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Mycobacterium ulcerans in wild animals

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Summary

Mycobacterium ulcerans infection, or Buruli ulcer, is the third most frequent mycobacterial disease in humans, often causing serious deformities and disability. The disease is most closely associated with tropical wetlands, especially in west and central Africa. Most investigators believe that the aetiological agent proliferates in mud beneath stagnant waters. Modes of transmission may involve direct contact with the contaminated environment, aerosols from water surfaces, and water-dwelling fauna (e.g. insects). Person-to-person transmission is rare. Trauma at the site of skin contamination by *M. ulcerans* appears to play an important role in initiating disease. Once introduced into the skin or subcutaneous tissue, *M. ulcerans* multiplies and produces a toxin that causes necrosis. However, the type of disease induced varies from a localised nodule or ulcer, to widespread ulcerative or non-ulcerative disease and osteomyelitis.

Although culture of *M. ulcerans* from a patient was first reported in 1948, attempts to culture the mycobacterium from many specimens of flora and fauna have been unsuccessful. Failure to cultivate this organism from nature may be attributable to inadequate sampling, conditions of transport, decontamination and culture of this fastidious heat-sensitive organism, and to a long generation time relative to that of other environmental mycobacteria. Nevertheless, recent molecular studies using specific primers have revealed *M. ulcerans* in water, mud, fish and insects. Although no natural reservoir has been found, the possibility that *M. ulcerans* may colonise microfauna such as free-living amoebae has not been investigated.

The host range of experimental infection by *M. ulcerans* includes lizards, amphibians, chick embryos, possums, armadillos, rats, mice and cattle. Natural infections have been observed only in Australia, in koalas, ringtail possums and a captive alpaca. The lesions were clinically identical to those observed in humans. *Mycobacterium ulcerans* infection is a rapidly re-emerging disease in some developing tropical countries. The re-emergence may be related to environmental and socioeconomic factors, for example, deforestation leading to increased flooding, and population expansion without improved agricultural techniques, thus putting more people at risk. Eradication of diseases related to these factors is difficult. Whether wild animals have a role in transmission is an important question that, to date, has been virtually unexplored. To address this question, surveys of wild animals are urgently required in those areas in which Buruli ulcer is endemic.

Keywords

Alpacas – Aquatic insects – Buruli ulcer – Environment – Fish – Koalas – Mycobacteria – Mycobacterium ulcerans – Possums – Water.

Introduction

Epidemiology and transmission

Mycobacterium ulcerans infection, also known as Buruli ulcer (BU), is the third most frequent mycobacterial disease in humans, after tuberculosis and leprosy. The causative agent, *M. ulcerans*, was first described in 1948 by MacCallum *et al.*, in Australia (22). Previously, the disease had been reported in Africa by Sir Albert Cook, who described large ulcers, almost certainly caused by *M. ulcerans*, in Uganda in 1897 (25). The disease was later described in a group of patients from Buruli County, Uganda, by Clancey *et al.*, who named the disease 'Buruli ulcer' (10).

In recent times, BU has emerged as an increasingly important cause of morbidity world-wide, partly related to environmental changes. In 1998, the World Health Organization (WHO) recognised BU as an important public health problem, and established the Global BU Initiative (51).

In the 1990s, increasing numbers of cases have been reported from West Africa (Benin, Ghana, Côte d'Ivoire, Togo, Guinea, Liberia, Burkina Faso and Nigeria) and from Australia and Papua New Guinea (51). Buruli ulcer has been reported from at least twenty-seven countries world-wide, principally in tropical areas (51). A few cases have also been reported in non-tropical areas, including the People's Republic of China (12), Japan (47, 48) and south Australia (20).

Seasonal variations in the frequency of the disease have been reported in Uganda (41), Papua New Guinea (39), Cameroon (40) and Australia (18).

