Unraveling an emerging disease associated with disturbed aquatic environments: the case of Buruli ulcer

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Buruli ulcer (***Mycobacterium ulcerans* infection) is an emerging disease of developing tropical and subtropical** countries. This environmental **mycobacterium causes severe morbidity in communities associated with rivers** and standing water bodies (**particularly those created as a result of** human **environmental disturbance such** as deforestation and agriculture) and **subsequent water quality changes.** Neither the reservoir nor the mode of transmission is known, but **data from laboratory studies suggest that** biting aquatic insects may be involved. *M ulcerans* has been shown to colonize and grow within specific water bugs (Naucoridae), which then transmit *M ulcerans* to mice through their bites. PCR evidence suggests that the mycobacterium is present in water, biofilms of aquatic plants, detritus, invertebrates, and fish; however, systematic ecological studies that would provide a more comprehensive understanding of *M ulcerans* distribution in the environment have been lacking. Several hypotheses are explored in relation to human impacts on aquatic food webs and *M ulcerans* ecology.

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"The ulcers were large and indolent, had widely undermined edges, and at the time were thought to be either tuberculous or syphilitic...The local people have a term, 'juwe okoro' or 'bile okoro', which means roughly 'the sore that heals in vain'" (Lunn *et al.* 1965).

We were wading in a turbid pond dressed in chest-high waders and latex arm gloves, and carrying dip nets to collect invertebrates. A woman gathered water next to us while her child was bathing. We were in a region of Africa near Accra, Ghana, an area endemic for Buruli ulcer (BU), a debilitating skin disease emerging in villages near aquatic habitats of western Africa and other tropical countries. Because some communities in Ghana have a 22% prevalence rate of BU (WHO 2000), we wondered whether the mother and/or child would become infected. For scientists this is a logical, albeit morbid, question, because the mode of transmission to humans remains unknown. The bacterium responsible for BU, *Mycobacterium ulcerans*, causes necrotic and sometimes massive, disfiguring ulcers that are occasionally mistaken for other tropical ailments (Horsburgh and Meyers 1997).

Historically, outbreaks of BU have been associated with domestic activities near rivers, marshes, human-made aquatic impoundments, and eutrophic water bodies adjacent to agricultural and deforested riparian lands (Figure 1). These habitats are home to many aquatic invertebrates, with some biting water bugs implicated in the transmission of *M ulcerans* to humans (Portaels *et al.* 1999, 2001; Marsollier *et al.* 2002b, 2003, 2004a). Our research was aimed at determining whether certain invertebrates could be potential reservoirs or vectors of *M ulcerans*, and how disturbed aquatic environments are related to BU incidence.

In 1998, the World Health Organization (WHO) established the Global Buruli Ulcer Initiative in response to the widespread prevalence and reported occurrence of this disease. The charge of the Initiative was to recognize BU as a public health problem, advocate support for endemic countries, seek partnerships for control and research, and coordinate global research efforts. As a result of this initiative, the 57th World Health Assembly adopted a resolution in 2004, encouraging participation in the Buruli Ulcer Initiative and intensifying research into diagnosis and prevention (WHO 2004b). Both the Initiative and resolution have spurred research interest, leading to a rapid increase in BU-related publications (Figure 2).

In a nutshell:

- After tuberculosis and leprosy, Buruli ulcer is the most common mycobacterial infection of humans
- Buruli ulcer is associated with rivers, swamps, and human-linked changes in the aquatic environment, such as dam construction and agriculture
- The source of infection and mode of transmission are unknown, but aquatic insects and other invertebrates are suspected reservoirs
- Understanding interactions between environmental disturbance and *Mycobacterium ulcerans* ecology may be important for identifying transmission pathways of Buruli ulcer

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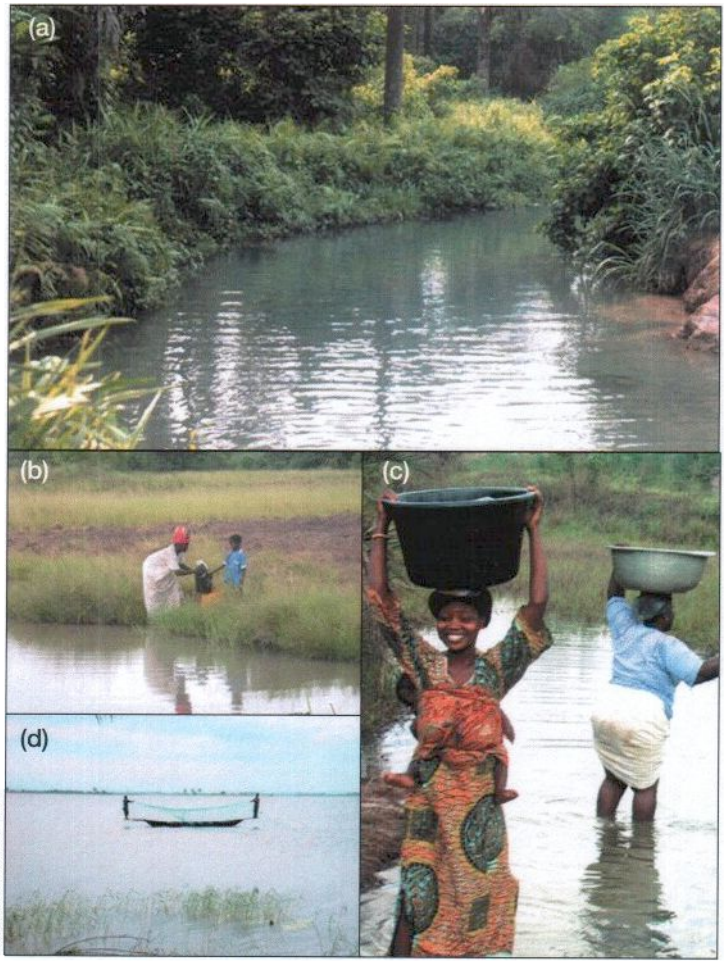

