Buruli Ulcer: History and Disease Burden

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1 History of a Mysterious Skin Disease

The first description of chronic skin ulcers consistent with the pathology of *Mycobacterium ulcerans* infection dates back to the end of the nineteenth century, when the British physician Albert Cook recorded his observations in *The Mengo Hospital Notes*, maintained in the library of the hospital in Kampala, Uganda [1]. In 1948, characteristics of similar skin ulcers were described by MacCallum and his colleagues in six patients from the Bairnsdale District in southeastern Australia [2], where the disease is therefore often referred to as Bairnsdale ulcer. The causative organism isolated from these ulcers was found to be an acid-fast mycobacterium, later named *M. ulcerans*. Noteworthy, the first isolation of the extremely slow-growing mycobacterium in culture was achieved by accidental incubation of culture plates in a faulty incubator [3], reflecting the low optimal growth temperature of the pathogen at 30–33 °C. This temperature preference is considered a major factor for the skin tropism and limited systemic dissemination of *M. ulcerans* infections. In the 1940s and 1950s a larger case series of 170 patients with necrotic skin ulcers, caused by an acid-fast mycobacterium, was recorded in the Democratic Republic of the Congo [4]. A high prevalence of *M. ulcerans* infections was noticed in the 1950s and 1960s in a geographically very limited area of the then sparsely populated Buruli County close to the Nile River in Uganda and as a consequence, the disease became more generally known as Buruli ulcer (BU) [5, 6]. In proximity to this initial infection focus, a second outbreak of the disease in Uganda was reported in Rwandans living in a refugee settlement that was opened in 1964 close to the Nile

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River. Intensive investigation of the 220 BU cases that occurred until 1969, when the community moved to a new locality, where case numbers declined, has provided much of the basic knowledge about the epidemiology of BU that is still valid today. Amongst other insights it became apparent that BU may affect individuals irrespective of sex and age, although the highest incidence was seen in children aged between 5 and 15 years. Furthermore, in contrast to a very low probability of person to person transmission, the involvement of an environmental reservoir in the transmission became obvious [7]. While until then clinical attention had been concentrated on ulcerative lesions, awareness of the disease in highly endemic regions has brought attention to pre-ulcerative forms of the infection including nodules, plaques and edema [8].

Until the end of the twentieth century, BU case series were reported mainly from West and Central African countries including the Congo [9], Nigeria [10], Gabon [11], Ghana [12, 13], Benin [14], and Côte d'Ivoire [15, 16] as well as from Australia [17, 18] and Papua New Guinea (PNG) [19]. Moreover, sporadic *M. ulcerans* infections occurred in a number of additional countries with tropical, subtropical and temperate climates [20], totalling up to 34 countries from which BU has hitherto been reported. However, limited awareness of the disease and the fact that it often affects poor populations in remote, rural areas has hampered the detection of new infection foci and thus the control of BU. In 1998, WHO launched the Global BU Initiative, a partnership of Member States, academic and research institutions, donors, nongovernmental organizations, WHO, and others with the intention to raise awareness and to coordinate global BU control and multidisciplinary research efforts. In addition, this Initiative was meant to strengthen BU surveillance systems and to assess the disease burden at local, national and global levels. More than 58,000 BU cases had been reported between 2002 and 2016 from 20 countries in Africa (Benin, Cameroon, Central African Republic, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Gabon, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, South Sudan, Uganda and Togo), the Americas (French Guiana), Asia (Japan), and the Western Pacific (Australia and PNG) (Table 1). Since 2008, a steady decline in the number of reported BU patients has been noticed (Fig. 1).

While this may in part be due to the establishment of effective national BU control programs, underreporting is considered likely, as there is lack of data from an increasing number of countries that have reported BU cases in the past. Moreover, surveillance activities in some of the highly endemic countries may have declined due to the decreased availability of specific funding. On the contrary, a steady increase in the number of BU patients has been reported from the BU endemic region of Victoria in southern Australia, with a peak incidence of 186 new BU infections in 2016. The geographic distribution of BU recorded by WHO in 2016 is illustrated in Fig. 2.

Table 1 Number of reported BU cases worldwide between 2002 and 2016. Data source: WHO

Country	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	Total
Côte d'Ivoire	750	768	1153	1564	1872	2191	2242	2679	2533	1659	1386	1039	827	549	376	21,588
Ghana	853	737	1157	1005	1096	668	986	853	1048	971	632	550	443	275	371	11,645
Benin	565	722	925	1045	1195	1203	897	674	572	492	365	378	330	311	312	9986
Cameroon	132	223	914	265	271	230	312	323	287	256	160	133	126	133	85	3850
DRC[a]	17	119	487	51	74	340	260	172	136	209	284	214	192	234	175	2964
Togo	96	38	800	317	40	141	95	52	67	52	51	37	67	81	83	2017
Congo	102	180	235	53	370	99	126	147	107	52	38	6	Nd[b]	Nd	Nd	1515
Guinea	Nd	157	146	208	279	Nd	80	61	24	59	82	96	46	72	72	1382
Australia	32	14	34	47	72	61	40	35	42	143	101	74	89	111	186	1081
South Sudan	568	360	4	24	38	8	3	5	4	Nd	Nd	Nd	Nd	Nd	Nd	1014
Gabon	Nd	Nd	43	91	54	32	53	41	65	59	45	59	47	43	39	671
Nigeria	Nd	Nd	Nd	Nd	9	Nd	Nd	24	7	4	40	23	65	114	235	521
Uganda	117	10	7	72	5	31	24	3	Nd	Nd	Nd	Nd	Nd	Nd	Nd	269
PNG	13	18	31	Nd	Nd	26	24	8	5	8	Nd	Nd	3	11	16	163
Liberia	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	21	8	Nd	105	Nd	134
French Guiana	24	7	17	2	2	2	8	2	7	3	2	3	1	Nd	Nd	80
Japan	Nd	Nd	1	1	1	3	2	5	9	10	4	10	7	4	2	59
Sierra Leone	Nd	Nd	Nd	Nd	Nd	Nd	1	Nd	Nd	28	Nd	Nd	Nd	Nd	Nd	29
CAR[c]	Nd	Nd	Nd	Nd	Nd	Nd	3	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	3
Equ. Guinea	Nd	Nd	Nd	3	Nd	Nd	Nd	Nd	Nd	0	Nd	Nd	Nd	Nd	Nd	3
Total	3269	3353	5954	4748	5378	5035	5156	5084	4913	4005	3211	2630	2243	2043	1952	58,974

[a]Democratic Republic of the Congo
[b]No data
[c]Central African Republic

Fig. 1 Number of BU cases reported worldwide between 2002 and 2016. Graph illustrating the downward trend of new *M. ulcerans* infections reported in the past years. Data source: WHO
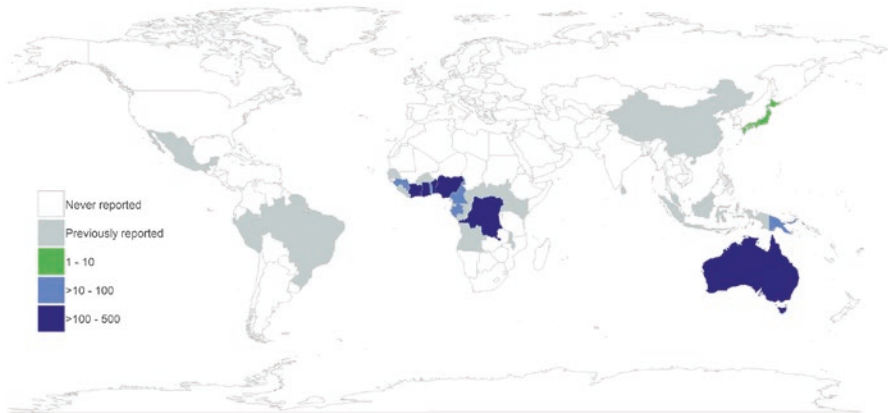
Fig. 2 Worldwide distribution of BU cases in 2016. Global map illustrating countries that have reported cases in 2016 (blue (classical lineage) and green (ancestral lineage)) and in previous years (grey). The map was kindly created by Dr. K. Ampah using 'R'. Data source: WHO

