Epidemiological Profile Of Buruli Ulcer And Its Management At The Buruli Ulcer Treatment Detection Center (CDTUB) In Pobè, Republic Of Benin

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Background :

The first clinical description of a new necrotizing skin disease appeared in 1948 with the work of Peter Mac Callum. He first described the causative agent when he found acid-fast bacilli (AFB) in a biopsy from a leg ulcer in a child in Bairnsdale, Australia in 1940. Eight years later, he also published his detailed report on six patients with ulcers on their arms or legs caused by this new mycobacterial infection. However, the bacterium responsible for this necrotizing skin disease was not named in the original publication; the name *Mycobacterium ulcerans* was not proposed until 1950 by Frank Johannes Fenner[1]

However, the disease was already known in Africa before 1948: suspected cases of infection with *Mycobacterium ulcerans* were reported as early as the middle of the 19th century. The detailed description of the infection 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 in the northeastern Congo (former Zaire), named Kleinschmidt, also observed skin lesions with sunken edges containing numerous acid-fast bacilli [3]. In 1960, many cases occurred in Buruli County, Uganda (now Nakasongola District), hence the most commonly used name for this disease Buruli ulcer [4].

Currently, Buruli ulcer is found in at least 33 countries in tropical, subtropical and temperate regions 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 worth noting that most of the reported cases are from West and Central African countries, such as Benin, Cameroon, Côte d'Ivoire, Ghana and the Democratic Republic of Congo (Figure 1). On the other hand, cases reported in Australia and Japan, countries with moderate non-tropical climates, have raised the interest of scientists in the biology of the bacterium responsible for the disease: different strains of *M. ulcerans* in different continents have been identified [6]. Every year, more than 7000 people are infected with Buruli ulcer, making it the third most common mycobacterial infection [7] in humans after tuberculosis (*Mycobacterium tuberculosis*) and leprosy (*Mycobacterium leprae*).

Figure 1: Global distribution of Buruli ulcer in 2015. Figure reproduced from ref 1 with permission. Copyright 2016 - *WHO*

***Abstract*—The aim of this study was to describe the epidemiological, clinical, therapeutic and evolutionary profile of Buruli Ulcer (BU) at 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 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 pre-ulcerative stage. The main locations were lower limbs (50.4%), upper limbs (32.6%) and trunk (13.3%). Lower limb locations were more frequent in patients older than 15 years (p < 0.001). In contrast, upper extremity (p = 0.002) and trunk (p = 0.03) locations were more common in patients under 15 years of age.**

All patients had received medical treatment with the combination of rifampicin and streptomycin for eight weeks. This treatment had been completed by surgical treatment in 30 patients. The evolution was marked by complications in 14 patients, limb amputation in 6 patients and sequelae in 10 patients. This study allowed us to confirm that UB is the prerogative of young subjects and occurs preferentially on exposed areas. Apart from these classical features, some original aspects, notably the age-dependent localization, are related to the pathogenesis of this condition.

Patients with BU are children (median age at diagnosis 14 years), presenting with a single (96%), large (more than 15 cm, 36%), ulcerative (66%) lesion of the lower limb (60%). We report an atypical clinical presentation of BU, in which patients present exclusively with M*. ulcerans* osteomyelitis. The sex ratio varies with age: boys predominate among children (57% male patients in children under 15 years old), and women among adults (33% male patients). Clinical presentation depended on age and sex. 9% of male patients had osteomyelitis compared to 4% of female patients. One year after the end of treatment, 22% of patients had fixed functional sequelae. A clinical presentation involving an oedematous, bony, large lesion or several lesions is significantly associated with the development of functional sequelae (OR 7.64, IC95% [8]). Understanding the pathophysiology of *M. ulcerans* infection is crucial to generate new therapeutic and vaccine leads.

Keywords—clinical epidemiological profile; Burili ulcer; therapeutics; Mycobacterium ulcerans; Treatment; Pobe

Introduction

Buruli ulcer (BU), a *Mycobacterium ulcerans* infection*, is* the third most common mycobacterial disease in the world and has been emerging rapidly since 1980, mainly in sub-Saharan African countries. Until now, epidemiological knowledge of BU has been based on series of non-laboratory confirmed clinical cases.

