History and Geographic Distribution of Buruli Ulcer

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39.1 Introduction

Cutaneous ulcers caused by *Mycobacterium ulcerans* were discovered more than 80 years ago, at nearly the same time, in two antipodal regions: in 1937 in southeast Australia and in 1942 in tropical Africa. At the time, these discoveries did not generate much interest in the medical/scientific world [1]. However, anyone interested in how a medical curiosity is transformed into a topic of worldwide interest should study the initial report of MacCallum, Tolhurst, Buckle, and Sissons, published in 1948, on their observations and pioneer findings in *M. ulcerans* infections [2]. In spite of the increased interest in this new disease, it remained largely ignored for decades by many national public health programs.

It was only in 1998 that WHO launched the Global Buruli Ulcer Initiative (GBUI), following the visit of its Director-General, Dr. Hiroyoshi Nakajima, to Côte d''Ivoire. Nakajima was impressed by the debilitating tropical disease that destroys the skin of its victims: Buruli ulcer (BU). The first international conference on BU was organized by WHO in July 1998. The GBUI was established to coordinate BU control and multidisciplinary research efforts in partnership with member states, academic and research institutions, nongovernmental organizations, and other foundations.

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The history of BU may thus be divided into two periods: before 1998 and after 1998.

39.2 Historical Overview

39.2.1 What Was Done Before 1998

The etiologic agent, *M. ulcerans*, was discovered in Bairnsdale, Victoria State, in a temperate zone of southeastern Australia. Searle, Clay, and Alsop, general practitioners in the Bairnsdale area, and Torode in Colac recognized indolent ulcers with undermined edges in the late 1930s. Biopsy specimens of these ulcers contained acid-fast bacilli (AFB). Unfortunately, these perceptive observations were never published.

The first patient reported by MacCallum et al. was a 2½-year-old boy hospitalized on 29 June 1940 in a private clinic in Bairnsdale, with an ulcer on his leg. A biopsy from the margin of the ulcer teemed with AFB.

M. ulcerans infection existed in central Africa for many years before the first published report by MacCallum et al. These infections were probably often considered a form of "tropical phagedenic ulcer" (TPU). Sir Albert Cook (1897) was perhaps the first expatriate physician to record a description of chronic necrotizing ulcers with undermined edges he saw in Uganda. During the years 1923 to 1964, Ralph E. Kleinschmidt, a missionary physician in northeastern Congo, also observed undermined ulcers rich in AFB [3].

Of particular relevance to BU was the long experience in Africa of treating TPU because of its importance for economic activities such as mining and various kinds of plantations. Programs for fighting TPU clearly stimulated interest in other cutaneous ulcerative diseases, including BU. In 1942, Prof Pieter G Janssens was involved with the TPU problem at the Kilo-Moto medical service in the far northeastern corner of the Congo (Ituri Province). He observed chronic necrotizing ulcers containing AFB and affecting mostly children in the Kakerifu encampment situated between the Kibali and Nzoro rivers. Janssens noted that *M. ulcerans* infection, although having some similarities with TPU, was a disease apart. In 1957, he became Director of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium, and was the first to bring BU and its importance as a tropical disease in Africa to the attention of the ITM. Before he died, at the request of F. Portaels, Janssens agreed to document his rich experience in the discovery of BU in Congo [1].

Unlike Janssens in Africa, scientists in Australia had ready access to both sophisticated laboratory facilities and BU patients and, thus, were able to contribute significantly to the early understanding of the disease and culture of the etiologic agent [2].

The most significant contributions to the knowledge of BU in Africa came from Uganda and the Democratic Republic of the Congo (DRC). The disease was named "Buruli ulcer" after the geographic area of the first large epidemic investigated in Uganda in 1961, in a county named "Buruli" (now called "Nakasongola"), near Lake Kyoga [4].

The Uganda Ministry of Health and the Makerere Medical School instituted the Uganda Buruli Group (UBG), with a mandate to investigate the BU situation and to advise the authorities on strategies for managing this significant public health problem. The UBG described the clinical aspects of the disease; emphasized the importance of early treatment, preferably in the pre-ulcerative stage [5, 6]; and produced important details on the epidemiology of BU [7]. The UBG also observed that BU was strongly associated with slow-flowing and stagnant waters; however, the Group was unable to isolate *M. ulcerans* from the environment but cultivated many other mycobacterial species [8]. Similarly, our attempts to culture *M. ulcerans* from more than 1000 environmental specimens collected in DRC between 1970 and 1974 failed. Many other environmental mycobacterial strains were cultivated, some of them new to science [9].