Photo (a) courtesy of P Elsen

Figure 1. *(a) A river in Benin, Africa, representative of common habitats associated with local incidence of Buruli ulcer. Other common aquatic habitats related to Buruli ulcer outbreaks in Ghana, Africa, include (b) ponds, (c) wetlands, and (d) lakes. Note that most habitats are frequently used for domestic purposes such as bathing, washing linens, and fishing.*

In this article, we review the history, background, and current research status of M *ulcerans* infection, more commonly referred to as Buruli ulcer disease. Our goals are to provide a synthesis of literature regarding the current status of BU and to identify research gaps in our understanding of the ecology of this emerging disease. We present hypotheses regarding the potential effects of environmental degradation and anthropogenic development on aquatic food webs and M *ulcerans* ecology. These factors have not been studied, but may provide suitable ecological conditions necessary for the proliferation, transmission, and infection of M *ulcerans* from aquatic invertebrate reservoirs to human hosts.

■ History

Although Buruli ulcer is the most common name of M *ulcerans* infection, it has also been referred to as Bairnsdale, Searles, and Kumusi ulcer, depending on the geographic region where it was historically reported (Radford 1974; Horsburgh and Meyers 1997). It is also sometimes called the "Mysterious Disease". In 1897, Sir Albert Cook documented patients with ulcers, described as what is now thought to be clinical BU (Horsburgh and Meyers 1997; WHO 2000). However, BU was first clinically identified in Australia during the late 1930s and 1940s as Bairnsdale ulcer (MacCallum *et al.* 1948), but was renamed after the Buruli district in Uganda following a surge of reported cases in the 1960s and 1970s (Uganda Buruli Group 1971). Throughout the 20th century, cases were consistently and increasingly reported from around the world, and during the past decade there has been both a rise in prevalence rates and an expanding geographic distribution, independent of increased surveillance (Horsburgh and Meyers 1997; WHO 2000; Amofah *et al.* 2002). Although the global extent of this disease is unknown, the worldwide burden is probably grossly underestimated, due to lack of reporting, difficult access to healthcare for infected individuals, and localized outbreaks in rural areas of developing nations (WHO 2000).

■ Disease significance and burden

Buruli ulcer is the third most frequent mycobacterial disease in humans after tuberculosis (TB) and leprosy, often causing serious ulcerations, deformities, and disability (Meyers 1995; Meyers *et al.* 1996; Asiedu and Etuaful 1998; Thangaraj *et al.* 1999; Portaels *et al.* 2001; WHO 2001). Unlike TB and leprosy, however, BU is a poorly understood disease, even though cases are reported from at least 31 countries, spanning Africa, Australia, Asia, Latin America, and the Western Pacific (Hayman 1991b, 1991a; Johnson *et al.* 1999; WHO 2001; Figure 3). Isolated cases, usually resulting from international travel, have also been reported in non-endemic areas such as North America and Europe (WHO 2001).

Buruli ulcer incidence is highest among developing West African nations (WHO 2001), with cases in some countries exceeding those of TB and leprosy (Amofah *et al.* 1993, 2002). Up to 16% of villages are affected in Côte d'Ivoire (Marston *et al.* 1995; WHO 2001), and Benin has recorded 4000 cases since 1989 (Lagarrigue *et al.* 2000). A 1999 national survey in Ghana identified over 6000 cases, making BU the second most prevalent mycobacterial disease (after TB) in that country (Amofah *et al.* 2002). In West Africa, nearly 25% of people infected are left permanently disabled (Johnson *et al.* 2005).

The impact of BU extends beyond the suffering of infected individuals. Often, family members will be

forced to stay away from home for long periods of time in order to care for afflicted kin, greatly reducing income generation. Moreover, families are often required to provide subsistence for the patient, inflating the high treatment costs that nearly always exceed average annual family income. The economic burdens of the disease therefore have potentially large negative effects on both the local economy and community development (Asiedu and Etuaful 1998; Thangaraj *et al.* 1999; Debacker *et al.* 2004b).