Buruli ulcer (BU) is a necrotizing infection of the 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 wanted 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 settings in 149 countries and affecting more than one 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 15 diseases are considered as 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. They include widespread diseases such as leprosy, rabies, and dengue fever, but also much less publicized ones such as Buruli ulcer. Buruli ulcer has been prioritized by WHO because of the limited knowledge about it and the social and economic burden it places on 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 as an emerging

disease. In 2002, at the 5th meeting of the WHO Buruli ulcer advisory group, all countries reported an increase in the number of cases. This is certainly due to better screening, given the implementation of national programmes, 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

Incidence	Cumulative cases	New case in 2011
Benin	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

Apart from 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: the strains originated from an ancient common ancestor, but were separated by continental drift and evolved independently in different environments.

In any case, it is in wetlands that infections caused by M. ulcerans are found. However, the incidence of the disease in wetlands is highly variable, ranging from zero in some places to 22% of the population in others [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, and that aquatic snails are able to graze on these grasses and become contaminated, thus carrying the germ. Attacked and eaten 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 multiplies. In endemic areas, children are most often affected. The lesions can develop all over their bodies. In adults, the ulcerations mainly affect the limbs.

Frame - Materials

The CDTUB (Buruli Ulcer Detection and Treatment Centre) in Pobè, financed by the Raoul Follereau France association, was founded in 2003 and employs about fifty people, including three doctors and a surgeon. It is located in a rural area in eastern Benin, near the Nigerian border. The hospital has a capacity of 58 beds. The operating theatre operates twice a week. The CDTUB has an analysis laboratory where the main biological tests can be carried out. X-rays are carried out at the nearby hospital in Pobè. The CDTUB coordinates inpatient and outpatient care in Pobè and in the 15 outposts in the heart of the endemic villages within a radius of about 30 kilometres. The organization of care includes three weekly medical rounds in the endemic villages. The annual number of patients treated is around 200. All patients are reviewed at a distance from the treatment to detect relapses and evaluate 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 disease: pre-ulcerating and ulcerating lesions. In the early stage, the disease begins with papules, resembling insect bites, or subcutaneous nodules (Figure 2a) [19]. These skin lesions, which cause some itching, gradually develop into irregularly edged sores more than 3 centimetres in diameter called plaques (Figure 2b). Diffuse swellings that may extend to an entire limb have also been observed in some patients (Figure 2c). These various pre-ulcer lesions are all painless, so infected persons usually ignore these symptoms. The disease then progresses to massive skin ulceration, the characteristic appearance of Buruli ulcer (Figure 2d). Discoloration of the skin may be seen around these ulcerating lesions, but again they remain painless. However, these open lesions may become painful when bacterial superinfection or severe edema occurs. A few deaths due to sepsis, tetanus or haemorrhage have been reported. An increasing number of bone infections, called osteomyelitis, which complicate case management have also been reported [20]. Furthermore, it is noteworthy that several spontaneous recoveries have been observed in patients after a long ulcerative phase, resulting in major disabilities due to retractions and bone destruction [21].

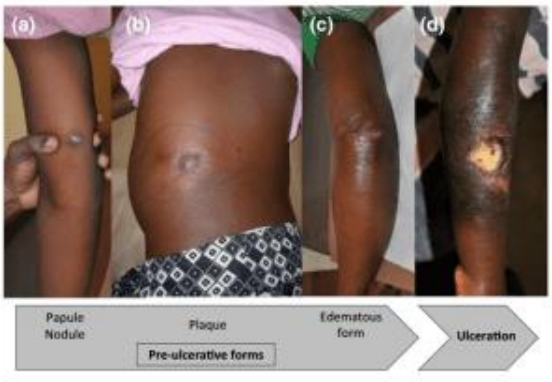

Figure 2: Clinical presentations of the different forms of Burili's Ulcer. Clinical manifestations of Buruli ulcer. Figure reproduced from ref. 12 with permission. Copyright 2017 - *WILEY*

(A) Ulcerative form located at the elbow. The periphery of the lesion is hyperpigmented, showing progressive tissue necrosis. Due to the destruction of the fatty tissue, the bottom of the ulcer is yellow with a cottony appearance.

