should be noted that all these tests require a sample of the ulcer or a tissue biopsy, and some require a fairly long analysis time (6 to 8 weeks for the culture of the bacteria). It should be noted that the replication time of the bacteria is also very long, its doubling has been estimated at more than 48 hours [33]. However, to comply with the standards recommended by the WHO, at least two of these tests must be positive for a conclusive diagnosis [34].

Treatment

The difficulties in early diagnosis of Buruli ulcer have resulted in difficult and unsatisfactory treatment of the disease. Indeed, complete surgical removal of infected tissues has long been the only way to effectively treat the infection, leaving the patient with significant scarring or even complete loss of limbs. Since 2004, the World Health Organization has recommended daily therapy for 8 weeks with a combination of oral rifampicin and intramuscular injections of streptomycin for the treatment of the infection (Figure 4). From a mechanistic point of view, it is currently considered that these bactericides inhibit, on the one hand, the production of the causative agent produced by *Mycobacterium ulcerans*, and on the other hand, *Mycobacterium ulcerans* itself [35]. However, despite its relative efficacy, there are still concerns about the use of streptomycin due to its invasiveness, therapeutic adherence and bioavailability. In addition, its side effects, such as hearing impairment and nephrotoxicity, are other concerns to consider, especially in children. Therefore, current efforts are focused on developing an oral antibiotic regimen alone, such as the combination of rifampicin with clarithromycin, which is currently recommended in Australia (Figure 4) [36].

To facilitate treatment and monitor progress in public health centers, WHO has introduced a classification system for disease lesions based on the size and position of the lesion. There are three different categories: category I corresponding to single lesions less than 5 cm in diameter, category II corresponding to single lesions between 5 and 15 cm in diameter, and category III grouping together single lesions greater than 15 cm in diameter or multiple lesions and lesion(s) at a critical site (eye, breast or

genitals) or a complication such as osteomyelitis. All category I lesions and some category II lesions heal with antibiotic treatment alone. For advanced cases, there is consensus among practitioners that surgery should be performed only after antibiotic treatment of at least 4 weeks, the minimum necessary to inhibit the growth of *M. ulcerans* [37].

1° Sensitivity to Antibiotics

The study of antibiotic sensitivity by the method described by Heifets [38] shows that M. ulcerans is resistant to isoniazid and ethambutol. It is sensitive to ansamycin (rifampicin rifabutin), amikacin and streptomycin, clarithromycin and fluoroquinolones. This *in vitro* sensitivity is not confirmed by experimental chemotherapy studies carried out in mice [39], since only amikacin and rifampicin are bactericidal taken in isolation and in combination. The use of isolated rifampicin allows, *in vivo*, the selection of resistant mutants requiring, for treatment, a dual therapy.

2° Surgical Treatment

It consists of the wide excision of healthy tissue of necrotic tissue, followed if necessary by skin grafting. This is necessary often because patients often come to consult too late, at the stage where the ulcers are largely formed, which not only poses immediate treatment problems but also causes sequelae, retractions, functional impotence, which will have to be treated by reconstructive surgery in the aftermath of the evolution.

The development of an effective and specific treatment for this infection, which is widespread in tropical countries, is essential. Buruli ulcer has attracted the interest of many research groups for several years, particularly to understand the mechanism of action of this mycobacterium, and to ultimately develop a means of combating the disease. In this context, since the 1960s, the clinical observation of necrotizing lesions developing at a distance from the site of infection [40] lead Connor and Lunn to propose the hypothesis of the existence of a toxin produced by *M. ulcerans* spreading in the organism [41].

Mycolactones: Virulence Factor of M. Ulcerans

The characteristic pathology of Buruli ulcer, ie the appearance of extensive ulcers without associated pain, is related to the formation of an exotoxin, of the polyketide macrolide type, called mycolactone. Due to the metabolic importance of mycolactone production, it is obvious that the toxin plays an important role in the survival and growth of *M. ulcerans* in its environment.

Clinical Features

M. ulcerans is essentially a germ with cutaneous tropism. However, in recent years it has been incriminated in apparently primary osteomyelitis.

Skin forms Evolve in Three Stages

a) Pre-ulcerative phase

Early, not painful, it is often neglected by patients. It can take several forms.

1° The nodular form: the most common, it is characterized by a single hard nodule adhering to the skin, painless and sometimes itchy. The epidermis covering this nodule is often hyperpigmented.
2° The edema form: less frequent but more serious from the outset, presents as an edema of sudden or progressive onset. The tissues are infiltrated. Sometimes hot and painful, it tends to spread, affecting a segment of the limb or even the entire limb. This is a necrotizing panniculus that is immediately worrying [42].
3° Other rare manifestations of the onset of Buruli ulcer can be observed. The papular and bullous forms and the banal skin plaque resting on an edematous base and within which an ulceration will appear.

b) The Ulcerative Phase

Gradually over a period of several weeks to several months, the nodule spreads, the epidermis softens and in a few days necrosis appears giving rise to an ulceration which progresses centrifugally. The dermis and the deep fascia are invaded, the subcutaneous fatty tissue lyses. The lesion oozes a necrotic fluid. The established ulceration has a characteristic appearance: its edges are irregular and largely detached from the underlying musculoaponeurotic plane, which means that the actual ulceration has a surface area much greater than that of the apparent cutaneous ulceration.

