Mycobacterium ulcerans disease

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Abstract Mycobacterium ulcerans disease (Buruli ulcer) is an important health problem in several West African countries. It is prevalent in scattered foci around the world, predominantly in riverine areas with a humid, hot climate. We review the epidemiology, bacteriology, transmission, immunology, pathology, diagnosis and treatment of infections. M. ulcerans is an ubiquitous micro-organism and is harboured by fish, snails, and water insects. The mode of transmission is unknown. Lesions are most common on exposed parts of the body, particularly on the limbs. Spontaneous healing may occur. Many patients in endemic areas present late with advanced, severe lesions. BCG vaccination yields a limited, relatively short-lived, immune protection. Recommended treatment consists of surgical debridement, followed by skin grafting if necessary. Many patients have functional limitations after healing. Better understanding of disease transmission and pathogenesis is needed for improved control and prevention of Buruli ulcer.

Keywords Mycobacterium ulcerans/pathogenicity; Mycobacterium infections, Atypical/etiology/epidemiology/therapy; Review literature; Meta-analysis; Africa, Western (source: MeSH, NLM).

Introduction Mycobacterium ulcerans disease, or Buruli ulcer, occurs in scattered foci around the world in riverine areas with a humid, hot climate (1–3). However, the disease may also occur in temperate climates, such as in coastal southeastern Australia (2). Although originally regarded as an unusual form of tropical skin ulcer, Buruli ulcer is now recognized as a distinct disease that places a major burden on affected populations and health facilities in endemic regions, particularly in West Africa. The typical presentation with indolent, painless, undermined ulcers is easily diagnosed, but atypical forms can be confounded with other causes of skin ulcers.

Unless super-infection has occurred, patients usually do not show signs of systemic inflammatory response. Early, pre-ulcerative lesions, usually in the form of nodules, may be easily managed by simple surgical excision and suturing (4). Surgical management is more complicated when the disease has advanced, and many patients in endemic regions present late because they live in rural areas and their families cannot afford the time to attend hospital, and also because they fear surgery (5, 6). Treatment of advanced disease is often difficult, and complicated by persistence and relapse (7). Surgery is still considered the main treatment option despite its poor acceptability, high costs, and failure to prevent recurrence. Over half of the people who have Buruli ulcers have functional limitations after treatment (8, 9), and suffer from social stigmatization (5), and loss of livelihoods (10). In 1998, WHO established the Global Buruli ulcer Initiative, and the importance of Buruli ulcer disease was again recognized by the 57th World Health Organization.
Assembly in 2004 (11). The Assembly called for increased surveillance and control of Buruli ulcer and intensified research to develop tools to diagnose, treat and prevent the disease, thereby reducing the burden in poverty-stricken communities affected by this disease.

We review the current understanding of the epidemiology and transmission of *M. ulcerans*. We summarize new developments in diagnostic tests, and describe what is known about the pathology and immunology of this infection and how these characteristics relate to current and potential treatments. We discuss pathogenesis, mediated by a toxin produced by *M. ulcerans*, which causes the extensive necrosis characteristic of the disease.

Search strategy: We extracted information from peer-reviewed publications retrieved from a MEDLINE search (English and French) with the search terms (Buruli OR (Mycobacterium AND ulcerans)) accessed on March 20, 2005, together with other published and unpublished data, presented at annual meetings of the Buruli ulcer ad hoc advisory group in Geneva.

**Epidemiology and Transmission**

Although the first report of Buruli ulcer from Africa dates back to 1897, when Sir Albert Cook described cases of chronic ulceration in Uganda, the first definitive description of *Mycobacterium ulcerans* was published in 1948 (12). The report describes lesions in different stages of Buruli ulcer disease in two Australian children and four adults in a riverine area in Bairnsdale, Victoria. Buruli ulcer has since been reported from several different regions. Most of these reports are of infections that occur in people living in riverine areas, in humid, hot climates. The area around Melbourne, Australia appears to be one of the few foci of disease in a temperate climate (13). Disease foci have been reported from tropical areas in Asia (Malaysia, Papua New Guinea, and Sri Lanka) and Latin America (Guyana, Mexico, Peru), but the largest numbers of patients with Buruli ulcer disease have been detected in sub-Saharan Africa (14, 15). The earliest reports came from the country that is now called the Democratic Republic of the Congo, from the area south-west of Kinshasa (16) where the disease is still prevalent (17). Later, there were reports of hundreds of patients in Uganda, from Kinyara, a refugee camp near the Nile river, in a county then called Buruli (18), hence “Buruli ulcer” (19). Today, many countries of sub-Saharan Africa are considered endemic for Buruli ulcer disease, but the largest number of patients have been reported from riverine areas in distinct regions of Benin, Côte d’Ivoire, and Ghana, where the number of detected cases has alarmingly increased in recent years (see Fig. 1).