(B) Massive ulceration of the elbow. The detached edges show the progression of the lesion.

(C) Disseminated form with bone involvement. An edematous form is seen in the eye of this young patient (arrow).

(D) *Staphylococcus aureus* superinfection on an ulcerated form. (E) Scar on the leg after surgical treatment.

(F) Disabling sequelae due to musculotendinous retractions (spontaneous recovery)

In general, all subjects, men and women equally and whatever their age, can be infected by the bacterium. However, it should be noted that in Africa it is children under 15 years of age who are most affected by the infection. The lesions are mainly located 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 bacterium under laboratory conditions is characterized by a remarkably narrow temperature range of 28-34°C with optimal growth of most strains 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 it is necessary for *M. ulcerans* to be inoculated into the dermis in order to multiply [24]. Given the predominance of Buruli ulcer cases reported in the vicinity of 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 areas *M. ulcerans* infections have only appeared after major ecological disturbances, such as deforestation, dam construction and

agriculture[26]. DNA fragments of *Mycobacterium ulcerans* have been identified in various aquatic insects, fish, animals, or directly in environmental water sources [27]. Nevertheless, the exact mode of transmission to humans was not well known, but has recently been fully elucidated. However, experimental trials show that the bacterium can be transmitted to mice through the bite of a contaminated aquatic insect [28].

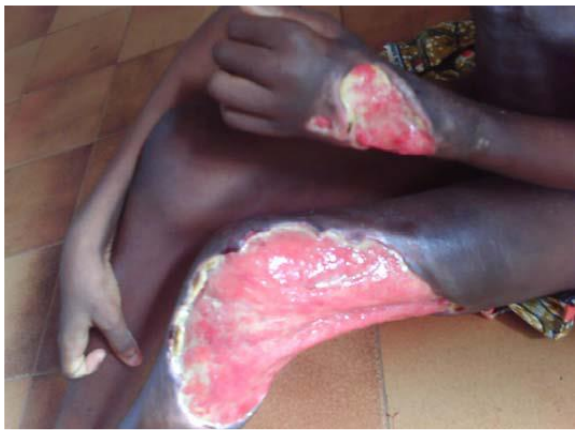

Figure 3: Buruli ulcer of the lower third of the thigh and upper third of left leg, and left wrist / Buruli ulcer at the union of lower third of the left thigh and upper third of the left leg, and left wrist

Figure 4: Plaque form of Buruli ulcer in early ulceration /Plaque form of Buruli ulcer with ulceration

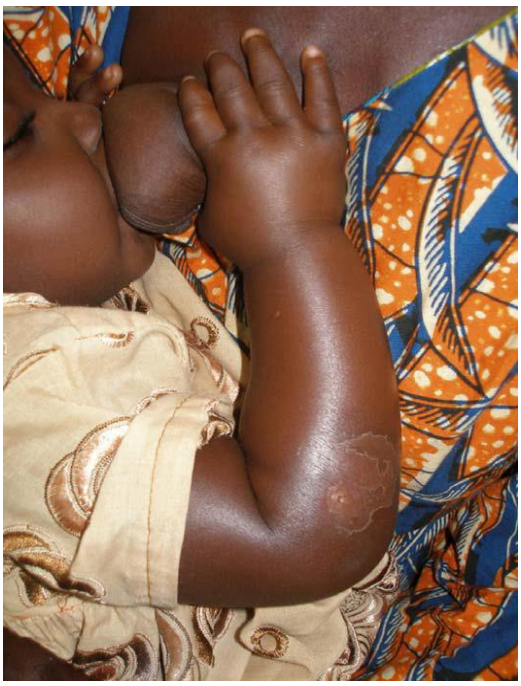

Figure 5: Edematous form of Buruli ulcer of the upper limb right / Edematous form of Buruli ulcer of the right upper limb

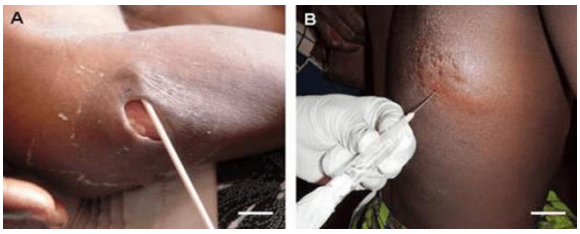

Figure 6 A and B. Fine needle aspiration of a nodule or plaque

(A) Swabbing of an ulcerated form. The swab should be taken deep under the detached edges. It is advisable to take several swabs from different locations.