From 1965 to 1973, in Lower Congo (DRC), Wayne M. Meyers was responsible for leprosy patients in Kimpese. During this period, he also treated many BU patients. Meyers was the first one to succeed in cultivating *M. ulcerans* in vitro from clinical specimens in a rural hospital and to successfully treat ulcerated BU lesions without surgery. The efficacy of oral rifampin in patients with early ulcerated lesions was demonstrated in 1971 and heat therapy in 1974 [10, 11]. Based on extensive clinical studies and detailed interviews of patients or their families, the role of trauma in transmission of *M. ulcerans* to humans was postulated [12].

In 1991, Dr. Augustin Guédénon, dermatologist and Director of the anti-leprosy control in Benin, contacted the Mycobacteriology Unit of the ITM to inform us of the increased importance of BU in his country. At that time, the real significance of the disease compared with tuberculosis and leprosy was not realized. With the motivation of Guédénon, and thanks to carefully archived materials of Sister Julia Aguiar and her extensive experience in diagnosing and treating BU, a descriptive study based on data from the records of 867 patients treated at the "Centre Sanitaire et Nutritionnel at Zagnanado" (Zou Department) was conducted. The patients came from four departments in southern Benin called at that time Atlantique, Mono, Ouémé, and Zou. The total number of BU patients detected exceeded those of leprosy and tuberculosis in some sub-prefectures [13].

It became clear that implementation of a Benin National Anti BU Program proved essential for education of populations and healthcare workers. In close collaboration with Benin, various aspects of BU, including its geographic distribution, incidence and prevalence, mode of transmission, pathogenesis and immunity, clinical manifestations, differential clinical diagnosis, laboratory diagnosis, and treatment, were studied [14]. For the first time, direct detection and identification of *M. ulcerans* in clinical specimens from Benin were performed using PCR [15].

The majority of microbiologically confirmed known BU foci in Africa were identified and described before 1998, in chronological order: Democratic Republic of Congo, Gabon, Uganda, Republic of Congo, Nigeria, Ghana, Cameroun, Côte d'Ivoire, Liberia, Sierra Leone, Benin, and Togo. Microbiologically confirmed cases were also described on other continents before 1998, in chronological order: Australia, Mexico, Malaysia, Papua New Guinea, Peru, French Guiana, and Japan [1].

Most of the data that we have available now on the clinical aspects and the epidemiology of BU were available before 1998, primarily because of investigations carried out in Uganda, DRC, West Africa, and Australia. Although the disease remained uncommon in Australia until the end of the twentieth century, John Hayman, a pathologist from Victoria, published several articles on clinical features, histopathology, and epidemiology of the disease [16–18].

Natural infections in mammals have been described for the first time in koalas from Australia. The lesions were clinically identical to those observed in humans [19].

Clinical trials published in 1969 and 1976 showed that the protective effect of BCG vaccination was short lasting and thus of limited value for BU control [5, 20]. A more effective BU vaccine has not become available so far. The first cases of BU patients with subclinical HIV co-infection have been described in 1992 [21]. Since then, accumulating evidence is indicating that HIV infection increases the risk of developing BU and that HIV co-infected BU patients tend to develop more severe and more frequently multifocal pathologies.

One of the most important advances in laboratory diagnosis of BU was the discovery of a repetitive DNA restriction fragment from *M. ulcerans* and the development of a specific and very sensitive PCR assay for the rapid diagnosis of *M. ulcerans* in clinical specimens [22] and the detection of *M. ulcerans* DNA in the environment for the first time [23]. This high-copy-number insertion sequence was designated IS*2404* [24].

39.2.2 Achievements Since 1998

Important findings and achievements were attained since the creation of the GBUI in 1998. The number of publications on BU has literally exploded. Of the approximately 1500 articles devoted to BU, 15% of them were published before 1998 (in 50 years) and 85% until the end of 2020 (in 22 years).

Of particular interest was the creation, beginning in 1998, of National Control Programs against BU, programs for education of populations and healthcare workers, resulting in better case finding, correct laboratory diagnosis, better treatment of the disease, and better understanding of its epidemiology and pathogenesis.