The bottom of the ulcer is more or less clean depending on the degree of superinfection. It is not very painful and is not accompanied by general signs. It is often at this stage that the patient comes to consult. The biopsy carried out on the peripheral skin tissue or the swab taken under the edges of the ulcer allows the detection of acid-fast bacilli. Several lesions can merge and extend to the whole of a limb.

c) Healing Phase

In the absence of treatment after a phase of extension of a variable, fleshy buds appear on a background of ulceration. The lesions stop spreading, healing begins. It is slow and can result in healing in a few months at the cost of sequelae that are all the more serious as the lesions were more extensive. They affect the functional and aesthetic prognosis. In other cases, the lesions evolve in a chronic way with frequent relapses.

2° Bone forms: Arthritis, Osteitis, Osteomyelitis

They are not rare. While it is easy to explain osteoarticular infections that develop near an ulceration, it is more difficult to explain the authentic primary osteomyelitis that has been described [43]. Indeed, diffusion by hematogenous or lymphatic routes in the depth of the bone are incompatible with the growth temperature of *M. ulcerans,* it is necessary to imagine a possible adaptation of the strain to higher temperatures. The same phenomenon has been noted with a certain number of deep infections caused by *M. marinum.* In any case, these bone infections can lead to amputation [44].

Bacteriological Characteristics

M. ulcerans is a very slow growing, non-pigmented species that is classified in Runyon Group III.

- After Ziehl staining, the bacilli are of variable size from 3 to 10 µm in the samples, they are often grouped in clusters comparable to the globi observed in leprosy.
- Cultural characteristics are important for the identification of the germ. Culture is difficult, slow (from 6 weeks to several months). *M. ulcerans* does not grow at 37° but between 29 and 32°. On egg medium *(Löwenstein Jensen),* the colonies are rough, slightly pigmented yellow. This scotochromogenic pigmentation is not constant. The same culture can give rise to pigmented and non-pigmented colonies. *M. ulcerans grows on 7H* 12 B medium but its growth is not faster than on egg medium.
- Biochemical identification characteristics vary according to the geographical origin of the strains. All strains grow in the presence of TCH. When they have catalase, it is thermostable. African and Australian strains grow in the presence of 250 µg of hydroxylamine. African strains can produce acid phosphatase. The presence of urease and accumulation of nicotinic acid is possible but uncommon [45].

Figure 9: Implementation of microbiological diagnostic methods based on samples likely to harbor M. ulcerans.

Mycobacteria of the terrae complex are sensitive to only a small number of antibiotics *in vitro*. In the study of Smith [45], all strains tested were sensitive to azithromycin MIC < 32 µg/ml to clarithromycin, only 57% to amikacin and 50% to streptomycin. Virtually all are resistant to rifampicin, isoniazid, clofazimine and fluoroquinolones.

In practice, treatment should always combine surgery with antibiotic therapy which could include: macrolide, azithromycin or clarithromycin combined with ethambutol or rifampicin.

Table 3: Biochemical characteristics of species belonging to the terrae Complex.

	M. non chromogenicum	*Mr. Terrae*	*Mr. Trivial*
Culture 25°C	+	+	+
Culture 37°C	+	+	+
Culture 45°C	-	-	-
Catalase > 45 mm	+	+	+
Thermostable catalase	+	+	+
Hydrolysis Tween 80	+	+	+
Acid phosphatase	+	+	±
Betagalactosidase	+	+	+
Aryl sulgatease 10d	+	±	±
Nitrate reductase	-	-	+
Culture Nacl 5%	-	-	+
Tellurite reduction 10d	-	+	-

Molecular Aspect

Given the difficulties of the nature and identification of the strains, molecular methods are of great importance for this germ. Represented in very many copies in the genome, the insertion sequence /S2404 is specific to M. ulcerans. It is not found in strains of the tuberculosis complex, nor in M. leprae, nor especially in 45 different species of mycobacteria [46]. PCR is therefore an easy, rapid means of identifying strains and detecting the germ in samples from animal tissues or the environment. After amplification of the hsp 65 KDA gene, the PRA method does not allow to differentiate M. ulcerans from M. marinum. Both show, after action of BstE II two fragments 245 and 220 bp and after digestion by Hae III, three fragments 160, 115, 80 base pairs. Strip hybridization cannot differentiate M. marinum from M. ulcerans.