(B) Fine needle aspiration. This minimally invasive and painless sampling method is recommended for closed lesions. *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 according to age and location					
Location of the lesions	Age of patients		RR	95% CI	*p*
	< 15 years	**≥ 15 years**			
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)			

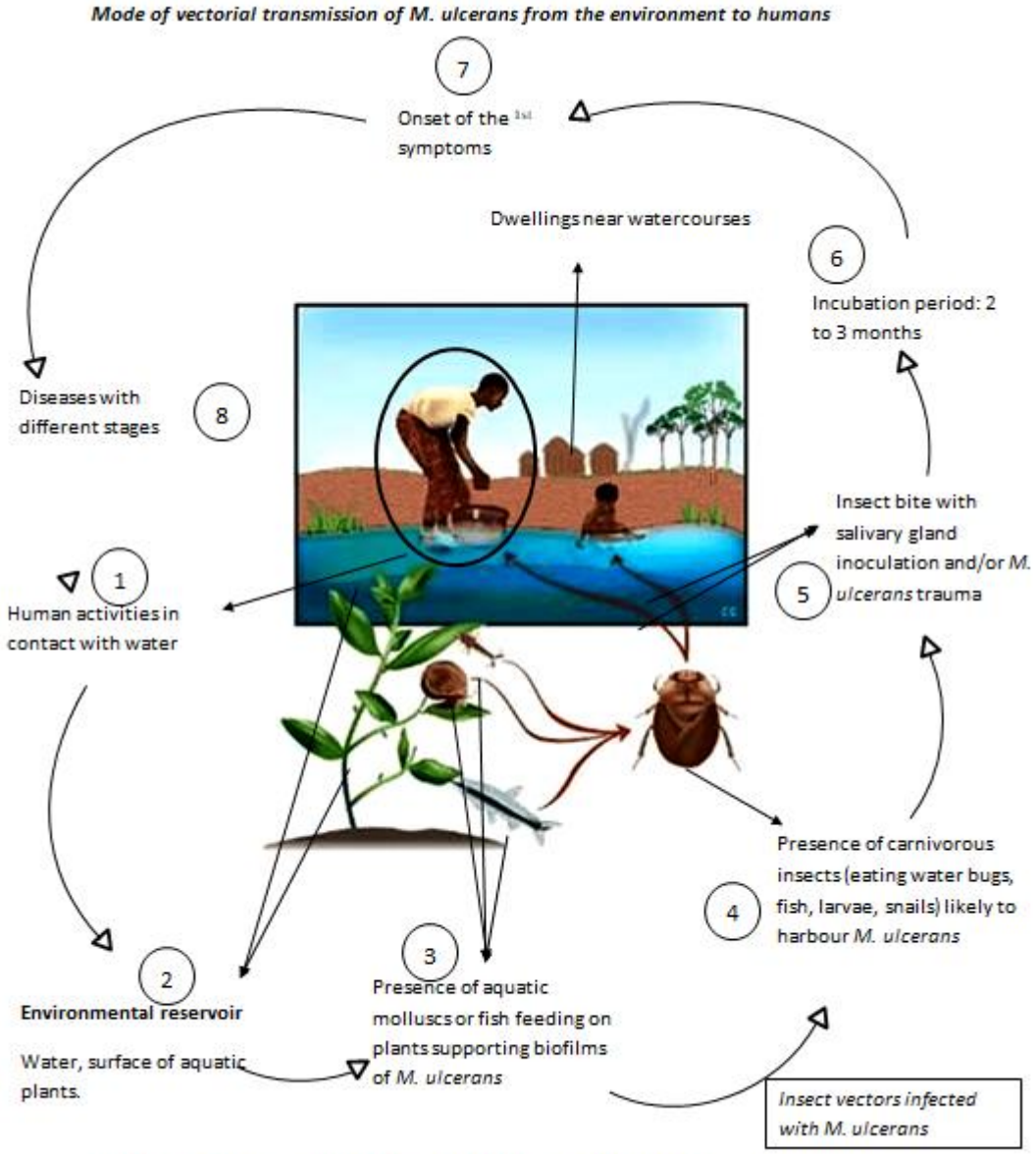

Figure 7: Cycle of transmission of *M. ulcerans* from the environment to humans

Description of the different points of the biological cycle of transmission of Mycobacterium ulcerans from the environment to humans.