2 Evolution, Niche Adaptation, and Transmission of Distinct *M. ulcerans* Lineages

The emergence of *M. ulcerans,* which has evolved from the fish and opportunistic human pathogen *M. marinum* [21] approximately a million years ago [22], was driven by horizontal transfer of genetic material [21]. Most notably, this included a virulence plasmid, carrying genes for the synthesis of macrolide toxins, whose cytotoxic and immunosuppressive properties account for much of the typical progressive skin necrosis and chronicity of *M. ulcerans* infections. Several structural variants of these mycolactones have hitherto been described [23–25]; for more information on the toxin of *M. ulcerans* the reader is referred to chapter

"Mycolactone: More than Just a Cytotoxin" of this book. Moreover, acquisition of high-copy number insertion sequences (IS*2404* and IS*2606*) and their expansion in the *M. ulcerans* genome has resulted in extensive gene loss, pseudogene formation, and modification of gene function, coining the likely adaptation to new, restricted, ecological niches [26]. For more detailed information on the population genomics and molecular epidemiology of *M. ulcerans* the reader is referred to chapter "Population Genomics and Molecular Epidemiology of *Mycobacterium ulcerans*" of this book. During the process of its genome reduction, *M. ulcerans* has diverged into at least two principal lineages [27], the ancestral and the classical lineage, which presumably have been adapting to distinct niche environments within the aquatic ecosystem [26]. Both lineages can cause severe tissue destruction if lesions are left untreated (Fig. 3).

Until today, the exact source of the infection is not clear, in part because cultivation of *M. ulcerans* from potential environmental sources is hindered by the extremely slow growth rate of the pathogen. The marked loss of gene function as compared to the genome of *M. marinum*, including genes required for pigment biosynthesis and anaerobiosis [26], indicates that *M. ulcerans* may exist as a commensal associated with a protective organism in the ecosystem [28–30]. Despite extensive research, the mode(s) of transmission from environmental reservoirs to mammalian hosts is unclear. It is thus likely that *M. ulcerans* may be transmitted by several distinct mechanisms, each enabling the entrance of a sufficient pathogen load into the susceptible layers of subcutaneous tissue.

2.1   Ancestral *M. ulcerans* Lineage

Strains of the ancestral lineage, which are closely related to *M. marinum*, mainly cause disease in ectotherms such as fish and frogs [25, 31–33], but certain subgroups sporadically infect humans. Due to their distinct host range, some mycolactone-producing ancestral strains were initially given different species designations such as *M. marinum* for globally detected fish pathogens [25], *M. pseudoshottsii* for mycobacteria isolated from diseased Chesapeake Bay striped bass [32], or *M. liflandii* for strains isolated during a mycobacteriosis outbreak in a laboratory colony of *Xenopus tropicalis* [31]. Furthermore, ancestral strains isolated from the lesions of BU patients from Japan are often referred to as *M. ulcerans* subspecies *shinshuense* [34]. Indeed, comparative genome analysis has shown that based on the sequences of fish and frog isolates on the one hand and human disease isolates from Japan on the other hand the ancestral lineage may be subdivided into two major sub-lineages [26]. The common feature of all of these isolated pathogens is the production of mycolactone and thus it was no surprise when comparative genome analysis of ancestral and classical *M. ulcerans* strains has revealed that all mycolactone-producing mycobacteria have evolved from a common *M. marinum*-like progenitor and should therefore be considered as ecovars of a single *M. ulcerans* species [26, 35]. Ecovars of the ancestral lineage appear to

Fig. 3 Ulcerative BU lesions in patients from West Africa (upper panel) and Japan (lower panel). Photographs from Japanese patients were kindly provided by Dr. M. Ohtsuka, Fukushima Medical University

be adapting to an ecological niche environment, from which they infect humans only occasionally. Environmental studies in regions where the ancestral lineage of *M. ulcerans* prevails are still in their infancy. However, in French Guiana, *M. ulcerans*-specific DNA sequences (IS*2404* and the ketoreductase-B domain of the *M. ulcerans* mycolactone polyketide synthase genes (KR)) were found in different freshwater bodies [36]. In Japan, an outbreak of BU among several family members was linked to a stagnant water channel in the backyard of the family's house, in which a sample of a crayfish contained an IS*2404* sequence identical to that of *M. ulcerans* [37].

Sporadic human infections caused by ancestral strains were reported from the Americas (French Guiana [36], Suriname [38], Peru [39], Brazil [40] and Mexico [41]) and from Asia (Japan [42] and China [43]). Between 2002 and 2016, French Guiana and Japan reported to WHO 80 and 59 BU cases, respectively. No official records are available for the other South-American and Asian countries. For more detailed information on BU in French Guiana and Japan the reader is referred to chapters "*Mycobacterium ulcerans* Infection in French Guiana; Current State of Knowledge" and "Buruli Ulcer in Japan" of this book.

2.2   Classical *M. ulcerans* Lineage

Strains of the classical lineage are responsible for the vast majority of human *M. ulcerans* infections, reported mainly from Africa [20], Australia [17, 18, 44], and PNG [19, 45, 46]. Considering the often very high local prevalence of BU in African and Australian endemic regions, the classical lineage may be present in an environmental reservoir that—compared to ecovars of the ancestral lineage—is associated with a higher risk for humans to contract an infection. Some environmental investigations in African BU endemic areas have shown that *M. ulcerans*-specific DNA sequences can be detected in many biotic components of aquatic ecosystems, such as plants, snails, fish, or insects pointing towards the ubiquitous presence of the pathogen in these ecosystems [47]. For reasons that are currently not clear, the detection rate was much smaller in other environmental studies in African BU endemic areas [48, 49]. Studies in southeastern Australia have revealed that possums are highly susceptible to *M. ulcerans* infection. Sporadic infections have also been found in other native wildlife and domestic mammals [50, 51]. For more detailed information on BU in animals the reader is referred to chapter "Buruli Ulcer in Animals and Experimental Infection Models" of this book. Infected possums were shown to harbour high loads of *M. ulcerans* DNA in their gastrointestinal tracts. However, *M. ulcerans* cultivation from faeces failed, while being successful from skin lesions of infected possums [50, 51]. Genome comparison of classical lineage *M. ulcerans* isolates from possums and humans revealed an extremely close

genetic relationship, supporting a role for these animals in the ecology of infection as a reservoir of the pathogen [26]. In Far North Queensland (FNQ), another BU endemic area of Australia, *M. ulcerans* DNA was detected in excreta of bandicoots [52]. These findings suggest that certain Australian marsupials may be highly susceptible to *M. ulcerans* infection. In contrast, until today, surveys of small mammals in African BU endemic areas have not led to the detection of an *M. ulcerans* animal reservoir [48, 53]. It has been suspected that in highly endemic areas of Africa, large chronic lesions of BU patients may contribute significantly to the dissemination of *M. ulcerans* in the environment.

Between 2002 and 2016, a total of 57,591 BU cases were reported from 16 African countries, 1081 from Australia and 163 from PNG. No official records are available for other countries, in which the presence of classical *M. ulcerans* infections was suspected, such as Kiribati [54] or Malaysia [55]. For more detailed information on BU in Africa and Australia the reader is referred to chapters "Buruli Ulcer in Africa" and "Buruli Ulcer in Australia" of this book.

3   Characteristics of BU Disease in Humans

In the following paragraphs, relevant data for BU disease in different BU endemic areas are compiled to enable a comparison of epidemiological and clinical features as well as environmental characteristics. For that purpose, PubMed was searched using the query "Buruli ulcer" OR "*Mycobacterium ulcerans*". All titles (N = ∼1180) and available abstracts were screened for relevant content. Studies including more than 30 BU cases were used to extract information on demographic and geographic features of BU in these areas. In view of the multitude of publications on African BU endemic settings, representative studies conducted in eight different countries were selected. We included at least one and not more than three studies from the seven countries that have reported the vast majority of BU cases in the past decade, namely Côte d'Ivoire [56], Ghana [57–59], Benin [60–62, 76], Cameroon [63, 64], Democratic Republic of the Congo [65], Togo [66] and Republic of the Congo [67] as well as a case series from Nigeria [68], one of the few countries, which has recorded an increase in the number of reported cases over the past years. Studies were selected based on a preference for (1) quantity of BU cases (large case series), (2) quality (preferentially studies including laboratory-confirmed patients), (3) content (data on many epidemiological aspects), and (4) "up-to-datedness" (recent publications, if available and complying with other preferences). Furthermore, a very detailed study of 220 BU cases that were identified within a community of Rwandan refugees between 1964 and 1969 near the river Nile in Central Uganda was included [7] (Tables 2 and 3). BU endemic regions outside of Africa included in this comparative analysis are French Guiana (chapter "*Mycobacterium ulcerans* Infection in French Guiana; Current State of Knowledge" of this book) [69], Japan [42] (chapter "Buruli Ulcer in Japan" of this book), PNG [19, 46], FNQ, Australia [44, 70] and Victoria, Australia [71, 72] (chapter "Buruli Ulcer in Australia" of this book).