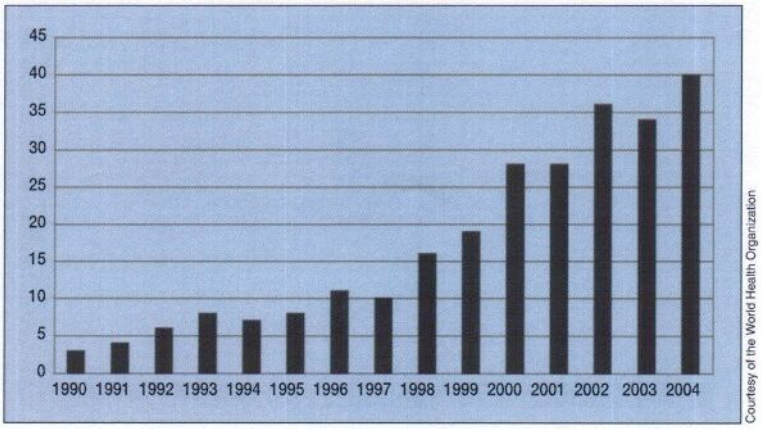

Figure 2. The number of Medline cited publications related to Buruli ulcer disease by year from 1990–2004. The Global Buruli Ulcer Initiative was created in 1998, reflecting a substantial and lasting increase in publications from 1998 to 2004.

■ Clinical features

Buruli ulcer presents in several ways (Figure 4). Most ulcers occur on the extremities, with lesions on the lower extremities being almost twice as common as on the upper body and arms (Marston *et al.* 1995; WHO 2000). The infection may begin with a painless raised skin lesion (papule), or with a hard subcutaneous nodule. Infection extends from the skin into the subcutaneous tissue and often invades underlying muscle tissue. In other cases, BU presents as an extensive area of edema or swelling. Tissue underlying these areas of edema is necrotic and the edematous region usually breaks down to form a large ulcer. The disease may also present as a firm, painless plaque of a well-demarcated lesion with irregular reddened or discolored edges (Figure 4). Ulceration can be extensive and disfiguring, often affecting 50% or more of a limb (Thangaraj *et al.* 1999; WHO 2001). Severe complications can arise with untreated lesions, including contracture deformities, amputation, and infections of the eye, breast, and genitalia, resulting in substantial social stigma and taboos associated with the disease (WHO 2001; Stienstra *et al.* 2002).

■ Diagnosis and treatment

Mycobacterium ulcerans is slow-growing and takes 2–6 months to form visible colonies on solid medium following primary isolation (WHO 2001). It grows optimally within the temperature range of 30–33°C (Horsburgh and Meyers 1997; WHO 2001), and many strains are incapable of growth at the normal core body temperature