Point prevalence estimates have varied between regions, but have been reported to be as high as 150 – 280/ 100,000 population in some highly endemic districts in Ghana (20, 21). Similar prevalence rates have been reported from Côte d’Ivoire (22) and Benin (23). Such figures are difficult to interpret because of methodological differences, but they may genuinely reflect the increasing burden of Buruli ulcer in some localities. Although Buruli ulcer may be found in almost all age groups, in most reported series the majority of patients are aged between 5–15 years, with an almost equal gender distribution (1–3).

It is commonly believed that *M. ulcerans* is an environmental mycobacterium. *M. ulcerans* has been recovered from several species in areas endemic for Buruli ulcer, including aquatic insects, molluscs, and fish (24, 25) but these animals do not appear to develop overt disease. Koalas (*Phascolarctos cinereus*) (26), ring-tailed possums (*Pseudochirurus peregrinus*), brushtail possums (*Trichosurus vulpecula*), an alpaca (*Llama alpaca*) and a potoroo (*Potorous longipes*) (Hayman JA, personal observation) have been reported to develop natural infections, but many other species that live in endemic areas appear to be resistant. Interestingly, certain aquatic insects (*Naucoridae*) appear to concentrate *M. ulcerans* in their salivary glands (27, 28). These insects are predators and may feed on molluscs that in turn feed on the biofilm of water plants that appear to contain *M. ulcerans* (28, 29). In a laboratory experiment, *M. ulcerans*-infected water bugs were able to transmit *M. ulcerans* disease to the tail of mice after a bite (28). Few if any patients recall having been bitten by an insect prior to developing disease however, and it is presently unknown whether insect bites represent a route of transmission to man. An alternative mode of transmission may involve penetrating skin injuries during fishing or farming activities that seed the micro-organism into subcutaneous tissues (30). Only two cases have been reported of human-to-human transmission (31, 32).

Clustering of cases among families has occasionally been observed. This may reflect an exposure to a common source of infection, and/or a common genetic susceptibility to infection with *M. ulcerans* (33). The analysis of detailed geographical information about rivers and streams, physico-chemical data, and reports of Buruli ulcer disease to health authorities, has implicated arsenic acid exposure as a confounding immunosuppressant in some cases (34). Foci of infection have been associated with water basins, and case control studies have shown that wading in water is a risk factor for contracting the disease (21, 22, 35). It has also been suggested that aerosols may play a role in transmission (36).

The relationship between naturally occurring swamps and human activity in the transmission of *M. ulcerans* disease was elegantly demonstrated when cases of Buruli ulcer occurred around a golf course irrigation system in Victoria, Australia (37). The golf course pumped water to sprinklers from a dam that contained ground water supplemented with recycled water from a sewage facility. Using polymerase chain reaction (38), *M. ulcerans* was demonstrated in the irrigation dam and a nearby swamp. After abandoning the use of water from this dam, there was a sharp reduction in cases in subsequent years.

In one study of a case series in Ghana, distribution of lesions over body surface area appeared to predominantly affect the right arm in children, and the left lower leg in adults, suggesting that Buruli ulcer was acquired by activities near the ground, during farming or playing (39). In an analysis of a larger case series, 15 years later, and elsewhere in the Ashanti Region of Ghana, the distribution of lesions was however evenly spread between right and left limbs (40). Different modes of transmission may be relevant, and probably play a role, but it is presently unclear which mechanism is most important.