Table 2 Demographic and clinical characteristics of BU patients

Continent (lineage)	Country/site	Time span (references)	Number of BU cases (included in this table)	Age in years	Gender distribution (by age)	Overall lesion site (characteristics by gender/age)	WHO category/type of lesions
Americas (Ancestral)	French Guiana	1969–2013 [69] Chapter "*Mycobacterium ulcerans* Infection in French Guiana; Current State of Knowledge"	Total: 245, 166 confirmed[a] (all)	Median (overall): 25, Median (1969–1983): 9, Median (1984–1998): 25, Median (1999–2013): 36	51% male, 49% female	Lower limbs: 66%, upper limbs: 24%, chest: 5%, face: 1%, Nd: 4% (chest + upper limb lesions more common/lower limb lesions less common in men than in women)	Ulcers: 83%, nodules: 8%, plaques: 7%, Nd: 2%; no osteomyelitis; multiple lesions: 8%
Asia (Ancestral)	Japan	1982–2011 [42]	Total: 32, at least 23 confirmed (all)	Tendency towards middle-aged adults	37.5% male, 62.5% female	Mainly extremities	Mainly category I lesions
		1982–2016 Chapter "Buruli Ulcer in Japan"	Total: 60, at least 41 confirmed (all)	Nd[b]	Nd	Mainly upper and lower limbs followed by head	Mainly category I lesions; multiple (small) lesions: 23%

(continued)

Table 2 (continued)

Continent (lineage)	Country/site	Time span (references)	Number of BU cases (included in this table)	Age in years	Gender distribution (by age)	Overall lesion site (characteristics by gender/age)	WHO category/type of lesions
Australia/ Oceania (Classical)	FNQ	1964–2008 [70]	92 confirmed (all)	Median: 42	58% male, 42% female	Extremities: 97% → 82% of those lower limbs	Ulcers: 90%, nodules: 5%, edema: 3%, mixed: 1%
		2009–2015 [44]	95 confirmed (all)	Median: 45	57% male, 43% female	Extremities: 94% → 73% of those lower limbs	Category I: 93%, category II: 3%, category III: 4%; multiple lesions: 1%
	Victoria	1998–2011 [71]	180 confirmed (all)	Median: 61	49% male, 51% female	Lower limbs: 61%, upper limbs: 34%, over joints: 40% (upper limb lesions more common/lower limb lesions less common in men than in women)	Ulcers: 87%, nodules: 8%, edema: 5%, osteomyelitis: 1%; multiple lesions: 5%
		1998–2015 [72]	579 confirmed (all)	Median: 55	54.5% male, 45.5% female	Lower limbs: 70%, upper limbs: 27%, trunk: 2%, head/neck: 1%, over joints: 36% (upper limb lesions more common/lower limb lesions less common in men than in women)	Multiple lesions: 5.5%
	PNG	1962–1973 [46]	112 suspected[c] (all)	Peak incidence in young children; Average: 9	63% male, 37% female	Lower limbs: 69%, upper limbs: 11%, trunk: 18%, head/neck: 2%; Majority over joints	Mainly ulcers; extension below deep fascia: 20%; multiple lesions: 4%
		1979–1983 [19]	46 confirmed (all)	Majority in children <10; Mean: 11	48% male, 52% female	Mainly extremities, occasionally face and trunk	Nd

Africa (Classical)	Benin	1997–2001 [60, 76]	Total: 1700, 906 confirmed (all)	Median: 15; Highest incidence in young teenagers and individuals >50	Overall balanced	Lower limbs: 60%, upper limbs: 24%, trunk: 13%, head/neck: 3% (Lower limb lesions more common in men than women) (Trunk/head/neck and upper limb lesions more common in children)	Multiple lesions: 9%; bone involvement: 13%
		2005–2011 CDTUB[d], Pobè [61]	1227 confirmed (all)	Median: 12	48% male, 52% female; patients ≤15: 57% male, >15: 33% male	Lower limbs: 60%, upper limbs: ~30%, trunk and other sites: ~10%	Category I: 22%, category II: 42%, category III: 36%; osteomyelitis: 7%; multiple lesions: 4%
		2005–2013 CDTUB, Allada [62]	476 confirmed (all)	Median: 12	51.5% male; 48.5% female; patients ≤15: 60% male, >15: 34% male	Lower limbs: 54%, upper limbs: 36%, head/neck/trunk: 10% (upper body lesions more common in younger ages)	Category I + II: 66%, category III: 34%; osteomyelitis: 1%; multiple lesions: 5%
	Cameroon	Survey in 2001 [63]	Total: 202 active, 135 confirmed (all)	Median: 14.5	57% male, 43% female	Lower limbs: 62%, upper limbs: 31%, head/neck/trunk: 7%	Ulcers: 93%, nodules: 2%, plaques: 3%, edema: 2%; bone involvement: 15%
		2010–2012 [64]	Total: 157, 88 confirmed (only confirmed)	Median: 12.5; Children <5 underrepresented; highest incidence in young teenagers and individuals >50	59% male, 41% female	Lower limbs: 56%, upper limbs: 31%, trunk: 11%, head/neck: 2%; proximity to joints: 52% (clusters around ankles and elbows) (lesions on trunk more common in men than in women)	Lesion category depending on health district; multiple lesions: 2%

(continued)

Table 2 (continued)

Continent (lineage)	Country/site	Time span (references)	Number of BU cases (included in this table)	Age in years	Gender distribution (by age)	Overall lesion site (characteristics by gender/age)	WHO category/type of lesions
	Congo	2007–2012 [67]	108 confirmed (all)	Patients <15: 56%	44% male, 56% female	Nd	Ulcers: 86%
	Côte d'Ivoire	2005–2010 [56]	1145 confirmed (all)	Patients <15: 53%, 15–49: 39%, ≥50: 8%	51% male, 49% female; patients <15: 55% male, 15–49: 46% male, ≥50: 51% male	Lower limbs: 67%, upper limbs: 26%, other sites: 7%	Category I: 29%, category II: 48%, category III: 23%; 45% ulcers, 17% nodules, 23% plaques, and 8% edema; bone involvement: 0.5%
	DRC	Survey in 2008 [65]	72 active, confirmed (all)	Patients ≤15: 38%, 16–49: 49%, >49: 14%	39% male, 61% female	Lower limbs: 53%, upper limbs: 40%, other sites: 7%	Category I: 50%, category II: 33%, category III: 17%; 76% ulcers, 18% non-ulcerated, 6% mixed
			187 active, suspected (all)	Patients ≤15: 33%, 16–49: 45%, >49: 23%	54% male, 46% female	Lower limbs: 73%, upper limbs: 17%, other sites: 10%	Category I: 48%, category II: 31%, category III: 21%; 66% ulcers, 29% non-ulcerated, 5% mixed

Ghana	1996–2002 [57]	750 suspected (all)	Median: 12	45.5% male, 54.5% female; patients <16: 48.5% male, ≥16: 40% male	Upper limbs: 46%, lower limbs: 46%, trunk: 7%, head/neck: 5%, over joints: 55% (Trunk/head/neck lesions less common, extremities more common with increasing age)	Ulcers: 63%, nodules: 27%, plaques: 6%, edema: 4%; multiple lesions: 6%
	2001–2004 [58]	Total: 99, 94 confirmed (all)	Median: 11	42% male, 57% female	Lower limbs: 51%, upper limbs: 41%, trunk: 8%	Pre-ulcerative: 48%, early ulcers: 33%, chronic ulcers: 19%
	2008–2016 [59]	Total: 2203, 1020 confirmed (only confirmed)	Median: 20; Patients ≤15: 40%;	49% male, 51% female	Lower limbs: 60%, upper limbs: 16%	Category I: 27%, category II: 15%, category III: 35%, unknown: 23%; 73% ulcers, 9% nodules, 3% plaques, 2% edema, 12% mixed; osteomyelitis: 1%
Nigeria	2004–2013 Treated in Benin [68]	127 confirmed (all)	Median: 10 (males), Median: 24 (females)	48% male, 52% female	Lower limbs: 57%, upper limbs: 28%, other sites: 5%, disseminated: 10%	Category I: 8%, category II: 34%, category III: 58%; osteomyelitis: 12% overall
Togo	2007–2010 [66]	109 confirmed (all)	Median: 12	52% male, 48% female	Limbs and shoulders: 91%	Category I: 39%, category II: 38%, category III: 23%