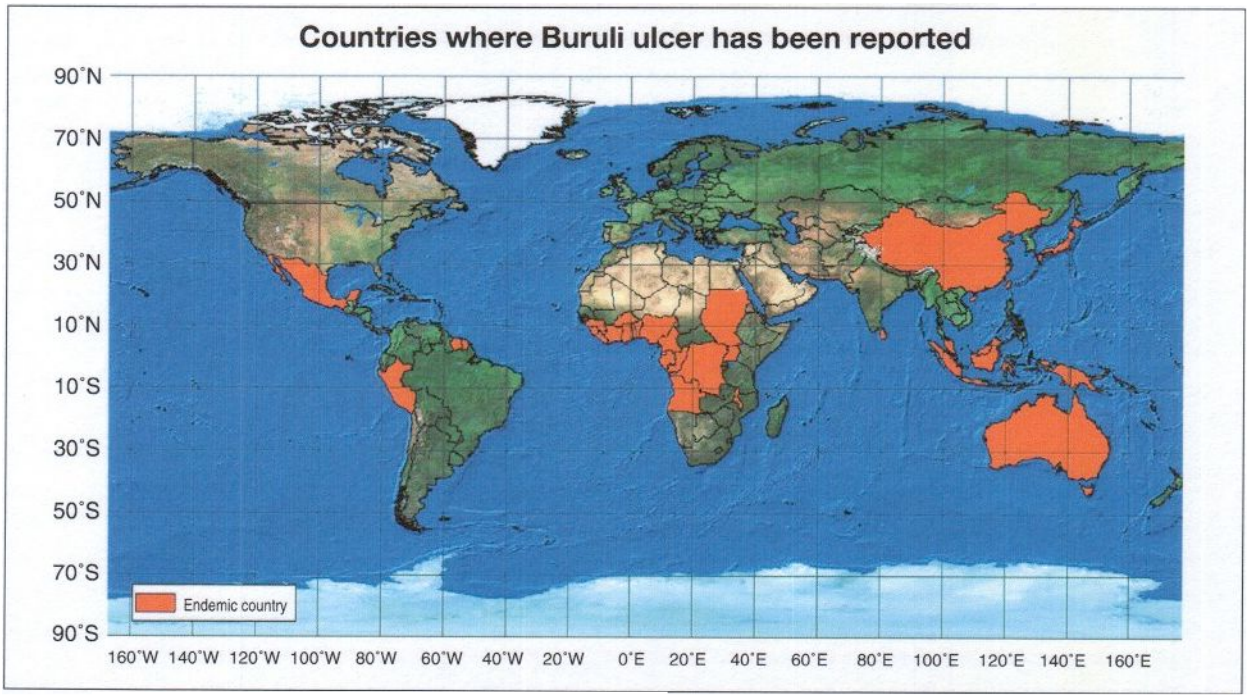

***Figure 3.** A global map representing countries that have reported cases of Buruli ulcer disease as of 2004.*

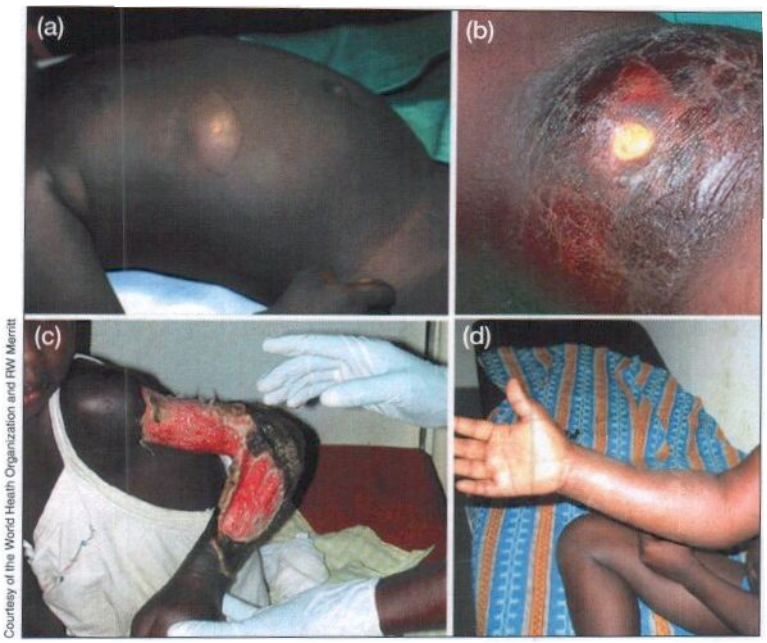

Figure 4. *Clinical symptoms of* M ulcerans *disease. (a) A nodule on the abdomen of a young child, (b) a plaque, (c) an ulcer covering most of a young child's arm, and (d) non-ulcerative edematous swelling of a child's hand.*

of 37°C (Uganda Buruli Group 1971). The restricted growth temperature is thought to limit infection to the cooler body extremities (Marston *et al.* 1995; WHO 2001). Growth is most efficient under low oxygen conditions (Horsburgh and Meyers 1997; Palomino and Portaels 1998), and ultraviolet light (UV) lowers cell viability (WHO 2000).

Many of the pathological features of BU are mediated by a polyketide-derived macrolide exotoxin called mycolactone, which is cytotoxic and immunosuppressive (George *et al.* 1999; Johnson *et al.* 1999). Early visual detection is limited because M *ulcerans* pathology shows nonspecific clinical manifestations (WHO 2000). The ulcers are usually painless until secondary bacterial infection sets in, with no constitutional symptoms or lymph node swelling. The burulin skin test (a new skin test involving an antigen for M *ulcerans*) is often ineffective because early active cases are typically burulin negative; however, skin is usually positive during healing and recovery (WHO 2000). Later detection through history or physical examination is useful for later-stage diagnosis using clinical features (WHO 2000, 2001). Because M *ulcerans* is slow growing, pure culture is difficult and biopsies are often inconclusive for acid-fast bacilli, making microscopic detection difficult (Ross *et al.* 1997; Guimaraes-Peres *et al.* 1999). Rapid diagnostic techniques using PCR methods show promise, and several have recently been improved (Ross *et al.* 1997; Guimaraes-Peres *et al.* 1999; Stienstra *et al.* 2003). A new dry-reagent PCR technique has shown good results (Siegmund *et al.* 2005). The WHO recommends that at least two of the above tests be used to confirm initial diagnosis of BU.

Several antimycobacterial agents have in vitro activity, but no single compound has proven regularly useful for treatment (WHO 2000). Recent studies suggest that combinations of anti-mycobacterial antibiotics (eg rifampicin and streptomycin) can kill M *ulcerans* cultured from human lesions (Johnson *et al.* 2005). Heat treatment and hyperbaric oxygen therapy have also had some success, but neither is practical for implementation in rural areas. The mainstay of treatment is surgical excision, but many patients do not seek medical attention until there is considerable tissue destruction, which often requires skin grafting (Thangaraj *et al.* 1999; WHO 2001).

■ Epidemiology

Buruli ulcer can affect all age groups, although children 15 years and younger are at proportionally higher risk (WHO 2000; Aiga *et al.* 2004; Debacker *et al.* 2004a; WHO 2004a). There is no evidence to suggest direct human transmission, nor that HIV-infected individuals are more susceptible (Johnson *et al.* 1999; WHO 2000). There have been a few reports of gender differences, but it is generally accepted that both sexes are equally affected (WHO 2000).