1. Human activities in contact with water or housing near water

2. Environmental reservoir**:** Water, aquatic plants.

3. Presence of aquatic molluscs or fish feeding on plants supporting biofilms of M. ulcerans

4. Carnivorous insects (eating water bugs, fish, larvae, snails) that may harbour M. ulcerans

5. Presence of carnivorous insects (eating water bugs, fish, larvae, snails) likely to harbour M. ulcerans

6. Incubation 2 to 3 months

7. Onset of the 1st symptoms

8. Diseases with different stages

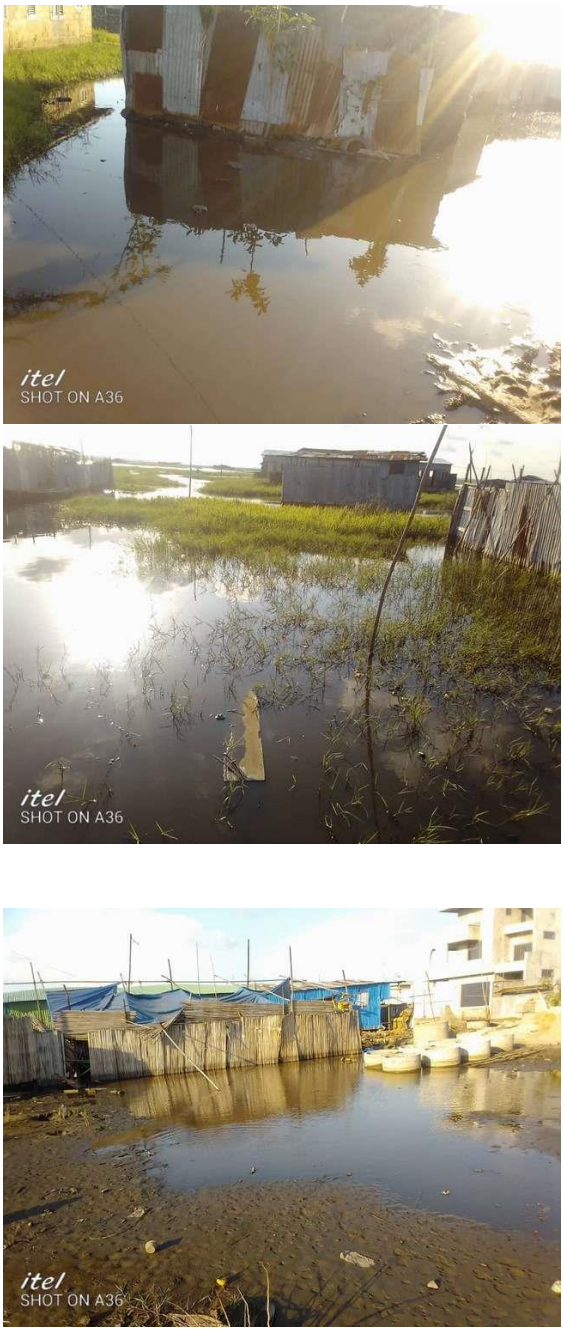

Figure 8 a, b, c: showing the increased proximity between houses and vector source water points.

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 mosquito transmission is favoured. An Australian case-control study found that patients more frequently recalled being bitten by a mosquito than controls and that the bite was localized to the site of ulceration [29]. M. ulcerans DNA has been identified in a small but non-zero proportion of mosquitoes captured in endemic areas (4.3 mosquitoes per thousand) [30]. This proportion is geographically correlated with the incidence of BU [31]. Nevertheless, a laboratory study fails to infect adult mosquitoes with M. ulcerans. Furthermore, while PCRs on mosquito grindings are positive after contact with contaminated meals, this is not the case for PCRs on salivary glands or intestines, suggesting a simple external contamination of the insect [32].