The disease is not contagious, and modes of transmission remain unclear. Aerosols may carry *M. ulcerans* and infect the host via the respiratory tract or contaminate the skin surface (19). Trauma is probably the most frequent means by which *M. ulcerans* is introduced deep into the skin or subcutaneous tissue from the contaminated surface of the skin (26).

The epidemiology of BU is poorly understood. Some evidence exists for an environmental reservoir associated with slow-flowing or stagnant water (34). However, culture of *M. ulcerans* from the environment has never been successful (34).

Studies of mycobacterial flora from various natural habitats in central Africa suggested that *M. ulcerans*, in common with other mycobacterial species, is present in very low concentrations in some natural wetlands (slow-flowing streams, stagnant ponds or swamps) of endemic regions (33). More recently, epidemiological data and molecular findings have been employed to investigate the source of *M. ulcerans*. Direct polymerase chain reaction (PCR) has been used to identify *M. ulcerans* in water and mud in Australia (42, 43, 44, 46).

Clinical manifestations

Clinically, BU is primarily a disease of the skin. Two broad categories of active clinical forms are recognised, namely: non-ulcerative (papules, nodules, plaques and oedematous forms) and ulcerative disease. A typical ulcer is illustrated in Figure 1.

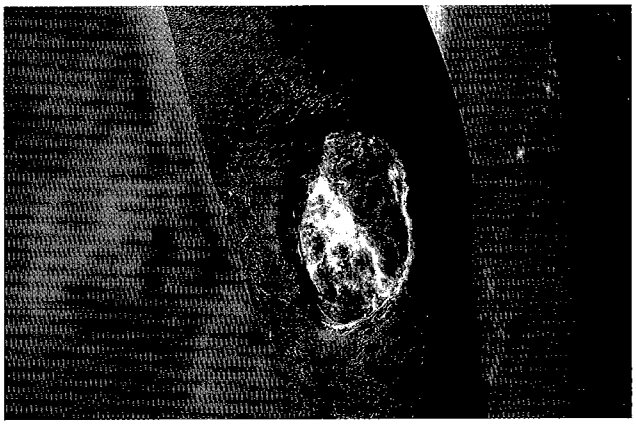

Fig. 1
Ulcerative lesion with typical undermined edges on the right leg of a boy aged twelve years from Togo
Photo: W.M. Meyers

Lesions are usually single and initially appear as firm, painless, non-tender, movable, subcutaneous nodules of 1 cm to 2 cm in diameter. Many patients complain of itching in the lesion. After one to two months, the nodule may become fluctuant and ulcerate, with an undermined edge of 15 cm or more in length. The skin adjacent to the lesion, and often that of the entire affected limb, may be indurated by oedema.

No regional lymphadenopathy or systemic manifestations are usually noted. However, it has been speculated that massive disseminated lesions may cause systemic toxic effects. Ulcers may remain small and heal without treatment, or may spread rapidly, undermining the skin over large areas, even an entire leg, thigh or arm. Important structures such as the eye, breast or genitalia are sometimes lost or severely damaged. Osteomyelitis may necessitate amputation or lead to other crippling disabilities. Most lesions heal spontaneously, but frequently leave extensive scarring and deformity, unless appropriate therapy is provided.

In some major foci, such as that in Benin, up to 10% of patients suffer severe osteomyelitis which frequently leads to amputation (21). A serious, life-threatening, disseminated form also exists, in which necrosis of massive areas of skin occurs, for example over the entire trunk of the body (1) or an entire limb.

A provisional classification of active *M. ulcerans* lesions can be proposed and is outlined in Figure 2. Contrary to the connotation of the name Buruli 'ulcer', microbiological and

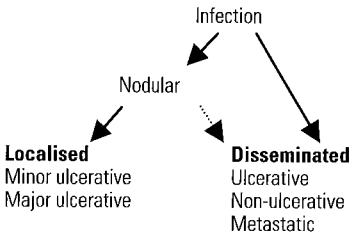

Fig. 2
Classification of active *Mycobacterium ulcerans* lesions

histopathological analyses of the cases from Benin confirm that non-ulcerative disease is now more frequent than ulcerative forms (60% in 1999, versus 40% in 1996) (F. Portaels, M. Debacker, A. Guédénon, W.M. Meyers, C. Zinsou and J. Aguiar, unpublished data). Inactive ulcerative forms are characterised by depressed stellate scars with or without sequelae such as contraction deformities.