(continued)

Table 2 (continued)

Continent (lineage)	Country/site	Time span (references)	Number of BU cases (included in this table)	Age in years	Gender distribution (by age)	Overall lesion site (characteristics by gender/age)	WHO category/type of lesions
	Uganda	1965–1969 [7]	220 suspected, partially confirmed (all)	Maximum incidence in children: 5–14	Patients <15: 59% male, 41% female, ≥15: 31% male, 69% female	(Children <5: any part of the body, 5–14 head and trunk less affected, limb lesions more frequent, ≥15: clear difference between sexes: lesions increasingly confined to lower limbs in men, in women arms and legs continue to be commonly affected)	1965 to mid-1967: majority presented with extensive, crippling ulcers, From mid-1967: people had become aware of pre-ulcerative stages, majority attended clinic at earliest sign of a lesion; multiple lesions: 5%

[a]Confirmed case: presence of a lesion clinically consistent with BU plus a positive result for any one of the available laboratory diagnostic tests (PCR, ZN, culture or histopathology)

[b]Nd = not defined

[c]Suspected case: presence of a lesion clinically consistent with BU. Inclusion of data in the table for either all suspected cases of BU or only for laboratory reconfirmed BU cases was dependent on the availability of data from the cited studies with a preference for data on only the reconfirmed BU cases

[d]CDTUB: Centre de Dépistage et de Traitement de l'Ulcère de Buruli

3.1 Demographic and Clinical Characteristics of *M. ulcerans* Infection

3.1.1 Age Distribution of BU Patients

BU has long been considered as a disease that mainly affects children under the age of 15 years living in rural areas of Africa. A comparison of the age distribution reported for various BU case series (Table 2) shows that this assumption still holds true for African BU endemic areas and for endemic sites in PNG. However, this generalized view lapsed when larger case series were reported from Victoria in southeastern Australia, where a high average age of BU patients was observed (Table 2). The peak incidence of BU among children in Africa and the elderly in Victoria may be partly related to the average age of the respective general populations in the affected areas. While the population in BU endemic areas in Africa is very young [64, 73, 74], the affected communities in Victoria are popular seaside holiday resorts, where many retired people have their homes. But even if taking the skewed age distribution of the study populations into account, the elderly were most affected in Australia [75] and a bi-modal distribution of the age-related risk of developing BU was observed in Africa with young teenagers and the elderly being overrepresented among cases [64, 74, 76]. Increasing risk of developing BU with age may be due to the gradual deterioration of the immune system in the elderly. Interestingly, a marked underrepresentation of BU patients among children below 4 years of age was found [64]. This observation was in line with subsequent sero-epidemiological studies in Ghana and Cameroon, indicating that young children are considerably less exposed to *M. ulcerans* than older children [77, 78].

In Japan, where BU disease is caused by strains of the ancestral *M. ulcerans* lineage, a tendency towards middle-aged adults was found [42]. In French Guiana, a marked transition of the most affected age groups was observed: over a period of 45 years the median age of patients increased significantly from 9 years of age in the study period between 1969 and 1983 to 36 years of age between 1999 and 2013 [69]. Taken together the age-related risk of developing BU appears to be determined both by differential exposure to *M. ulcerans* and by higher susceptibility of certain age groups.

3.1.2 Gender Distribution of BU Patients

A comparison of various BU case series (Table 2) reveals an overall balanced male:female ratio among patients, although in some studies the proportion of one of the sexes was higher than that of the other. While more female than male patients were recorded in Japan, more men than women tended to be affected in FNQ, Australia. Several studies conducted with large numbers of African BU cases revealed significant differences in the male:female ratio, when the study population was stratified by age. All of these studies have consistently reported that in children below the age of 15 years, *M. ulcerans* infection is more common in boys than in girls and conversely that in individuals above the age of 15 years the infection is more frequent in females than in males [7, 56, 61, 62] (Table 2). It appears likely

that observed differences are due to different environmental contact patterns associated with the movement radius of children or different occupational exposure to environmental reservoirs.

3.1.3 Distribution of BU Lesions on the Human Body

While the mode of transmission of *M. ulcerans* remains unclear, it is commonly assumed that infection takes places via inoculation of the bacteria into the skin through direct trauma or bites of insects such as water bugs or mosquitoes [47, 51]. The site of inoculation is thought to be also the site of infection, not least because about 95% of BU patients present with a single lesion. A disseminated disease progression may only be found in those 5% of the patients presenting with multiple lesions (Table 2). Therefore, the location of BU lesions on the body of patients has been extensively studied, with the hope of detecting specific distribution patterns, such as a clustering of lesions at preferred feeding sites of biting arthropods vs. common sites for mechanical skin injuries, favouring any of the discussed mechanisms of infection. BU lesions, irrespective of whether they are caused by strains of the ancestral or the classical *M. ulcerans* lineage, occur mostly on parts of the body, which are not commonly protected by clothing (Table 2). The vast majority of studies found that lesions occurred in 90% or more of the cases on the limbs. Around two-thirds of the lesions occurred on the lower limbs, followed by the upper limbs, and less frequently the trunk, and sites on the neck/head (Table 2). While overall patterns observed in different regions, such as for example in Cameroon and Victoria, Australia appear to be surprisingly similar [64, 72], several studies have reported that the anatomical site of the lesions may vary with both gender and age. Interestingly, in studies conducted in French Guiana and Victoria upper limb lesions were more common in men than in women, who were more frequently affected on the lower limbs than were men [69, 71, 72]. Conversely, in African BU endemic areas lower limb lesions were more common and upper limb lesions were less common in men than in women [7, 76]. The most likely explanation for this differential pattern of lesion distribution is that women in Africa more commonly protect their lower limbs with clothing as compared to women in South America or Australia. Along the same line, men tend to be more often affected on the chest than women [64, 69]. Similarly, differential exposure to *M. ulcerans* may serve as an explanation for differences between age groups. It was reported that in children below 5 years of age any part of the body may be affected, while with increasing age the head and the trunk seem to be less commonly affected and limb lesions become more dominant [7, 57, 62, 76]. Failure to wear protective clothing has been identified as a risk factor for BU in several case-control studies [79–81]. In summary, the distribution of BU lesions appears to be strongly correlated with exposure of body parts to *M. ulcerans*, which may in turn be related to insect bites, skin injuries, or both.

3.1.4 WHO Categories and Clinical Manifestations of BU

M. ulcerans infections have been classified by WHO into three categories: category I is defined as a single, small lesion <5 cm in diameter, category II includes single lesions between 5 and 15 cm in diameter, plaque and edematous forms, and

category III is comprised of single extensive lesions >15 cm in diameter, multiple lesions, lesions at critical sites (e.g. eye, genitalia, joints), and osteomyelitis [82]. There are marked differences in the proportion of BU patients presenting at health facilities with lesions of one of these categories for different geographical regions (Table 2). In Japan and Australia, where healthcare is readily available, the vast majority of BU patients present with category I lesions, for which standard national antibiotic regimens are highly effective. On the contrary, a significant proportion of BU patients in Africa and in particular those detected by active case search, present with more advanced stages of the disease, for which time required for healing is prolonged and healing is often accompanied by permanent disability. The percentage of patients with category II and III lesions is strongly dependent on the degree of remoteness of a population and consequently access to healthcare as well as with awareness of the disease among populations at risk. A high degree of awareness among the Rwandan population in the Kinyara refugee settlement as well as the establishment of a BU treatment center, has for example led to reporting at early stages of the disease [7]. On the contrary, due to a lack of adequate health infrastructure for the diagnosis and treatment of BU, a large cohort of patients from southwestern Nigeria presented to a health center in Benin with 58% category III and only 8% category I lesions [68]. Osteomyelitis is mainly observed in African populations [61, 63, 68] and only occasionally in Australia [71]. The fact that ulcers are the most common form of BU lesions in all of the affected geographical areas is related to the relatively unspecific, nodular onset and the often reported painlessness [84] of *M. ulcerans* disease.