**Bacteriology**

*Mycobacterium ulcerans* is a slow-growing mycobacterium that may be cultured in vitro at 32 °C on the usual media for mycobacterial culture (12). Isolation from the environment has been unsuccessful, and isolation success from clinical samples has varied among laboratories, with some reference laboratories reporting high success rates in clinically confirmed cases, using...
improved transport media and decontamination methods (41). The development of PCR for quick identification of \textit{M. ulcerans} in clinical and environmental samples has greatly improved the diagnostic yield as well as our understanding of the epidemiology of Buruli ulcer. The most extensively studied PCR has been a nested PCR of a DNA repeat sequence of the \textit{M. ulcerans} genome, IS\textit{2404} (38, 42, 43). \textit{M. ulcerans} resembles \textit{M. marinum} in many aspects but there is a major difference in that \textit{M. ulcerans} appears to produce a secreted toxin, or class of toxins, chemically identified as ketolide — usually referred to as mycolactone (44). When injected in experimental animals, mycolactone molecules alone are able to produce massive necrosis similar to what is observed if these animals are inoculated with \textit{M. ulcerans}. Three of the polyketide synthases involved in the biosynthesis of mycolactones appear to be coded by genes located on a giant plasmid (45). Strains of \textit{M. ulcerans} isolated within certain regions show remarkable similarity, but differences between geographical regions have been identified with important differences in type of mycolactone production, perhaps reflecting regional differences in clinical presentation and virulence of \textit{M. ulcerans} disease. Another mycobacterium, referred to as \textit{M. liflandii}, has been isolated from frogs. These frogs were imported from West Africa and showed signs of disease mimicking the oedematous and ulcerative forms of \textit{M. ulcerans} disease in humans (46). This mycobacterium tested positive for the IS\textit{2404} that was previously considered species-specific for \textit{M. ulcerans}, and appears able to produce mycolactones (7). This finding may impact future studies into the natural reservoir of \textit{M. ulcerans}.

Pathology and Immunology

The predominant pattern in the pathology of \textit{M. ulcerans} disease is that in early, pre-ulcerative and ulcerative lesions, large numbers of extra-cellular mycobacteria are seen, with extensive necrosis and very little inflammatory response, and no granuloma formation. In surgical specimens resected in later stages during healing, bacilli are scanty or even absent, with granuloma formation (12, 48, 49). Many healthy individuals in Buruli ulcer-endemic areas show specific immune responses to \textit{M. ulcerans} (50), suggesting that, in analogy with leprosy and tuberculosis, the disease develops only in a limited proportion of those infected with \textit{M. ulcerans} (31). Further evidence that a cellular immune response may protect individuals with Buruli ulcer is provided by case reports that describe disseminated, overwhelming \textit{M. ulcerans} disease in patients co-infected with HIV.

Several research groups, using different models, have observed that in early stages of disease, specific immune protection seems to be lost. Almost 30 years ago, Stanford et al. observed that cell-mediated immune response, as evidenced by skin testing using burulin, a protein derivative of \textit{M. ulcerans}, showed low responses in initial stages of Buruli ulcer disease that appeared to improve over time, in later stages of the disease (51). Gooding et al. described a similarly low specific immune protection in early Buruli ulcer in Australian patients but this effect seemed to persist after patients were cured (52). Prévot et al. (53) showed that in active \textit{M. ulcerans} disease in patients from Guyana, in vitro IL-10 production in peripheral mononuclear cells after stimulation with \textit{M. ulcerans} was markedly
increased compared to tuberculin skin test positive control subjects, while interferon gamma (IFN-γ) production was significantly lower. In resected tissues, using reverse transcriptase PCR after stimulation with whole heat-killed M. ulcerans, messenger RNA (mRNA) IFN-γ production was higher, and IL-10 mRNA was lower in pre-ulcerative (nodular) lesions, compared to ulcerative lesions. In Ghana, immune protection seemed to be restored in patients with later stages of Buruli ulcer, at least at the systemic level. IFN-γ production appeared significantly lower after non-specific stimulation in patients with early lesions compared to those with late lesions. Stimulation with tuberculin resulted in low IFN-γ production in patients with early lesions, but it was significantly higher in patients with later lesions, and higher than levels in healthy controls (54). When no highly M. ulcerans-specific stimulation was used, an increase in IL-10 or IL-4 production could not be detected in any of the stages of M. ulcerans disease compared to controls.

In the study by Prévot et al. (53), evidence emerged that systemic immune phenomena were mirrored by local, intra-lesional cytokine profiles. High IFN-γ with low IL-10 mRNA levels were present in early, nodular lesions, while high IL-10 with low IFN-γ mRNA levels were present in ulcerative lesions. Intra-lesional IL-4 and IL-13 mRNA levels were low, and were only detected in patients with the ulcerative form.

These data, although using different models, generally support the hypothesis (30) that in early M. ulcerans disease, Th1 response is indeed down-regulated — either by Th2 preponderance, or by IL-10 overproduction, or by Th1 down-regulation per se.