Pathogenesis and pathology

After inoculation into the skin, *M. ulcerans* proliferates and produces a toxin that causes necrosis of the dermis, panniculus and deep fascia. Studies by George *et al.* have established that a mycolactone (a polyketide-derived macrolide) is responsible for the cytotoxic effects observed in BU lesions (13, 14).

More recent studies have suggested that phospholipase C (15) and haemolysis (16) may also play a role in the pathogenesis of BU. Early lesions are closed, but as the necrosis spreads, the overlying dermis and epidermis eventually ulcerate, with undermined edges and a necrotic slough in the base of the ulcer.

Histopathological sections reveal a contiguous coagulation necrosis of the deep dermis and panniculus, with destruction of nerves, appendages and blood vessels. Interstitial oedema is noted. Clumps of extracellular acid-fast bacilli are abundant and are frequently limited to the base of the ulcer and adjacent necrotic subcutaneous tissue. Bone is sometimes involved, and specific osteomyelitis is common. Histologically, local and regional lymphadenitis may be observed, with invasion by *M. ulcerans*. In active lesions, inflammatory cells are conspicuously few, presumably as a result of the immunosuppressive activity of the toxin. In the course of healing, a granulomatous response occurs with fibrosis, and the ulcerated area is eventually replaced by a depressed scar. Although information on the topic is limited, human immunodeficiency virus (HIV) infection does not appear to predispose to BU or render infection with *M. ulcerans* more aggressive.

Buruli ulcer in wild animals and humans

Since domestic and wild animals can be exposed to the same *M. ulcerans*-contaminated environment as humans, animals would be expected to become contaminated or infected. However, very few reports exist of natural infection in animals.

This paper describes investigations which detected the presence of *M. ulcerans* in wild animals and reports cases of disease due to *M. ulcerans* in wild animals. The discussion is therefore divided into the following two main sections:

a) detection of *M. ulcerans* in wild animals
b) *M. ulcerans* infection in wild animals.

Detection of *Mycobacterium ulcerans* in wild animals

The discovery of cases of BU in humans in some endemic regions, and the suspicion that the source of *M. ulcerans* is the environment, have led several investigators to propose certain wild animals as possible reservoirs of *M. ulcerans*. Several studies have been conducted to investigate the role of these animals in the epidemiology of *M. ulcerans*.

Vertebrates

In endemic areas, a wide variety of aquatic and non-aquatic vertebrates that share the same environment as humans have been examined. Mammals, frogs, reptiles and fish have been proposed as possible reservoirs of *M. ulcerans*. Investigation for *M. ulcerans* in these animals in Australia yielded no positive findings (39). Bats, which are particularly common in some of those parts of Australia in which BU is endemic, were also investigated without positive findings (7).

In Uganda, over 700 wild rodents were captured in regions of both low incidence and high incidence for BU in humans. Although a few of these had mycobacterial infections, none proved to be caused by *M. ulcerans* (41). Studies of fish in Uganda failed to yield *M. ulcerans* (5). Similarly, in the Democratic Republic of the Congo (DRC; formerly Zaire), attempts to culture *M. ulcerans* from fish in BU endemic and non-endemic areas were not fruitful, although numerous other mycobacterial species were cultured (32, 33, 34). While most environmental mycobacterial species were ubiquitous, a group of unclassified mycobacteria were detected only in fish collected in BU endemic areas. Two strains were isolated from the intestinal tracts of two fish (*Claria* sp.), and twenty-one strains from the gills of *Tilapia* sp. These unclassified strains probably existed in very low concentrations in the water in these BU endemic areas, and were concentrated either by a filtration mechanism in the gills, or in the intestines by ingestion of food contaminated with mycobacteria. *Tilapia* are microphagous, and the very fine lamellae of the gills can trap particles as small as bacteria (17). Hypothetically, if *M. ulcerans* is present in the water in these BU endemic areas, the bacterium could be concentrated by fish in a similar manner.