3.2   Geographical Features of *M. ulcerans* Infection Foci

3.2.1   Environmental and Climatic Characteristics of BU Endemic Areas

Environmental and climatic factors are highly diverse in different *M. ulcerans* infection foci, not only if areas are compared where either the ancestral or the classical lineage prevails, but also among areas affected by the same lineage (Table 3).

French Guiana is located at the Atlantic Ocean in northern South America between Suriname and northern Brazil. Most of the inhabitants reside along a 50 km wide coastline, which is mainly composed of marshy savannah and mangroves, whereas the rest of the country consists of dense, barely accessible rainforest. Located between latitudes 2° and 6° N and rising only to modest elevations, the climate in French Guiana is tropical (hot and humid) all year round. While rainfall may be heavy from January to June/July, the period between August and December is considered the main dry season [85]. The highest mean incidence of BU was detected in the western coastal area around the towns of Sinnamary and Mana adjacent to the Sinnamary and La Mana rivers, respectively [69]. On the contrary, Japan extends through a wide latitude from 30° to 45° N, with climate conditions ranging from humid subtropical to humid continental. BU cases are sporadically distributed throughout the large, mountainous Honshu Island, which separates the Sea of Japan

Table 3 Geographical characteristics of BU endemic areas

Continent (lineage)	Country/site	Refs.	Location/environment	Climate	Seasons	Seasonality of infections	Linkage to environmental factors
Americas (Ancestral)	French Guiana	[69] Chapter "*Mycobacterium ulcerans* Infection in French Guiana; Current State of Knowledge"	Coastal, surrounded by marshy savannah and mangroves	Tropical (hot and humid)	Dry season: August–December, rainy season: January–July	After peak in rainfall increase in the number of BU cases during a subsequent dry period (short-term and long-term rainfall patterns)	Construction of a dam (Petit-Saut Dam) on the Sinnamary river: decline in case numbers; Deforestation and creation of rice fields in Mana: increase in incidence
Asia (Ancestral)	Japan	[42] Chapter "Buruli Ulcer in Japan"	Sporadically distributed throughout the large Honshu island	Temperate (humid, subtropical in most of Honshu; humid, continental in northern Honshu)	Four distinct seasons including a period with higher rainfall between mid of June and end of July	80% of BU cases diagnosed during autumn/winter (indicating infection during/shortly after the hot rainy season in summer)	Due to wide distribution of cases no general associations; one case of familial occurrence connected with water channel in the backyard of their house

Australia/Oceania (Classical)	FNQ	[44, 70]	Daintree River catchment and adjacent coastal lowlands	Tropical (hot and humid)	Dry season during winter: May–November, rainy season during summer: November–April	Cases presented in 2011 during the late dry season after an unusually wet rainy season	Flooding after cyclones or exceptionally long and wet rainy seasons associated with BU cases
	Victoria	Chapter "Buruli Ulcer in Australia"	Low-lying, coastal towns, focal distribution	Temperate	Four distinct seasons; rainfall spread throughout the year	Diagnosis mainly during cooler months (April–September) between autumn and spring, with a peak in winter (indicating infection during warmer summer months)	Outbreak on Phillip island was suspected to be connected with golf course irrigation system; strong association of recent cases on Bellarine and Mornington Peninsulas with animal reservoir (possums)
	PNG	[46]	Coastal Oro Province, endemic villages around Kumusi River[a]	Tropical (hot and humid)	Rainfall high throughout the year, relatively drier months: May–November	Higher incidence in drier months (May–November)	Great flood following the eruption of nearby volcano Mount Lamington associated with BU cases

(continued)

Table 3 (continued)

Continent (lineage)	Country/ site	Refs.	Location/ environment	Climate	Seasons	Seasonality of infections	Linkage to environmental factors
Africa (Classical)	Different African countries	[56–60, 63–68] Chapter "Buruli Ulcer in Africa"	Highly focal distribution in river valleys, often affecting rural villages and therefore exact distribution within affected countries is often unkown	Tropical (hot and humid)	Region-specific dry and rainy seasons	Analysis of seasonal patterns of *M. ulcerans* infections complicated by delayed reporting of patients and combined analyses of country-wide data, which may obscure local climate variation; necessity of future investigations on seasonal patterns of the infection in areas with high quality surveillance systems	Proximity to rivers and tributaries
	Uganda	[7]	Community of refugees close to Nile River		Rainy seasons: March–May and September–November	Diagnosis mainly from September–November and to lesser extent January and February	Proximity to the Nile River (but not actual contact)

[a] BU has been reported from many parts of PNG (e.g. one large focus along Sepik River; information is mainly available for BU focus in villages along the Kumusi River ("Kumusi ulcer"))

from the North Pacific Ocean. Much of Honshu belongs to the temperate zone with humid, subtropical climate characterized by four distinct seasons including a period with higher rainfall between mid of June and end of July. Humid, continental climate may be encountered in northern Honshu, with cold winters commonly experiencing snowfall [86]. The 60 sporadic BU cases reported between 2003 and 2016 in Japan were distributed over 17 of the 47 prefectures of the country (chapter "Buruli Ulcer in Japan" of this book). Interestingly, in some of the northern regions reporting BU cases, the temperature can be below zero degrees Celsius during the coldest season of the year. In Australia, *M. ulcerans* infections mainly occur in two geographically distinct areas in a temperate climatic zone in the south-eastern state of Victoria, and in tropical FNQ. In Victoria, *M. ulcerans* infections are consistently associated with low-lying coastal areas, where rainfall is spread throughout the year [75]. After first BU cases were described in the Bairnsdale region [2], the pathogen appeared to migrate westwards [87] causing larger outbreaks in the 1990s in Phillip Island [18] and Frankston/Langwarrin [17]. Since 1998 new foci have continuously appeared on the Bellarine and Mornington Peninsulas [71, 80, 88]. The second significant BU endemic area of Australia in FNQ comprises a rim of coastal valleys and lowlands surrounding the Dagmar Range, and extending from Daintree in the North to Mossman in the South [44]. The climate in FNQ is characterized by two distinct seasons with a warm, dry season during the winter period in May–October/November and a warm, wet season during the summer period in November/December–April [52]. Information on the distribution of BU in PNG is sparse, although infections have sporadically occurred in different parts of the country. One main BU focus was seen in the coastal Oro province in villages along the Kumusi River, where the climate is hot and humid and rainfall is high throughout the year with a relatively drier period from May to November [46]. BU endemic areas of West and Central Africa are mainly located in remote, rural areas and are characterized by a focal distribution with often high local prevalence rates. Affected regions have a tropical climate, typically with distinct dry and rainy seasons and are often associated with slow flowing and stagnant water bodies in proximity to large rivers. In West Africa the dry period lasts generally from November to February and is followed by region-specific rainy seasons. The southern areas of West Africa in the Guinea Coast region, where most of the BU cases occur, commonly experience two rainy seasons, one from April to July and a shorter one in September/October. In contrast, only one rainy period between July and September is observed in the North [89]. Distinct, region-specific dry and rainy seasons are also observed in Central African regions reporting BU cases [90].

Taken together *M. ulcerans* infection foci may be located in tropical or temperate climatic zones with or without distinct seasons, are connected with coastal lowlands in French Guiana, Australia and PNG, and with water bodies in remote, rural inland or coastal areas of Africa (Table 3).