The overwhelming epidemiological trend for BU incidence is human activity associated with water bodies (Barker and Carswell 1973; Radford 1975; Hayman 1991b, 1991a; Horsburgh and Meyers 1997; Thangaraj *et al.* 1999; WHO 2001; Aiga *et al.* 2004; Duker *et al.* 2004; Raghunathan *et al.* 2005), but these observations have never been rigorously tested in systematic or quantitative studies. In a recent case-control study from Ghana, Aiga *et al.* (2004) showed that swimming and activities on riverbanks were important risk factors, and this was confirmed by another research group (Raghunathan *et al.* 2005). Both found that swimming in rivers was a decisive water-related risk factor. However, Marston *et al.* (1995) showed that activities such as swimming and fishing were not significant, although other domestic water-related activities were associated with increased risk. Disease prevalence was higher in villages close to the river. Swimming was not clearly defined in these studies, which makes interpretation of conflicting results difficult. Nevertheless, activities in aquatic habitats are clearly related to BU incidence.

It has been reported that BU outbreaks result from: (1) unprecedented flooding of lakes and rivers during heavy rainfall; (2) the damming of streams and rivers to create artificial lakes and wetlands; (3) resorts (eg recreational facilities) that modify wetlands; (4) deforestation practices leading to increased flooding; (5) agricultural practices and associated irrigation systems; and (6) population

expansion, resettlement, and migration closer to water bodies that put more people at risk (Hayman 1991b; Marston *et al.* 1995; Meyers *et al.* 1996; Johnson *et al.* 1999; Portaels *et al.* 2001).

Many water bodies associated with increased sedimentation and eutrophication have low dissolved oxygen, which has been shown to enhance M *ulcerans* growth (Palomino *et al.* 1998). Hayman (1991b) speculated that M *ulcerans* enters surface waters through deforestation and run-off contamination where environmental conditions facilitate growth and proliferation, much like an algal bloom. But because of the slow growth rate, the author speculated that it takes from several weeks to months to achieve population numbers that increase human infection rates (Hayman 1991b).

Based on more recent evidence, we hypothesize that M *ulcerans* is part of the normal epiphytic microbial flora (ie biofilms on aquatic plants) and is maintained in relatively low numbers through competitive interactions with other microbes and algae in undisturbed conditions. We suggest that increased sediment runoff changes water quality (eg increasing nutrients such as phosphorus) and vegetation conditions in a manner that increases the competitive advantage of M *ulcerans*, leading to increased abundance in these habitats. The mechanism of this potential competitive advantage has not been previously studied.

Human impacts such as deforestation lead to lost riparian cover, resulting in increased water temperatures that may facilitate bacterial growth. Associated sedimentation (and dissolved organics) might increase UV attenuation and protect submerged M *ulcerans* biofilms. Thus, deforestation and high-impact agriculture may promote increased nutrients, higher water temperatures, lower UV penetration, and lower dissolved oxygen – environmental conditions known to facilitate M *ulcerans* growth (Horsburgh and Meyers 1997; Palomino *et al.* 1998; WHO 2000).

Increased human activities (eg mining) and urban development remove riparian forests and also change water quality through both point and non-point source pollution; these factors may in turn be tied to ecological changes and BU incidence. Duker *et al.* (2004) found significant spatial relationships among villages in BU-affected areas and arsenic-enriched surface waters and adjacent farmlands. The authors suggested that increased BU risk was related to immunosuppression resulting from the consumption of arsenic-enriched drinking water and food crops. This hypothesis has not been clinically tested.

Although there have been reports of a seasonal distribution in BU cases related to rainfall-influenced patterns in village use of water bodies (Revill and Barker 1972), it is difficult to understand changes in foci because of the long and variable incubation period of the disease (Meyers *et al.* 1974). Correlating cases with particular seasons without adjusting for this lag is therefore difficult. In a study that examined the distribution of cases in relation to domestic water sources, it was shown that the number of cases increased in families using permanent (53%) compared to seasonal (25%) swamps and boreholes (6%) (Barker and Carswell 1973).

In early studies, cases were spatially associated with the regional distribution of an aquatic plant (*Echinocloa pyramidalis*) typically found in rivers and swamps in Uganda (Barker 1972; Barker *et al.* 1972). However, these studies were descriptive and anecdotal. Meyers *et al.* (1974) and Barker *et al.* (1972) speculated that direct inoculation resulting from contact with vegetation might occur, but neither study directly tested this hypothesis. More recently, a case-control study by Marston *et al.* (1995) found that farming activities near the Lobo River in Daloa, Côte d'Ivoire, was a significant risk factor for the disease. The authors also reported that wearing long pants substantially lowered the risk of disease, but they did not speculate about the potential mechanisms of protection.

Taken together, these studies imply transmission of M *ulcerans* via skin contact with aquatic plants and/or water through lesions related to skin trauma or, arguably, through insect bites (see below; Portaels *et al.* 1999; Marsollier *et al.* 2002b, 2004a; Kotlowski *et al.* 2004). Research is urgently needed to identify the natural reservoirs of this mycobacterium and to focus on how its distribution and abundance are affected by environmental disturbances.

■ Environmental reservoirs and transmission

Direct human transmission of M *ulcerans* has never been reported, with the exception of a single infection following a human bite (Debacker *et al.* 2003). To date, epidemiological studies have not identified specific vectors or reservoir species, and there is only anecdotal information on the ecology of endemic water bodies routinely used by local villages (WHO 2000; Portaels *et al.* 2001).