Recent studies performed in BU endemic and non-endemic areas of Benin have demonstrated that, of twenty small fish collected in endemic regions, three specimens were positive for *M. ulcerans* by PCR using primers specific for *M. ulcerans*, i.e. the insertion sequence IS*2404* described by Ross *et al.* (44, 45). Neither these fish, nor those examined in the DRC, presented any evidence of mycobacterial disease. In both locations, fish appear to be colonised by unclassified mycobacteria or *M. ulcerans*, but do not become infected or diseased.

Invertebrates

Molluscs and leeches

Analysis of aquatic snails and leeches collected in the DRC revealed no evidence of *M. ulcerans* in these invertebrates.

Insects

Radford suggested that some arthropods (mosquitoes, flies and scorpions) may be vectors of *M. ulcerans* (39). Blood-sucking insects, such as bedbugs (*Cimex*), have also been proposed as possible vectors of BU, but *M. ulcerans* has never been cultivated from these insects (7). A small black biting fly (*Simulium griseicolle*) was also thought to be a potential vector because of the similarity in geographical distribution of this fly and BU within Uganda (41).

A recent study using PCR has detected *M. ulcerans* in aquatic insects for the first time (38). In this study, ninety-five specimens, comprising ninety samples of aquatic plants (roots, stems and leaves) and five insects, were collected in endemic areas. Only the five insects were positive for *M. ulcerans* by PCR.

These encouraging findings led to a more extensive study with a larger number of environmental specimens collected in Benin, in both endemic and non-endemic regions. The samples collected included aquatic insects, prawns, snails and roots of aquatic plants, gathered from three endemic foci (Lalo, Abomey and Zagnanado) and from two sites near Cotonou where BU is not endemic. Mycobacteria were cultured from all types of samples, and included species frequently isolated from the environment (*M. gordonae*, *M. terrae*, *M. nonchromogenicum* and *M. avium* complex) (34). Mycobacterial species were cultured from samples obtained in both endemic and non-endemic areas, but *M. ulcerans* was not recovered. Testing of all samples by PCR for *M. ulcerans* gave a positive result only in aquatic insects from endemic areas.

Of a total of 123 aquatic insects sampled from BU endemic areas, twenty-six (23.6%) were positive in culture for mycobacteria other than *M. ulcerans*, and twenty (16.3%) were positive by PCR for *M. ulcerans*. Among these twenty samples, thirteen were water bugs belonging to the Belostomatidae and Naucoridae families, three were firefly larvae (order: Odonata; suborder: Anisoptera) and four were aquatic beetles belonging to the Hydrophilidae family.

These insects are all aggressive predators of other species of aquatic arthropods and crustacea. These other aquatic invertebrates may be water-filtering organisms capable of concentrating *M. ulcerans* from water or mud in swamps and ponds.

Discussion and conclusion

Inability to culture *Mycobacterium ulcerans* from the environment

Attempts to detect *M. ulcerans* in thousands of vertebrates and invertebrates from BU endemic and non-endemic regions by culture have revealed numerous environmental mycobacteria belonging to species frequently cultivated from the environment (34). Despite the failure to culture *M. ulcerans* from the environment, strong evidence exists to suggest that *M. ulcerans* is present in the environment. Suggested explanations for the inability to culture *M. ulcerans* from environmental specimens are discussed below.

Sampling

Sampling may be inadequate. Environmental specimens should be collected from sites in which *M. ulcerans* is suspected to be concentrated (e.g. by filtering organisms), and from sites in which *M. ulcerans* is demonstrably able to survive.