Discussion

This study confirms that UB is common in young subjects and is preferentially located in exposed areas. During the study period, 138 (58%) of the 238 clinically suspected cases of BU received at the CDTUB were confirmed by PCR. The median age of the patients was 14 years. The proportion of children (age < 15 years) was 56.3%. The sex ratio (male/female) was 1.3. The mean duration of symptom evolution before consultation was 11.4 ± 4 months (range: four days to seven years). At admission, 170 patients were in the ulcerative stage and 68 patients in the preulcerative stage (30 cases of nodular form, 9 cases of edematous form, 10 cases of plaque form and 10 cases of popular form). All patients were in their first episode of BU. The main localizations were the lower limbs (50.4%), including 14.8% in the leg alone, followed by the upper limbs (32.6%), including 12.6% in the forearm alone, and the trunk (13.3%). 24 patients had multiple localizations. Localizations in the lower limbs were more frequent in patients aged over 15 years ($p < 0.001$). On the other hand, localizations in the upper limbs ($p= 0.002$) and the trunk ($p = 0.03$) were more observed in patients aged under 15 years.

The proportion of children (age < 15 years) was 56.3% in our study, comparable to the 56.9% found by Kanga and Kacou in Ivory Coast [47]. As in our study, UB is preferably located in exposed areas [47], linked to the fact that these parts of the body are exposed to micro cutaneous trauma and contact with water from rivers, ponds and lakes but also to insect vectors of M. Ulcerans. The second particularity of our study is the preferential location of lesions in the lower limbs in patients aged over 15 years, linked to the fact that the latter practices much more agricultural work or fishing, in comparison to patients aged under 15 years and therefore that contact with the microbial reservoir is with the lower limbs. Clinically, the predominance of the ulcerative form in our study, as in most other series [48], is due to the delay in consulting patients linked to the painless nature of BU. The first-line treatment of BU with CDTUB, which is the combination of rifampicin and streptomycin for eight weeks, complies with the WHO recommendations in force since 2004 [49,50]. This antibiotic treatment is very effective, as demonstrated by the low number of patients with an unfavorable outcome after four to six weeks and the low number of patients who did not achieve a cure at the end of treatment. The effectiveness of this treatment also explains the low rate of complications observed in our study compared to that of Ecra et al. [51], and the low rate of sequelae in our study

compared to that of Kanga and Kacou [52] who found 13%. It should be noted that these two Ivorian studies covered periods when the treatment of BU was essentially surgical. However, serious complications, including the occurrence of squamous cell carcinoma on UB reported in Ivory Coast [52], were not noted in our series. It should also be added that one of the evolutionary profiles of UB is recurrence after cure, which was not observed in our study, probably due to the fact that the follow-up is short.

Conclusion

The results of this study confirm that UB is the prerogative of young subjects and that it causes after-effects that have a considerable impact on the schooling and social reintegration of these children. To reduce this risk of after-effects, it is necessary to rapidly expand UB care services, create care centers by region to be closer to the populations and intensify awareness in order to reach all affected patients requiring early and appropriate care.

UB is exceptionally fatal, although some deaths are reported in very specific circumstances (septic shock due to superinfection, tetanus, severe malnutrition). It is the major and permanent functional and aesthetic sequelae that make this disease serious, affecting a young population. Healing is accompanied by the formation of tendon bands and retractions, amyotrophy, lymphedema and ankylosis that limit joint range of motion [53]. Various studies report amputation rates of 2 to 10% with a peak of 19% in a series of 106 bone forms in Benin. Two recent studies in Ghana reveal that approximately 60% of patients have a long- term limitation of joint range of motion (mainly elbow, knee, wrist). All these limitations do not translate into incapacity in the gestures of daily life, which explains why other studies less detailed in their description of the disability report lower rates of sequelae between 15 and 25%, but corresponding to an obvious incapacity [53]. On the functional level, approximately 30% of patients report an inability or difficulty in fetching water from the well; 20% in pouring water into a cup, 15% in washing themselves, 20% in walking on a flat surface, 40% in running, 45% in carrying their harvest [54].

Whatever the criteria used, it is indisputable that UB significantly affects the life trajectory of many patients since approximately 50% of adult patients must suspend their professional activity (of which approximately 80% do not find work), and approximately 25% of children interrupt their schooling. There is also a certain stigmatization of the disease, since in a study in Ghana, 40% of those questioned would not accept a former patient as a village chief [55]. Twenty percent of cured patients, a third of patients currently receiving treatment and half of the witnesses questioned consider that UB is a probable or proven obstacle to marriage. That said, this stigmatization is not absolute since 90% of those questioned would accept a patient occupying the position of teacher. Patients are not physically sidelined or generally rejected, as was and still is the case for leprosy [55]. Ideally, the development of a vaccine to prevent infection would offer the best solution and seems feasible. Indeed, knowing that the active form of Buruli ulcer only develops in some people exposed to *M. ulcerans* [56] and that the risk for young adults to develop the disease is much lower than for children, these observations suggest that the development of protective immunity against Buruli ulcer is possible. However, attempts in this area have had limited success so far [56].

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