3.2.2 Seasonality of BU Transmission

The detection and interpretation of seasonal fluctuations in the incidence of *M. ulcerans* disease is complicated by a combination of different factors, including

the extremely long incubation period of the infection and the lack of knowledge on the mode of transmission and accordingly the timing of the infection, unless the patient has visited an endemic area only once and for a short time. Furthermore, features of early lesions are relatively uncharacteristic, causing delays in detection of the disease and reporting to health facilities. Well-documented records of BU cases among short-term visitors to BU endemic sites represent a unique source for the estimation of the time from exposure to disease onset. This approach was applied in south-eastern Australia by systematic identification of BU patients with a single visit exposure to one of the well-known focal BU endemic areas. The mean incubation period determined for patients infected in Victoria was 4.5 months with a wide variation from 32 to 264 days [91, 92]. In the Daintree region of FNQ, a large spike of BU cases was observed in September and October 2011, 7–8 month after an exceptionally long and very wet rainy season with peak rainfall in February 2011 that was suspected to be connected with the occurrence of the infections [44]. While this may speak for a longer average incubation period in FNQ as compared to Victoria, in the Kinyara refugee camp in Uganda, the period between short stays of visitors and the development of BU was estimated to be between 1 and 3 months [7]. These apparent differences in the mean incubation period may be due to mode of transmission-related differences in the inoculation dose of *M. ulcerans*. All of these studies have shown that the time interval between exposure and onset of symptoms and finally to the diagnosis of BU can vary substantially on an individual basis, which may obscure potentially distinct seasonal variations in transmission intensity. Nevertheless, seasonal patterns of BU incidence have been reported for infection foci caused by both ancestral and classical *M. ulcerans* strains (Table 3). In French Guiana, *M. ulcerans* was found to be correlated with short-term (6 months) and long-term (a decade) rainfall patterns and the El Niño-Southern oscillation [85], (chapter "*Mycobacterium ulcerans* Infection in French Guiana; Current State of Knowledge" of this book). In Japan, approximately 80% of the reported BU cases were diagnosed during autumn and winter [42] (chapter "Buruli Ulcer in Japan" of this book), indicating contraction of the infection during or shortly after the hot and rainy season in summer. Between 2009 and 2015, most BU cases in FNQ were diagnosed during the dry season from July to November, which is likely explained by a surge in transmission during the wet season [44]. For the BU endemic focus in villages along the Kumusi River in PNG, a consistently higher incidence of cases in the drier months (May–November) was recorded [46].

Analyses of seasonal patterns of *M. ulcerans* infection are particularly difficult for remote BU endemic regions in Africa, where very long delays in reporting of patients to the public health system have to be taken into account. In addition, several studies assessing seasonal patterns of the disease in Africa have used country-wide data, ignoring local differences in rainfall patterns. In a study assessing seasonal patterns of BU in an endemic area of Cameroon, case incidences between 2002 and 2012 peaked in March. Assuming a delay between infection and diagnosis of 5–6 months, this suggested that the risk of infection is highest during the high rainy season from August to October [93].

Similarly, transmission in the Kinyara refugee camp, was estimated to be greatest from July to September [7].

Seasonal patterns of BU incidence may result from differences in the environmental presence of the pathogen or potential vectors, as well as from behaviour-associated variations in exposure of populations to *M. ulcerans*. These variations may be triggered by climatic conditions and connected landscape dynamics as well as by seasonal changes in agricultural and other activities intensifying environmental exposure. Considering the general association of BU endemicity with aquatic ecosystems, one of the main drivers of increased transmission may be flooding and the seasonal appearance of stagnant temporary water bodies and swamps, providing a breeding ground for potential reservoirs/vectors and/or the pathogen itself.

3.2.3 Linkage of the Emergence of BU Foci to Environmental Changes

Altered environmental conditions may be favourable for the growth and dissemination of pathogens and may lead to increased contact of populations to pathogens, i.e. their vector(s) and/or environmental reservoir(s) [94, 95]. Therefore changes in land use including intensification and changes in the nature of agricultural activities, water management projects, deforestation and urbanization as well as natural phenomena, such as cyclones, flooding, or volcanic eruption can lead to ecological edge effects that promote disease emergence. It is suspected, that the emergence of some of the *M. ulcerans* infection foci was related to different types of ecological and environmental disturbances (Table 3).

In French Guiana, an increase in the incidence of BU in Mana between 1984 and 1988 was observed just after the creation of rice fields [69]. On the other hand, construction of the hydroelectric Petit-Saut Dam on the Sinnamary River in 1994 upstream of an adjacent BU endemic area has been associated with a significant decline in case numbers, possibly due to a better regulation of water flows. In Japan, a BU outbreak affecting several members of a family was linked to a stagnant agricultural water channel [37] and in Victoria an outbreak in Phillip Island in the 1990s was suspected to be triggered by a newly formed lake and/or a golf course irrigation system [96]. The population in BU endemic areas along the Kumusi River in PNG has claimed that infections had only occurred after a great flood following the eruption of the nearby volcano Mount Lamington. In FNQ, a spike of cases seen in 2011 was connected with the aforementioned exceptionally long and very wet rainy season. The subsequent decrease in the number of cases since 2011, may be due to much drier conditions in the following years [44]. In African BU endemic areas associations have been suspected between BU incidence and damming of rivers or streams leading to the creation of artificial lakes; e.g. a small stream on the University campus in Ibadan, Nigeria [97], the Mapé River in Cameroon [98], the Densu River in Ghana, or the Bandama River in Côte d'Ivoire [99]. High risk zones for the contraction of BU were also connected with land cover changes; e.g. establishment of irrigated rice and banana fields in Côte d'Ivoire [100] or areas of mining and agricultural activity in Ghana [101].

4   Risk/Protective Factors for BU

In order to identify risk factors for BU, a number of case-control studies were conducted in areas endemic for the classical lineage of *M. ulcerans*. In view of a limited reliability of the clinical diagnosis, laboratory confirmation of patients enrolled in these studies is a crucial prerequisite for the quality of obtained results. Due to the multitude of studies conducted on behavioural risk factors for BU, only those with laboratory-confirmed patients were included here. On the other hand, considering that only very limited information is available to date on potential host genetic factors and on the impact of BCG vaccination and HIV co-infection, also studies with only partly reconfirmed cases are discussed in the corresponding paragraphs. The BU case confirmation status of all of these studies is listed in Table 4.

Table 4 Assessment of risk factors for the contraction of BU caused by classical *M. ulcerans* strains identified by comparative case-control studies

Country/ site	Time span of study (Ref.)	No of confirmed[a] BU cases	No of controls (matching factors)	Assessed risk factors	Risk factors identified	Protective factors identified
Behavioural risk factors						
Australia (Victoria)	1998– 2005 [80]	49	609 (community-based)	Lifestyle and insect exposure	Mosquito bites on lower legs and lower arms	Use of insect repellent, wearing protective clothing, washing of wounds
Benin	2006– 2008 [105]	104	312 (community-based; matched by age, sex and village of residence)	Water sources, family relationships	BU history in the family, contact with natural water sources	
Côte d'Ivoire	2012 [102]	51	102 (hospital/ health center-based; matched by age (±5 years), sex, and type of residency)	Socio-sanitary, environment, and behaviour	Regular contact with unprotected surface water and absence of protective equipment during agricultural activities	Good knowledge about the risks that may result in BU and perception about the disease causes

Table 4 (continued)

Country/ site	Time span of study (Ref.)	No of confirmed[a] BU cases	No of controls (matching factors)	Assessed risk factors	Risk factors identified	Protective factors identified
Ghana	1999 [104]	51	51 (hospital-based; matched by age group, sex, BCG)	Water-related	Swimming in rivers on a habitual basis	
	2000 [79]	116	116 (community-based; matched by age and village)	Environment and behavior	Wading in a river or stream	Wearing a shirt while farming, sharing indoor living space with livestock, bathing with toilet soap
	2010–2011 [81]	113	113 (community-based; matched by age, sex and village)	Demography, socio-economy, health and hygiene as well as environment	Presence of wetland, insect bites in water, use of adhesive when injured, and washing in river	Use of alcohol for injuries, covering limbs during farming
	2013–2015 [103]	176	176 (community-based; matched by age (±5 years), sex, and place of residence)	Demography, environment, and behaviour	Farming in swampy areas, farming while wearing short clothing, insect bites, and application of leaves on wounds	Farming in long clothing, washing wounds with water, and application of adhesive bandage on wounds

Table 4 (continued)