The majority of known BU foci in Africa and elsewhere were described before 1998. Since 1998, only six new countries have been added to the list of endemic countries where microbiologically confirmed cases were discovered, five in Africa (Burkina Faso, Central African Republic, Equatorial Guinea, Kenya, and South Sudan) and one in Asia, China where the first confirmed case of *M. ulcerans* subspecies *shinshuense* was described in 2000 [25].

Since the creation of the GBUI, significant progress has been made in the field of BU treatment and laboratory diagnosis. These advances are developed in Chaps. 45 and 41.

Significant progress has also been made in the field of scientific research, partly thanks to international collaborations stimulated by the GBUI.

In 1999, George et al. isolated a cytotoxic factor from *M. ulcerans*. The chemical structure of this toxin was deciphered. This polyketide-derived macrolide, required for the virulence of *M. ulcerans*, named mycolactone, destroys tissues by apoptosis and necrosis and suppresses host immune responses [26, 27]. In 2004, Stinear et al. demonstrated that *M. ulcerans* carries the giant plasmid pMUM001 that harbors genes encoding the polyketide synthases required for mycolactone synthesis [28]. The loss of this plasmid, after several in vitro subcultures, makes the strain non-pathogenic [29]. The possible role of plasmids in the virulence of *M. ulcerans* had already been pointed out in 1989 [30].

A real-time PCR assay for quantification of *M. ulcerans* DNA was developed in 2003 [31], and two multiplex real-time PCR assays for the detection of *M. ulcerans* in clinical and environmental samples were developed in 2007 [32].

The first complete 5.8-Mb genome sequence of a Ghanaian *M. ulcerans* isolate was published in 2007 and showed >98% nucleotide sequence identity with the genome of *M. marinum* [33]. However, in addition to the acquisition of the virulence plasmid, *M. ulcerans* has accumulated multi-copy insertion sequences, many pseudogenes, and multiple DNA deletions. The reductive evolution indicates that *M. ulcerans* has evolved from a generalist to a niche-adapted specialist. All mycolactone-producing mycobacteria represent a single clonal group, which has diverged into several ecovars [34]. Among *M. ulcerans* isolates from human lesions, two principal lineages have been identified: the classical lineage responsible for BU in Africa, Papua New Guinea, and Australia and the ancestral lineage isolated from patients from Asia, Mexico, and South America [35]. Comparative whole genome analyses have furthermore revealed that, in many African BU endemic regions, local clonal complexes of *M. ulcerans* have developed [36, 37]. The strong spatial segregation of these complexes is speaking against the existence of highly mobile reservoirs.

The possible role of insects in the epidemiology of BU was evoked for the first time in 1999 [38]. Aquatic insects (Hemiptera) were suspected to be vectors of BU in Africa [39], and mosquitoes were suspected to play a role in the transmission of BU in southeastern Australia [40]. Case-control studies in Africa and Australia have also suggested insects may play a role in transmission [41, 42]. The first cultivation of *M. ulcerans* from a water strider, an aquatic insect that does not bite humans, was also reported. Hemiptera should, however, be considered as passive reservoirs [43].

While the local incidence of BU caused in Africa and Australia by classical lineage strains is high, cases caused by the ancestral lineage in Asia, Mexico, and South America occur only sporadically, which may reflect differences in environmental reservoirs of the two ecovars. For short-term visitors to BU endemic areas in Victoria, Australia, the mean incubation period for BU was estimated to be 135 days, with 34 days recorded as the shortest and 264 days as the longest [44]. Sero-epidemiological studies are indicating that in BU endemic areas of Africa, only a small minority of individuals exposed to *M. ulcerans* are developing clinical BU disease and that exposure to *M. ulcerans* intensifies at an age of about 4 years [45].

The major role for mammals in the ecology of *M. ulcerans* in Australia was highlighted in 2010 [46], and two domestic animals were recently found infected by *M. ulcerans* in Benin suggesting that animals may also play a role in the ecology of *M. ulcerans* in Africa [47]. However, the environmental reservoir of *M. ulcerans* and its exact mode of transmission still remain unknown. Over 70 years ago, Tolhurst and Buckle wrote the following: "Whatever the reservoir of the organism, the method of transfer to man has still to be elucidated" [2]. This just proves that "there is nothing new under the sun!"