In Africa, no studies have reported finding M. ulcerans in mosquitoes. The protective effect of the net does not discriminate against these vectors. A mammalian reservoir is also sought. Overall, it seems clear that the water bug is a vector of the disease in Africa. Vector transmission is the most active. The hypothesis of passive transmission from the environment through a skin lesion remains valid.

Diagnosis

The unambiguous identification of *M. ulcerans* infections is very difficult at health care facilities because of the number of other nontuberculous mycobacterial infections and the general level of technical equipment in endemic areas. Several detection methods exist but are not suitable for all regions where the disease occurs. Indeed, the development of early, easy-to-use and minimally invasive detection methods, such as dermal or serological tests, remains a priority, particularly in view of the young age of patients and the rural and isolated nature of some 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 about 98%. Direct examination has a sensitivity of only about 40%, culture between 20 and 60%, and histology around 90%. Nevertheless, it should be noted that all these tests require a sample of the ulcer or a tissue biopsy, and some of them require a fairly long analysis time (from 6 to 8 weeks for the culture of the bacteria). It should be noted that the replication time of the bacterium is also very long, and its duplication has been estimated at more than 48 hours [33]. Nevertheless, to meet the standards recommended by the WHO, at least two of these tests must be positive for a conclusive diagnosis [34].

Treatment

The difficulties of early diagnosis of Buruli ulcer have resulted in difficult and unsatisfactory treatment of the disease. Indeed, complete surgical removal of infected tissue was for a long time 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 advocated 8 weeks of daily therapy with a combination of oral rifampicin and intramuscular streptomycin injections for the treatment of the infection (Figure 4). Mechanistically, it is currently considered that these bactericides inhibit the production of the causative agent produced by *Mycobacterium ulcerans on the one hand,* and *Mycobacterium ulcerans* itself on the other [35]. However, despite its relative efficacy, there are still concerns about the use of streptomycin due to its invasiveness, adherence and bioavailability. In addition, its side effects, such as hearing impairment and nephrotoxicity, are further concerns, especially in children. Therefore, current efforts are focused on the development of an oral-only antibiotic regimen, such as the combination of rifampicin with clarithromycin, which is currently recommended in Australia (Figure 4) [36].

In order to facilitate treatment and monitor progress in public health centres, WHO has introduced a classification system for the disease 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 single lesions larger 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 and some Category II lesions heal with antibiotic treatment alone. For advanced cases, there is a consensus among practitioners that surgery should be performed only after at least 4 weeks of antibiotic treatment, which is the

minimum necessary to inhibit the growth of *M. ulcerans* [37].

1° Sensitivity to antibiotics

Antibiotic susceptibility testing 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 in mice [39] since only amikacin and rifampicin are bactericidal taken alone and in combination. The use of rifampicin in isolation allows, in vivo, the selection of resistant mutants requiring, for the treatment, a dual therapy

2° The surgical treatment

It consists of a large removal of the necrotic tissues in healthy tissue, followed if necessary by a skin graft. This is often necessary because patients often come to the clinic too late, at the stage when the ulcerations are largely constituted, which not only poses immediate problems of treatment but also causes sequelae, retractions, and functional impotence, which will have to be treated by reconstructive surgery after 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, in particular to understand the mechanism of action of this mycobacterium, and to eventually develop a means of combating the disease. In this context, in the 1960s, the clinical observation of necrotizing lesions developing at a distance from the site of infection [40] led Connor and Lunn to propose the hypothesis of the existence of a toxin produced by *M. ulcerans* that spreads throughout the body. [41]

Mycolactones: Virulence factor of M. Ulcerans

The characteristic pathology of Buruli ulcer, i.e., the appearance of extensive ulcers without associated pain, is related to the formation of a polyketide macrolide exotoxin called mycolactone. Because of the metabolic importance of mycolactone production, it is evident 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 skin-tropic germ. However, in recent years it has been implicated in apparently primary osteomyelitis.

The cutaneous forms evolve in three stages

a) Pre-ulcer phase

Early, painless, it is often neglected by patients. It can take several forms.