Country/ site	Time span of study (Ref.)	No of confirmed[a] BU cases	No of controls (matching factors)	Assessed risk factors	Risk factors identified	Protective factors identified
Togo	2014–2015 [106]	83	128 (community-based; matched by sex and place of residence)	Socio-demography, environment and behaviour	Bathing with water from open borehole, frequently crossing or swimming in a river, and receiving cuts, scratches or insect bites near a river	Using detergents for washing clothes or dishes
Host genetic factors						
Benin	2005–2013 [111]	208	300 (community-based; matched by age, sex, water contact habits, and ethnic background)	Autophagy-related genes	rs1333955 SNP in PARK2	rs2241880 SNP in ATG16L1
Ghana	2006 [110]	102 of 182	191 (community-based; matched by age)	Polymorphisms in the natural resistance-associated macrophage protein gene (SLC11A1)	D543N in SLC11A1	
	2011 [109]	96	384 (community-based; matched by age, sex, ethnicity and home village)	Polymorphisms in genes known to be associated with susceptibility to Tuberculosis and Leprosy	rs9282799 SNP in iNOS and rs2069705 in IFNG	

Table 4 (continued)

Country/ site	Time span of study (Ref.)	No of confirmed[a] BU cases	No of controls (matching factors)	Assessed risk factors	Risk factors identified	Protective factors identified
BCG vaccination						
Benin	2002–2003 [117]	134 of 279	988 (community-based; matched by age and sex)	BCG vaccination	None	None
Côte d'Ivoire	2001 [119]	56 of 116	116 (community-based; matched by age, sex, and village)	BCG vaccination (and other risk factors not discussed here)	No history of BCG vaccination	
DRC, Ghana and Togo	2010–2013 [118]	401	826 (mostly family members and neighbours)	BCG vaccination	None	None
Ghana	2000 [79]	116	116 (community-based; matched by age and village)	BCG vaccination (and other risk factors discussed above)	None	None
HIV co-infection						
Benin	2002–2003 [120]	258 of 426	613 (community)	HIV infection	HIV infection	
Ghana	2000 [79]	116	116 (community-based; matched by age and village)	HIV infection (and other risk factors discussed above)	None	None

[a]Laboratory confirmation of clinically diagnosed patients by at least one diagnostic test

4.1 Behavioural Risk Factors

Failure to wear protective clothing [102, 103] and activities close to or in certain unprotected water sources [79, 81, 102–106] have repeatedly been connected with an increased risk of contracting BU. Conversely, wearing protective clothing [79–81, 103], wound care [80, 81, 103] and good hygiene [79, 106] have been identified

as factors conferring some degree of protection (Table 4). In southeastern Australia, where an involvement of mosquitoes in the transmission of *M. ulcerans* infections as either biological or purely mechanical vectors of the pathogen has been suggested, mosquito bites were identified as a risk factor. In addition to using insect repellent, immediate cleansing of wounds has also been associated with a decreased risk of contracting an *M. ulcerans* infection [80]. In African settings, insect bites, but also cuts and scratches near or in water bodies have been linked with an increased risk of infection [106]. One of the case-control studies came to the conclusion that good knowledge about BU and the risk factors involved in infection had a protective effect [102].

4.2   Host Genetic Factors

With the availability of a large number of human genome sequences, millions of polymorphic markers have been identified [107]. For case-control studies aiming at uncovering genetic factors that influence disease susceptibility, there are two main principal approaches; one based on testing candidate genes and the other based on genome-wide association studies. While candidate gene studies have higher statistical power, they cannot discover new markers or marker combinations. Even with several thousands of samples, genome-wide association studies can on the other hand be underpowered [108] and in the case of infectious diseases the element of exposure adds complexity and can contribute to statistical noise.

In view of the limited number of BU patients that can be enrolled for geographically restricted case-control studies, it is not surprising that only candidate gene studies have so far been conducted for BU. To date, single nucleotide polymorphisms (SNPs) in the inducible nitric oxide synthase gene *iNOS* and in the interferon gamma gene *IFNG* [109], the natural resistance-associated macrophage protein gene *SLC11A1* (*NRAMP1*) [110], and the autophagy-related E3 ubiquitin-protein ligase gene *PARK2* [111] have been linked to susceptibility to BU (Table 4). However, these initial findings should be reconfirmed in future studies enrolling larger BU patient cohorts.

The fact that SNPs, which reduce the promoter activity of *iNOS* and *IFNG* implicated in macrophage activation, were found to increase susceptibility to BU, support the hypothesis that macrophages may be of crucial importance for the containment of *M. ulcerans* infections. Also, the influence of polymorphisms in SLC11A1 implicated in the transport of divalent cations to late endosomes/lysosomes [112] and in the ubiquitin ligase PARK2 implicated in resistance to intracellular pathogens [113], hint to a key role of macrophages in the early stage of an *M. ulcerans* infection, when low mycolactone levels may still permit elimination of the inoculated mycobacteria. For more detailed information on the immunology of BU the reader is referred to chapter "The Immunology of Buruli Ulcer" of this book. It should be noted however, that selection of candidate genes investigated so far was strongly biased towards host polymorphisms that have previously shown significant associations with susceptibility to intracellular mycobacteria, such as *M.*

tuberculosis and *M. leprae.* Genome-wide association studies for susceptibility to BU could thus potentially reveal even more robust gene-disease association data for genes that are relevant for other immune effector mechanisms.

4.3   BCG Vaccination

No specific vaccine is currently available to prevent *M. ulcerans* disease, but the protective effectiveness of *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) vaccination has been controversially discussed [114]. Two randomized controlled trials, one at the settlement of Rwandan refugees in Kinyara between 1967 and 1969 [115] and a second in another BU endemic area of Uganda in the 1970s [116] indicated a certain protective effect of BCG vaccination, although protection appeared to be short-lived [116]. Results of three case-control studies showed no evidence of a protective effect of BCG vaccination on the risk of developing BU [79, 117, 118], whereas no history of BCG vaccination appeared to be associated with BU in another study [119] (Table 4). Uncertainty about the BCG vaccination status among study populations with missing vaccination records should be considered as a potential confounder in these investigations.

4.4   BU-HIV Co-infection

BU-HIV co-infection is not uncommon due to the high HIV prevalence in many BU endemic areas. But until today, there is paucity of information on epidemiological and clinical relationships of the two infections. A case-control study conducted in Benin found that the prevalence of HIV in BU patients (2.6%) was significantly higher (P = 0.003) than that of the local control population (0.3%) [120], indicating that HIV increases the risk of BU. Similar percentages were reported in another case-control study performed in Ghana, with 5% of the BU patients and 0.9% of the control individuals testing positive for HIV, although—probably due to the small number of study participants—this association was not statistically significant [79] (Table 4). Furthermore, the prevalence of HIV among BU patients was significantly higher than that of the regional estimated prevalence [121] or that of other patients or pregnant women attending the same health facilities [122]. HIV infection weakens the immune system and seems to also affect the clinical presentation of BU, as co-infected patients tend to have more severe and more often multifocal lesions than HIV-negative patients [121, 122]. This is also indicated by a number of BU-HIV case reports describing aggressive, multifocal BU disease progression [123–128]. Management of BU-HIV co-infection is challenging [129]. It is recommended that all BU patients should be tested for HIV co-infection and that HIV-positive individuals should receive early antiretroviral treatment to reduce mortality (for more information on the management of BU-HIV co-infection see chapter "Management of BU-HIV Co-infection" of this book), which seems to be higher in BU-HIV co-infected than in HIV-negative patients [121, 122, 126, 128].

5   Diagnosis and Treatment of BU in Different Geographical Settings

The diagnosis of *M. ulcerans* infections is not trivial. The disease has a wide spectrum of clinical manifestations including ulcerative lesions as well as non-ulcerative forms such as nodules, plaques, and edema. As a consequence, the differential diagnosis of BU is broad, particularly in tropical endemic areas, where the prevalence of skin conditions with similar presentations is high [130]. Findings from a recent retrospective assessment of the diagnosis of BU in Ghana between 2008 and 2016 has revealed that in over 50% of all cases, a clinical suspicion of BU could not be confirmed by laboratory testing [59]. While surgical excision of lesions had long been the only treatment option for BU, pre-treatment laboratory confirmation of *M. ulcerans* infections has gained further in importance after the introduction of antibiotic therapy in 2004. In recent years, PCR targeting the IS*2404* element of *M. ulcerans* has become a gold standard for the diagnosis of BU in reference laboratories of the endemic countries. PCR-based laboratory confirmation of suspected BU cases is routinely performed in countries with resource rich healthcare systems and good laboratory infrastructure such as Japan [131] and Australia [132]. In French Guiana, the proportion of laboratory-confirmed BU cases increased from 52% before PCR became available in 2000 to 92% after 2000 [69]. However, the main drawback of PCR-based diagnosis for rural African endemic areas is its limited accessibility. To date, the only laboratory test locally available is microscopy of Ziehl-Neelsen stained smears from lesion specimens, a method with low sensitivity. In-country PCR reconfirmation in Africa can only be performed at a few reference laboratories. Samples are often stored for bulk shipments to these laboratories, leading to delayed delivery of results. Initiation of antibiotic therapy is thus often based on clinical diagnosis only, bearing the risk that some of the BU patients (and also misdiagnosed non-BU patients) do not receive appropriate treatment. A low-tech, accurate, rapid diagnostic test for the diagnosis of BU is of urgent need. For more information on the laboratory diagnosis of BU see chapter "Laboratory Diagnosis of Buruli Ulcer: Challenges and Future Perspectives" of this book.