Th2 preponderance might conceivably be induced by common helminthic infestations like schistosomiasis (33). In a study from Benin, only an association with more disseminated Buruli ulcer disease was found (55), but generally, no clear link appears to exist between schistosomiasis and Buruli ulcer (55, 56).

Immune protection by M. bovis BCG lasting six months has been found in an earlier study in Uganda (57). In a case control study in Ghana, BCG scars were no more common in control subjects than in Buruli ulcer patients (21) but in a study in Benin, BCG was shown to be protective against more severe M. ulcerans disease — notably, osteomyelitis (58). Based on these data, a study has been designed to explore the potential impact of repeat-BCG vaccination in endemic regions in West Africa. This study will be implemented as soon as the necessary financial support and logistics have been obtained.

It is not known whether natural resistance to M. ulcerans is inherited or acquired in later life (30). This is an important area of research as an unknown proportion of disease progression or spontaneous healing may be due to genetic polymorphisms.

**Differential diagnosis and diagnostic tests**

The clinical diagnosis may be straightforward in patients living in Buruli ulcer-endemic areas, especially in those who present with chronic, indolent ulcerated lesions with undermined edges and a necrotic slough (1–3). The differential diagnosis depends on the stage at presentation, and the relevant conditions that occur in the area where the patient lives. In some endemic countries, particularly in West Africa, M. ulcerans disease may be confused with onchocercosis, keratin (sebaceous retention) cyst, lipoma, and lymphadenitis or lymphadenopathies. The plaque and oedematous presentation of M. ulcerans disease may be mimicked by cellulitis or deep fungal infection. Ulcerative lesions may be confused with tropical (phagedenic) ulcer. However, tropical ulcers are usually painful, and found only on the lower legs. Leishmaniasis is an important differential diagnosis in South America, and squamous cell carcinoma can also present as ulcerating lesions.

In addition to clinical evaluation, there are four tests that can be employed to confirm a suspect case:

1) smear for direct detection of acid-fast bacilli; this test may be useful in ulcerative stages, but in some studies the diagnostic yield was low (21); in pre-ulcerative lesions, a smear may be taken from a biopsy;
2) histopathological examination of tissue obtained during surgery (48, 49);
3) culture of smears, or of tissue; the diagnostic sensitivity used to be very low (12), but laboratories that use special transport media have acceptable diagnostic yield (42); and
4) PCR from biopsy material; most groups now use the high-copy insertion sequence IS2404 (39, 43, 44). The test is still being improved for specific purposes and circumstances, and a dry-reagent PCR has been developed that could be used in laboratories in the region (59). One other mycobacterium that might test positive is M. liflandii — but in clinical studies in M. ulcerans-endemic areas, this may not necessarily confound the findings.

In clinical practice, cases are usually managed without microbiological confirmation of the diagnosis. For studies however, case confirmation is highly desirable. WHO is reconsidering its earlier recommendation (3) that two confirmative tests should be obtained to establish a definitive diagnosis; one positive test result in the context of high clinical suspicion might also be considered sufficient.

Serological testing for Buruli ulcer disease has been done in a case control study in Ghana (50). In the IgG class there was considerable overlap of immune recognition between sera of patients diagnosed with Buruli ulcer disease and matched healthy controls, but in the IgM class, 85% of Buruli ulcer patients tested positive, with only 4.5% of healthy controls testing false-positive to M. ulcerans culture filtrate proteins; none of the 25 patients with active tuberculosis tested false-positive. Clearly, these promising results justify further experiments, perhaps with more purified M. ulcerans antigens, and further testing in larger patient populations, with appropriate controls from endemic regions.

**Therapy**

Although surgical debridement, comprising radical excision of all necrotic tissues and a surrounding rim of normal tissue, followed by skin grafting, has been widely promoted and practised (1, 3), no formal studies have been done of surgical efficacy, recurrence rates, and functional limitations after healing. There is circumstantial evidence that local control is best achieved by radical excision and grafting, when compared with a more limited surgical approach (7). Excellent healing rates were achieved in the largest treatment centre in Benin (23). However, extensive surgery may unnecessarily damage healthy tissues, and it does not prevent recurrences. Even simple excision of early lesions carried a recurrence rate after one year of...
Physiotherapy should be considered as an important adjunct of surgery to prevent contractures (3). Other topical treatments have been proposed, including heat treatment (67), hyperbaric oxygen (67, 68), medicinal clay, phenytoin powder (69), and nitrite ointment. The case for hyperbaric oxygen treatment in *M. ulcerans* infection has its proponents but large-scale implementation in endemic regions is unlikely to be feasible. Heat treatment was proposed long ago, and seems simple and affordable, but has not been shown to have a large beneficial effect; in fact, the first report made mention of several recurrences (67). Nitrite ointment has been studied in 37 people with a clinical diagnosis of Buruli ulcer, and a beneficial clinical response was demonstrated (70). The disadvantage of all such localized treatments is that the antimicrobial effect may be incomplete. Recurrent infections are problematic, especially in immuno-compromised hosts and in patients with disseminated disease, as well as in those who have developed *M. ulcerans* osteomyelitis, which is not uncommon in Benin (23, 58). As patients with lesions involving joints are prone to develop contractures, these individuals may benefit most from physiotherapy.