Non-human mammals and reptiles have tested negative (Radford 1974), as have several arthropods (ie bedbugs, black flies) known to vector other disease agents (Revill and Barker 1972; Portaels *et al.* 2001). However, only a few populations of these arthropods, taken from localized areas, have been screened. Cases in wild and domesticated animals from Australia (ie koalas, possums, and an alpaca) confirm that M *ulcerans* can infect non-human vertebrates (Mitchell *et al.* 1984), but such infections have not been reported from other endemic regions.

In Australia, it has been postulated that aerosols arising from contaminated water may disseminate M *ulcerans* and infect the host via the respiratory tract, or through skin lesions and minor abrasions (Hayman 1991a), but this has not been proven. The reported cases were associated with the installation of an irrigation system that drew water from a human-made aquatic impoundment that tested PCR positive for M *ulcerans* (Stinear *et al.* 2000a).

Portaels *et al.* (1999) suspected that aquatic bugs (Hemiptera) could be reservoirs of M *ulcerans*. A study of 123 aquatic insects of the orders Hemiptera (water bugs), Odonata (dragonfly larvae), and Coleoptera (beetle lar-

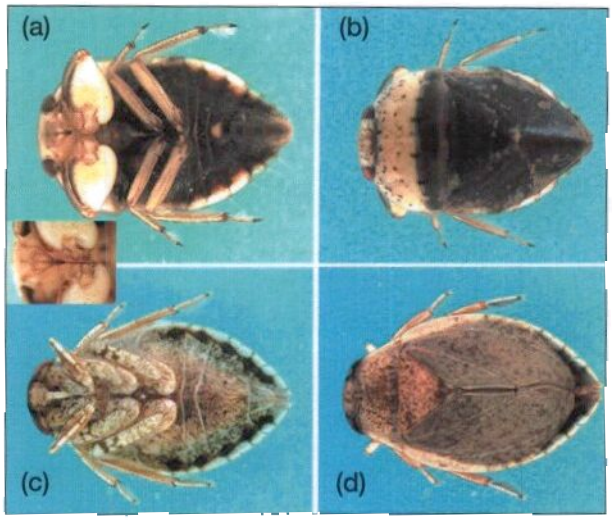

Figure 5. *Two families of Hemiptera that have tested positive for* M uclerans. *(a) Ventral and (b) dorsal views of* Macrocoris *sp (Naucoridae), 1.0 cm body length. (c) Ventral and (d) dorsal views of* Appasus *sp (Belastomatidae), about 2.5 cm body length. The inset shows the piercing stylet of* Macrocoris *sp.*

vae) collected in endemic swamps confirmed their findings; further research has demonstrated that small fish also test positive for M *ulcerans* (Portaels *et al.* 2001; Eddyani *et al.* 2004; Kotlowski *et al.* 2004). Marsollier *et al.* (2002a,b, 2004a) demonstrated experimentally that M *ulcerans* could survive and multiply within the salivary glands of aquatic bugs (Naucoridae: *Naucoris cimicoides*; Figure 5). They also showed that *N cimicoides* could transmit the mycobacteria by feeding on inoculated insect prey and then biting mice, following which the mice presented with clinical BU (Marsollier *et al.* 2002b). Interestingly, even after identical exposure to other mycobacteria (M *marinum*, M *fortuitum*, and M *kansasii*), none were found in the salivary glands of the bugs.

The role of non-insect aquatic invertebrates as intermediate hosts for M *ulcerans* has been suggested by several authors (Portaels *et al.* 1999; Marsollier *et al.* 2002b, 2003; Kotlowski *et al.* 2004). It was experimentally confirmed that aquatic snails could acquire M *ulcerans* after feeding on inoculated aquatic plant biofilms; cells of M *ulcerans* were found in the guts and feces of grazing snails (Marsollier *et al.* 2004a). Aquatic plant extracts stimulated biofilm formation, and doubled the growth rate of M *ulcerans* in laboratory experiments (Marsollier *et al.* 2004b). In the field, Kotlowski *et al.* (2004) recorded M *ulcerans* in aquatic snails from endemic regions of Ghana and Benin, and other studies reported that average estimates of M *ulcerans* increased by two orders of magnitude in detritus compared to water (Stinear *et al.* 2000a). Based on these studies, it is evident that M *ulcerans* is associated with aquatic plants, within biofilm on the plant surface, and as part of decaying organic matter, all of which serve as food for certain aquatic invertebrates and fish, suggesting reservoirs and movement throughout the aquatic food web.

A recent finding in coastal Australia linked a localized human BU outbreak to a major emergence of the southern salt marsh mosquito (*Ochlerotatus camptorhynchus*). Scientists investigating this outbreak identified M *ulcerans* by PCR in a small number of adult salt marsh mosquitoes from the outbreak area. However, localization of M *ulcerans* within these mosquitoes and the role they may play in human transmission have yet to be established (PD Johnson pers comm).

■ Conceptual model of *M ulcerans* in nature

We suggest a possible scenario (Figure 6), expanded and modified from an original hypothesis proposed by Portaels *et al.* (1999), for the movement of M *ulcerans* among aquatic reservoirs, as well as potential routes of inter-water body dispersion unrelated to aquatic habitat connections (eg flooding). This is a hypothetical scenario, intended to provide the conceptual framework for developing and testing explicit hypotheses in future research endeavors. It is based on the few environmental studies identifying M *ulcerans* in nature and also in suspected, albeit less than adequately tested, reservoirs and modes of movement in the aquatic food web.