39.3 Geographic Distribution

Cases of BU have been reported in 34 countries [48]. Of these 34 countries, cases have been confirmed microbiologically in 27 countries.

There are laboratory-confirmed *M. ulcerans* infections in the following **tropical** countries:

- *Africa*: Angola, Benin, Burkina Faso, Cameroon, Central African Republic, Côte d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Ghana, Guinea, Kenya, Liberia, Nigeria, Republic of Congo, South Sudan, Togo, and Uganda
- *The Americas*: French Guiana, Mexico, Peru, and Suriname
- *Asia*: Malaysia
- *Oceania*: Papua New Guinea and northern Australia

The **nontropical** countries with confirmed BU are southern Australia, China, and Japan.

Cases in the remaining seven countries (Brazil, Indonesia, Kiribati, Malawi, Senegal, Sierra Leone and Sri Lanka) lack convincing microbiological confirmation, and the clinical features are not in favor of BU.

In recent years, the number of BU cases reported to WHO has decreased in several countries, especially in most of the highest prevalence African countries (Ghana, Côte d'Ivoire, and Benin) [49]. This decrease was also observed in French Guiana [50] and on the Bellarine Peninsula in Australia [51].

Reasons for decrease remain unknown, but several hypotheses have been proposed: environmental changes; improvement of living conditions (access to safe water); the increasing use of antibiotics for the treatment of BU, which may impact the human reservoir [52]; and the increasing confirmation of cases by PCR reducing the overdiagnosis of the disease that may have occurred previously [53]. It is however unlikely in countries such as French Guiana or Australia where dermatologists are BU experts since several decades [50]. With a decline in surveillance activities, underreporting may be an issue in some African BU endemic areas.

Some "so-called" epidemics of BU may be due to the lack of clinical experience in the differential diagnosis of BU and the lack of laboratory confirmation of the

cases. For example, Guinea reported to WHO, between 2002 and 2017, a total of 1480 cases, but none of them were laboratory confirmed. During the same period, South Sudan reported 1014 cases to WHO, but only a few cases were confirmed by laboratory tests [54]. In Uganda, the disease was believed to have disappeared in 1976 [20]. However, a survey carried out in 2003 revealed 117 suspected cases in the Nakasongola district (formerly Buruli district), but none of them were confirmed by laboratory tests [55].

Conversely, the number of cases has increased on the Mornington Peninsula in Australia [51], and an increasing number of cases has been reported to WHO in Nigeria [55]. Reasons for increase in Nigeria may be partly related to the increasing awareness of BU and better detection of the disease.

39.4 "To Be or Not to Be" a Buruli Ulcer Case, "That Is the Question"!

Any clinical feature of BU can be mistaken for another skin condition, particularly in areas where other skin diseases are frequent (see Chap. 43). Studies on the differential diagnosis of BU seem to indicate that its clinical diagnosis may sometimes be more difficult than usually recognized even in experienced hands.

Thus, microbiological confirmation remains essential to confirm (or to invalidate) BU. It is generally based on only one test, IS*2404*-PCR, which is the most sensitive test among the presently available laboratory tests (see Chap. 41). However, false-positive or false-negative PCR results may be due to laboratory errors. In view of this, an External Quality Assessment Program (EQAP) of PCR has been established by WHO. The third EQAP round has revealed that 20% of the participating laboratories had false-positive results, probably due to DNA contaminations [56].

Consequently, laboratory errors or the absence of microbiological confirmation may be responsible for inadequate treatments of patients, non-reliable epidemiological data, and the description of "new" (unlikely) BU foci despite clinical features not being in favor of BU!

It is essential to ensure that all laboratory tests be accurate and reliable. Internal quality control and external quality assessment systems are detailed in Chap. 41.

To avoid a misdiagnosis caused by false-positive or false-negative results, it is recommended that two different tests have positive results before a definitive diagnosis is made. The development of rapid point-of care diagnostic tests would also be a precious tool for the differential diagnosis of BU (see Chap. 41).

Overdiagnosis or underdiagnosis of BU? "*Errare humanum est, perseverare diabolicum*" ("*To err is human but to persist in error is diabolical*") says an old Latin proverb. Good laboratory practices, self-criticism, and collaboration with a multidisciplinary team should allow us to limit bias due to human errors and get a more reliable picture of the actual geographical distribution and real burden of BU worldwide.

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