WHO treatment recommendations include an eight-week course of a rifampicin-based combination of antibiotics, and—if needed—adjunct debridement and skin grafting. Until recently, rifampicin—the most effective drug against *M. ulcerans*—was prescribed in combination with intramuscular doses of streptomycin. However, administration of streptomycin through injections has a major impact on patient acceptance and adherence, and is often not practical in rural BU endemic areas. Moreover, prolonged use of streptomycin has been shown to cause permanent ototoxicity and transient nephrotoxicity in BU patients [133]. Therefore, the WHO Technical Advisory Group on Buruli ulcer recommended in 2017 to replace streptomycin with oral clarithromycin for BU treatment. Current first-line eight-week treatment regimens include rifampicin combined with clarithromycin, moxifloxacin or ciprofloxacin in Australia [134], a triple combination of rifampicin, levofloxacin, and clarithromycin in Japan (chapter "Buruli Ulcer in Japan" of this book) and

rifampicin combined with amikacin or clarithromycin in French Guiana (chapter "*Mycobacterium ulcerans* Infection in French Guiana; Current State of Knowledge" of this book). WHO has developed a policy of free supplies of antibiotics to the affected countries on request from national BU control programs, which should in turn ensure that BU treatment facilities have an uninterrupted supply of the antibiotics [82]. However, the logistics involved in the provision of medication to remote, rural health facilities is challenging and hence antibiotics are not always available to patients in these areas. Alternative options for the treatment of BU, in particular the potential of new tuberculosis drug candidates are currently being investigated. Furthermore, local thermotherapy at the site of the lesions with simple phase change material devices showed promising results in a phase II clinical trial [135] and may be suitable for treatment at community level. For more information on the treatment of BU see chapters "Antimicrobial Treatment of *Mycobacterium ulcerans* Infection" and "Thermotherapy of Buruli Ulcer" of this book. Another important component to improve the healing process of lesions and to prevent secondary infections is adequate wound management. Interested readers are referred to chapter "Secondary Infection of Buruli Ulcer Lesions" of this book dealing with secondary infection and management of BU lesions.

6 Socio-Economic Burden of BU for Patients and their Families

The diagnosis and treatment of BU patients in Australia, Japan, and French Guiana is secured by well-resourced universal healthcare systems. In contrast, health services in remote BU endemic communities of Africa and PNG are often very limited. Although in many BU endemic countries, antibiotic treatment of BU is free of charge, other expenditures and required efforts may prevent patients from seeking care at the formal health system. The economic burden arising from transport, accommodation and food for patients and caregivers, lost earnings and work force may be too high for the affected families [136, 137], in particular if long hospital stays are required. Cultural beliefs, perceptions regarding the effectiveness of BU treatment, and stigma derived from the perceived mystical origin of the disease further aggravate the situation [138, 139] (see chapter "Social Science Contributions to BU Focused Health Service Research in West-Africa" of this book for more information on social science aspects of BU). In many BU endemic areas, patients prefer to first consult traditional healers and only refer to hospitals as a last resort [137]. Although BU is a slowly progressing disease, delays in receiving adequate medical care is detrimental to the treatment outcome. Chemotherapy is highly effective for early stages of BU, whereas the management of advanced stages is often complicated by delayed wound healing requiring prolonged hospitalization. Moreover, treatment of advanced BU may not prevent permanent functional disabilities.

Neglected tropical diseases (NTDs) have a substantial, cumulative effect on the economy and the health of affected populations, imposing a devastating social and

economic burden on affected individuals, households and communities. A number of studies in BU endemic African countries have revealed that BU can push households into poverty, whether patients were hospitalized or not. For instance, the median cost burden of hospitalization of BU patients in Central Cameroon was reported to correspond to 25% of the annual earnings of a household due to high non-medical costs and productivity loss. More than half of the families were forced to withdraw financial and hence social support to the patient, resulting in the patient's isolation at the hospital. Indeed, social isolation of in-patients has been mentioned as the principal cause for abandonment of biomedical treatment [140]. On the other hand, for non-hospitalized BU patients in Ghana, transportation and other costs added up to 45% of the annual income of households. For these outpatients, social isolation was also an issue, particularly for children, who were often not accompanied for treatment [141]. In a study conducted in Nigeria it was revealed that costs before a definite diagnosis of BU was established (including costs for medications, drugs, wound care, hospitalization, transportation, food, and others), accounted for the brunt of the total costs for the treatment of BU. Costs were catastrophic for 50% of all affected households [142].

Taken together, new, socially more compatible intervention strategies, such as a more decentralized system of diagnosis and care as well as improved community mobilization and education of populations concerning BU to reduce care-seeking delays are of urgent need. Results of a recent pilot BU outreach campaign and decentralized care program in one of the most endemic districts of Benin demonstrated the great value of such interventions, reflected by a strong community support and a dramatic increase in the detection of BU cases that was associated with immediate, free, and accessible care [143]. However, a major challenge will be the mobilization of both financial and human (organization, training, health staff etc.) resources to implement and sustain decentralized care at a larger scale and on a long-term basis.

7   Outlook

Launching of the Global BU Initiative in 1998 and adding BU to the WHO list of NTDs, has helped to increase awareness of the disease among affected populations, health staff, potential donors, and researchers. As a result, national BU control programs were established in the affected countries, healthcare provision to BU patients was improved, and new diagnostic tools and treatment modalities have been developed. The decline in the number of new infections reported to WHO in the past years might on the one hand be a result of the progress made in the fight against the disease. Active case finding and treatment of patients with chronic ulcerative BU lesions, which may represent significant reservoirs of the pathogen, may be an important corner stone for the control of the disease. But on the other hand, the declining number of reported BU patients has also led to a decrease in awareness of and interest in the disease. The reduction in the number of reported cases may therefore be in part related to a decline in active case search activities in

known BU endemic regions and also to a lack of efforts to identify new BU endemic areas. The large BU burden in Nigeria only became apparent when Nigerian patients presented to established BU health facilities in Benin [68, 144], indicating that the true number of BU cases in regions where no fully functional BU control programs are in place, may be vastly underestimated. While the detection and treatment of BU in Australia, Japan, and French Guiana will continue to be covered by their universal healthcare systems, a major task for the next years will be the sustained control of BU in Africa. For that purpose, funding for control activities and research has to be mobilized to maintain established healthcare infrastructure in the affected countries and to implement newly developed diagnostic tests and treatment regimens. Support by NGOs has played in many settings an important role in the development of BU control activities and treatment centers and was crucial for BU research funding (an example for funding of a BU research consortium by an NGO is provided in Chapter "Transdisciplinary Research and Action to Stop Buruli Ulcer: A Case Study from Philanthropy"). This has major implications for sustainability as reductions in program support by major partners may lead to severe drops in performance of BU control activities [145] and a loss in research output and capacity development.

Fragmentation of health interventions and services is a widely discussed issue, since narrowly targeted interventions can generate in particular in low resource settings inequity and substantial extra costs. Therefore, it has been suggested to integrate BU control into the broader context of care for skin NTDs [146]. The co-endemicity of several of these diseases, such as BU, yaws, scabies, mycetoma, and leprosy may allow maximizing financial and human resources by the implementation of integrated approaches, including training of health workers in differential diagnosis of skin NTDs, basic dermatology and wound management [147].

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