First, M *ulcerans* has been reported from mud, detritus, water column, and plant biofilms. Secondly, aquatic insects (eg midges, mosquito larvae) or other invertebrates (eg snails, crustaceans, plankton) may acquire and concentrate mycobacteria through feeding activities (grazing, gathering, and/or filtering fine particles). Thirdly, predatory aquatic vertebrates such as fish and invertebrates (eg bugs, beetles, and dragonfly larvae) feed on other invertebrates or small fish, serving to move M *ulcerans* from prey to biting insects. Finally, fish, adult aquatic insects capable of flight, and birds that prey on fish and/or aquatic invertebrates may potentially disseminate M *ulcerans* to other aquatic habitats that have the necessary environmental characteristics. If terrestrial vertebrates are found to be reservoirs, then fecal droppings may also be an alternate mode of inter-water body dissemination.

Certain water bugs (eg Naucoridae, Belostomatidae) are aggressive predators of aquatic invertebrates and fish, but often also bite humans and other animals visiting freshwater habitats (Merritt and Cummins 1996). In this way, they may be mechanically inoculating M *ulcerans* into the skin, or depositing cells onto the skin surface near lesions or minor abrasions. If insects are confirmed to transmit M *ulcerans* to humans, it would be the first report of insect-mediated transmission of a mycobacterial disease (Portaels *et al.* 1999). Alternatively, individuals with recent skin lesions may be inoculated as a result of contact with infected plant biofilms or suspended sediment particles in water.

■ Future research directions

Most field studies of BU have been short term, and were conducted in disparate regions of the world. No studies

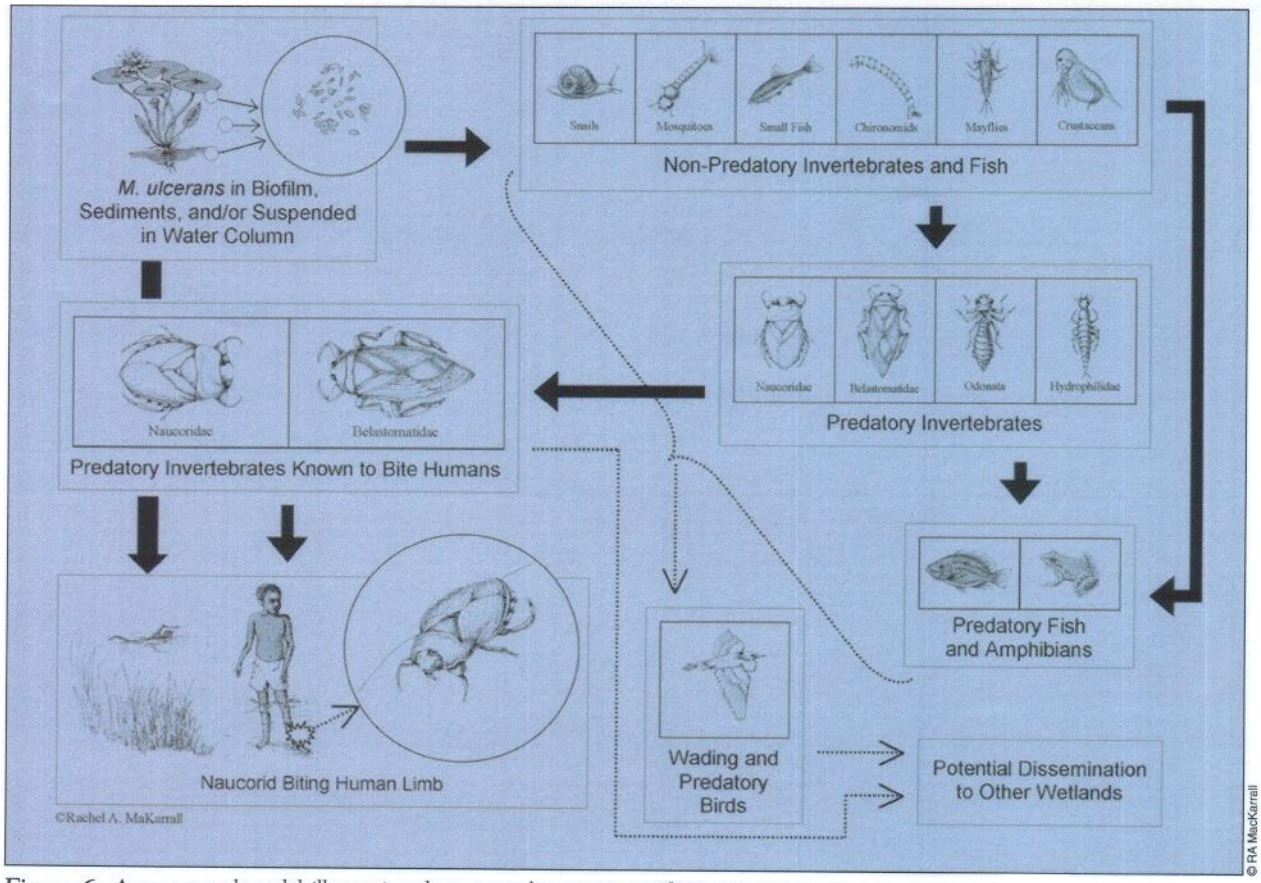

Figure 6. *A conceptual model illustrating the potential reservoirs and movement of* Mycobacterium ulcerans *within and among aquatic environments. The dark arrows indicate potential movement directions within a water body; dashed lines and arrows represent potential dissemination pathways to other water bodies. This model has been expanded and modified from an earlier hypothesis proposed by Portaels* et al. *(1999).*