1° The nodular form: the most frequent, it is characterized by a single hard nodule adhering to the skin, painless and sometimes pruritic. The epidermis covering this nodule is often hyper-pigmented.

2° The oedematous form: less frequent but immediately more serious, presents as an oedema of sudden or progressive appearance. The tissues are infiltrated. Sometimes hot and painful, it tends to spread, involving a segment of limb or even the whole limb. It is a necrotizing panniculus of immediate concern [42].

3° Other rarer manifestations of the onset of Buruli ulcer can be observed. The papular and bullous forms and the banal cutaneous placard resting on an oedematous base and within which an ulceration will appear.

b) The ulcerative phase

Progressively, 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, and the subcutaneous fatty tissue becomes infected. The lesion oozes a necrotic fluid.

The ulceration that has formed has a characteristic appearance: its edges are irregular and largely detached from the underlying musculoaponeurotic plane, which means that the actual ulceration has a much larger surface area than the apparent skin 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. Biopsy of peripheral skin tissue or swabbing under the edges of the ulcer reveals acid-fast bacilli. Several lesions may coalesce and extend to an entire limb.

c) Healing phase

In the absence of treatment, after a variable extension phase, fleshy buds appear on the bottom of the ulceration. The lesions stop spreading and healing begins. Healing is slow and can lead to recovery in a few months, at the cost of more serious sequelae as the lesions are more extensive. These sequelae 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 genuine primary osteomyelitis that has been described [43]. Indeed, diffusion by the haematogenous route or by the lymphatic route into the depth of the bone is incompatible with the growth temperature of *M. ulcerans, and* it is necessary to imagine a possible adaptation of the strain to higher temperatures. The same phenomenon has been noted with a number of deep infections caused by *M. marinum.* However, 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's staining, the bacilli vary in size from 3 to 10 µm in the samples, and are often grouped in clusters comparable to the globi observed in leprosy.

- The cultural characteristics are important for the identification of the germ. The 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 yellow pigmented. This scotochromogenic pigmentation is not constant. The same culture may give rise to pigmented and unpigmented colonies. *M. ulcerans* grows on 7H12B medium but does not grow 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 possess catalase, it is thermostable. African and Australian strains grow in the presence of 250 µg hydroxylamine. African strains can produce an acid phosphatase. Urease and nicotinic acid accumulation are possible but not common [45].

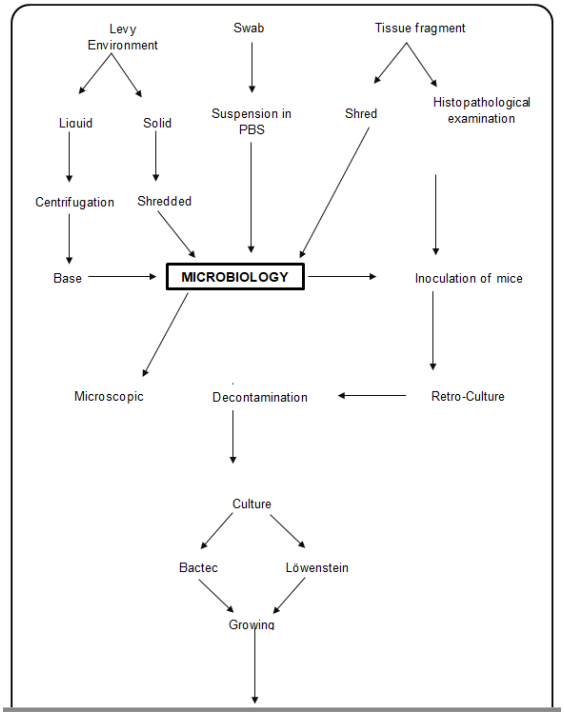

Figure 9: Implementation of microbiological diagnostic procedures on samples that may harbour M. ulcerans.

Mycobacteria of the terrae complex are susceptible to only a few antibiotics in vitro. In Smith's study [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, izoniazid, 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	*M. terrae*	*M. trivial*
Culture 25°C	+	+	+
Culture 37°C	+	+	+
Culture 45°C	-	-	-
Catalase > 45 mm	+	+	+
Thermostable Catalase	+	+	+
Hydrolysis Tween 80	+	+	+
Acid phosphatase	+	+	±
Betagalactosidase	+	+	+
Aryl sulgatase 10d	+	±	±
Nitrate reductase	-	-	+
Nacl 5% culture	-	-	+
Tellurite reduction 10d	-	+	-

Molecular aspect

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

Discussion

This study confirms that BU is common in young subjects and occurs preferentially on the exposed areas.