### Conclusion

Buruli ulcer disease has re-emerged in the last decades in a number of west African countries. The causes of this emergence are unknown. The WHO Global Buruli Ulcer Initiative has been instrumental in developing and providing materials that have been used to improve early detection and intervention. Despite the progress made in recent years, major gaps remain. The reservoir of *M. ulcerans*, its mode of transmission to humans, and its immunopathogenesis are still poorly understood. Current treatment options discourage patients from reporting early; and no available treatment can prevent recurrence. Drug treatments need to be tested for effectiveness and toxicity, and some patients require surgery and physiotherapy — the long term outcomes of which also need further study. While antimycobacterial treatment may prove to be effective, studies are needed to ascertain that any such treatments can be used successfully in endemic areas. Improved treatment options would encourage patients who currently present late; afraid of mutilating surgery, reluctant to seek treatment outside their own community, and unable to cope with long, expensive hospitalisations (5). There is a dire need for better treatment and understanding of this devastating disease.

### Competing interests: none declared.

Résumé

**Infection à Mycobacterium ulcerans**

L’infection à *Mycobacterium ulcerans* (ulcère de Buruli) constitue un important problème sanitaire dans plusieurs pays d’Afrique de l’Ouest. Elle est prévalente sous forme de foyers dispersés à travers le monde, principalement dans les zones fluviales soumises à un climat humide et chaud. L’article examine l’épidémiologie, la bactériologie, la transmission, l’immunologie, la pathologie, le diagnostic et le traitement de cette infection. *M. ulcerans* est un microorganisme omniprésent. Il est hébergé par des poissons, des escargots et des insectes d’eau. Le mode de transmission n’est pas connu. Les lésions touchent le plus souvent les parties exposées du corps, en particulier les membres. Une guérison spontanée est possible. Dans les zones endémiques, de nombreux malades se présentent tardivement, avec des lésions à un stade avancé et grave. La vaccination par le BCG apporte une protection immunitaire limitée, de durée relativement courte. Le traitement recommandé consiste en un débridage chirurgical des lésions, suivi si nécessaire, d’une greffe de peau. De nombreux malades souffrent de limitations fonctionnelles une fois guéris. Pour combattre et prévenir plus efficacement l’ulcère de Buruli, il convient de mieux comprendre la transmission de cette maladie et sa pathogénèse.

**Infection à Mycobacterium ulcerans disease**
Resumen
Enfermedad por *Mycobacterium ulcerans*

La enfermedad causada por *Mycobacterium ulcerans* (úlcera de Buruli) constituye un grave problema de salud en varios países de África occidental, pero es frecuente en focos dispersos en todo el mundo, predominando en zonas fluviales con clima cálido y húmedo. Examinamos aquí la epidemiología, bacteriología, transmisión, inmunología, histopatología, diagnóstico y tratamiento de este tipo de infección. *M. ulcerans* es un microorganismo ubicuo que se alberga en peces, caracoles e insectos acuáticos. Se desconoce el modo de transmisión. Las lesiones aparecen sobre todo en las partes expuestas del cuerpo, en particular en los miembros. A veces se produce una curación espontánea. Muchos de los pacientes de las zonas endémicas acuden al médico cuando presentan ya lesiones muy avanzadas y graves. La vacunación con BCG confiere una protección inmunitaria limitada y relativamente breve. El tratamiento recomendado consiste en el desbridamiento quirúrgico, seguido de injerto cutáneo si es necesario. Muchos pacientes sufren limitaciones funcionales aun después de curados. Es preciso comprender mejor la transmisión y la patogenia de la enfermedad para poder prevenir y controlar más satisfactoriamente la úlcera de Buruli.