have attempted to quantify the geospatial and ecological distribution of *M ulcerans* in nature. Specific hypotheses are necessary to direct future research priorities, and we propose the following:

H_1 – Poor water quality influences biological communities, leading to increased growth and proliferation of *M ulcerans* in aquatic habitats.
H_2 – Invertebrates and fish concentrate *M ulcerans* through feeding and act as environmental reservoirs.
H_3 – Biting aquatic insects infected with *M ulcerans* can act as transmission vectors to humans.
H_{A1} – Direct contact of open lesions with infected vegetation biofilm and suspended sediment in water leads to human infection.
H_{A2} – Humans become infected from aerosols of water originating from *M ulcerans*-contaminated water bodies.

From these hypotheses, we propose the following ecological research priorities: (1) describe the aquatic biodiversity and food webs of endemic versus non-endemic water bodies using standardized methods and analyses; (2) systematically establish the distribution of *M ulcerans* within and among food webs; (3) experimentally identify *M ulcerans*–invertebrate interactions and transmission; (4) determine terrestrial–aquatic linkages of landscape disturbance and water quality, ecological condition, and disease occurrence using GIS and remote sensing technologies; (5) spatially map and model quantitative patterns of human BU infection with *M ulcerans* distribution along land-use (ie ecological) disturbance gradients; and (6) determine geographic differences (eg Africa versus Australia) in the ecology of endemic water bodies.

A first step to testing the hypotheses is to conduct systematic and quantitative studies identifying landscape changes that define ecological attributes along gradients of landscape disturbance (Figure 7). These studies should focus on the distribution of *M ulcerans* within and among aquatic habitats and geographic regions, *M ulcerans*–invertebrate interactions, and how organisms might facilitate the movement of *M ulcerans* in the environment; they should also provide data for modeling how all of these factors relate to human infection incidence (Figure 7).

While comprehensive studies on the ecology of *M ulcerans* are necessary, additional information on the

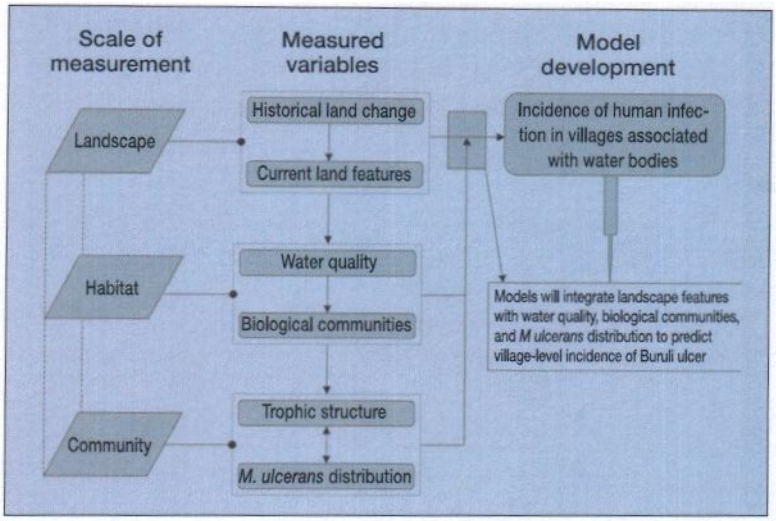

Figure 7. A flow diagram showing the scale of measurement and linkages of potential research interest for determining ecological connections important to BU incidence and future control efforts. Studies identifying and linking landscape features that influence water quality through habitat-scale biological communities and food web organization with community-level M ulcerans *distribution can be used to develop models for predicting human incidence rates in villages associated with water bodies where these variables have been quantified.*

basic microbiology and molecular pathology of M *ulcerans* is needed to test transmission models. Without tools to accurately identify M *ulcerans* from environmental samples, repeatable transmission experiments and ecological distribution analyses cannot be conducted. M *ulcerans* biovars show geographic heterogeneity (Portaels *et al.* 1996; Stinear *et al.* 2000a, 2000b), and M *ulcerans* from different geographic regions (ie Africa and Australia) produce different forms of the mycolactone toxins that may be relevant both to the pathology and epidemiology of infection (Mve-Obiang *et al.* 2003). Furthermore, a connection has only very recently been made between PCR-positive insects in these two continents.

By better understanding specific water-body systems and how human land-use disturbances affect the ecological interactions associated with Buruli ulcer, scientists will be able to provide the missing epidemiological links necessary to unravel the mysteries of this emerging disease.

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