During the study period, 138 (58%) of the 238 clinically suspected BU cases received at 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 symptoms before consultation was 11.4 ± 4 months (extremes: four days and seven years). On admission, 170 patients were in the ulcerative stage and 68 patients in the pre-ulcer stage (30 nodular, nine edematous, 10 plaque and 10 papular). All patients were first-time BU patients. The main sites were the lower limbs (50.4%), of which 14.8% were on the leg alone, followed by the upper limbs (32.6%), of which 12.6% were on the forearm alone, and the trunk (13.3%). 24 patients had multiple locations. Lower limb locations were more frequent in patients older than 15 years ($p < 0.001$). In contrast, upper limb ($p = 0.002$) and trunk ($p = 0.03$) locations were more common in patients under 15 years of age.

The proportion of children (age < 15 years) was 56.3% in our study, comparable to the 56.9% found by Kanga and Kacou in Côte d'Ivoire [47]. As in our study, BU occurs preferentially on the uncovered areas [47], related to the fact that these parts of the body are exposed to skin microtrauma and contact with water from rivers, ponds and lakes but also to insect vectors of M. Ulcerans. The second peculiarity of our study is the preferential localization of lesions in the lower limbs in patients aged over 15 years, linked to the fact that the latter are much more involved in agricultural work or fishing, compared to patients aged under 15 years, and therefore the 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 consultation of the patients related to the painless character of BU. The first-line treatment of BU in 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 evidenced by the low number of patients with an unfavourable evolution after four to six weeks and the low number of patients who did not obtain a cure at the end of the 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.

But serious complications, notably 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 short follow-up time.

Conclusion

The results of this study confirm that BU is a disease of the young and that it causes sequelae that have a considerable impact on the schooling and social reintegration of these children. To reduce this risk of sequelae, it is necessary to rapidly extend UB management services, to create management centres by region to be closer to the population and to intensify awareness in order to reach all affected patients requiring early and adapted management.

UB is exceptionally fatal, although a few deaths have been reported in very specific circumstances (septic shock on superinfection, tetanus, severe malnutrition). The seriousness of this disease, which affects a young population, lies in its major and permanent functional and aesthetic sequelae. Healing is accompanied by the formation of tendon bands and retractions, amyotrophy, lymphoedema and ankylosis which limit joint range of motion [53]. Various studies report amputation rates of 2-10% with a peak of 19% in a series of 106 bony forms in Benin (2008). Two recent studies in Ghana show that about 60% of patients have a long-term limitation of joint amplitude (mainly elbow, knee, wrist). Not all of these limitations translate into disability in daily life, which explains why other studies, less detailed in their description of the disability, report lower rates of sequelae of between 15 and 25%, but corresponding to an obvious disability [53]. In terms of function, about 30% of patients report an inability or difficulty in fetching water from a well, 20% in pouring water into a cup, 15% in washing, 20% in walking on a flat surface, 40% in running, 45% in carrying their harvest [54].

Whatever the criteria used, it is indisputable that BU significantly affects the life trajectory of many patients, since about 50% of adult patients have to suspend their professional activity (of which about 80% do not find a job), and about 25% of children interrupt their schooling. In addition, there is a certain stigma attached to the disease, since in a study in Ghana, 40% of the people interviewed would not accept a former patient as village chief [55]. Twenty percent of cured patients, one third of patients undergoing treatment and half of the controls surveyed considered BU to be a probable or proven barrier to marriage. However, this stigma is not absolute, as 90% of those interviewed would accept a patient as a teacher. The patients are not physically isolated or globally 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, given that the active form of Buruli ulcer only develops in certain individuals exposed to *M. ulcerans*[56] and that the risk of young adults developing the disease is much lower than for children, these observations suggest that the development of protective immunity against Buruli ulcer is possible. Nevertheless, attempts in this area have had limited success to date [56].

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