In tropical regions, surface samples are subjected to high temperatures and ultraviolet light, which have been demonstrated to adversely affect the viability of the bacilli in the laboratory. In sites such as the bottom of swamps, ultraviolet rays do not penetrate and the temperature is lower and more stable. Moreover, oxygen concentration is markedly reduced in deeper parts of swamps, and microaerophilic conditions (2% to 5% O_2 concentration) favour the multiplication of *M. ulcerans in vitro* (30). Thus, environmental specimens should be collected in sites compatible with these physical conditions and should include water-filtering organisms and the predators of these organisms.

Transport to the laboratory

Mycobacterium ulcerans grows optimally on conventional mycobacteriological media at 32°C, and is very sensitive to higher temperatures. A temperature of 41°C over a period of 24 h kills more than 90% of the bacilli, and ten days at 37°C kills most of the strains (F. Portaels, K. De Ridder and W.M. Meyers, unpublished data). Temperature during transportation to the laboratory is therefore critical, especially for specimens collected in tropical countries in which the temperature may exceed 37°C for long periods. During transport of specimens, temperatures should never exceed 32°C.

Decontamination methods

Mycobacterium ulcerans is sensitive to decontamination methods. All decontamination methods currently used for the isolation of *M. ulcerans* from clinical specimens (Petroff method or N-acetyl-L-cysteine-sodium hydroxide

[NALC-NaOH]) or for the isolation of mycobacteria from environmental specimens (Petroff or oxalic acid) (37) have a detrimental impact on the viability of *M. ulcerans* (29). This fact alone contributes to the difficulty often experienced in cultivating this organism from clinical specimens that are known to contain the aetiological agent in large numbers. The influence of decontamination is further exaggerated in environmental specimens that, by definition, are heavily contaminated with other micro-organisms and thus require drastic methods for decontamination, and are undoubtedly less rich in *M. ulcerans* than clinical specimens. Smears from clinical specimens stained by the Ziehl-Neelsen method usually reveal clumps of acid-fast bacilli (4+ according to the scale of the American Thoracic Society) (2), whereas environmental specimens are rarely smear-positive (F. Portaels, unpublished data). Application of harsh decontamination methods on specimens that contain few or rare organisms would be expected to be detrimental to the successful culture of *M. ulcerans*.

Generation time

Environmental mycobacteria are abundant at all latitudes in nature (34). Some of the species frequently found in the environment are classed as rapidly growing mycobacteria (e.g. *M. fortuitum*), while other species are slowly growing mycobacteria (e.g. *M. gordonae*, *M. terrae*, *M. nonchromogenicum* and *M. scrofulaceum*). The generation time of *M. ulcerans* is longer than that of these slowly growing mycobacterial species. Primary cultures of smear positive sputum specimens from tuberculous patients are positive after less than eight weeks incubation, while primary cultures of smear positive tissue fragments from patients with BU are generally positive after seven to ten weeks incubation. According to David, the generation time of mycobacteria can vary from 2.3 h in *M. phlei* (a rapidly growing mycobacterial species) to 15 h in *M. tuberculosis* (11). Using the radiometric BACTEC 460 system, a generation time of 23 h was determined for *M. ulcerans* (K. Chemlal, J.C. Palomino, J. Chauca, M. Debacker, A. Martin and F. Portaels, unpublished data). In specimens that contain both other slowly growing mycobacteria and *M. ulcerans*, the other bacteria always appear in primary culture before *M. ulcerans*, adding to the difficulty in isolating *M. ulcerans* in pure culture. Over the past thirty years, many attempts to culture *M. ulcerans* from the environment have been confounded by the presence of rapidly growing mycobacteria that overgrow the culture media (4, 32, 33, 34, 43). The development of selective methods is required to isolate *M. ulcerans* in primary and pure culture. The media commonly used to culture slowly growing mycobacteria (e.g. Löwenstein-Jensen, Ogawa and Middlebrook media) are also suitable for *M. ulcerans*. However, better growth is obtained in primary culture on Löwenstein-Jensen, compared to agar media or liquid media such as Middlebrook media used by the BACTEC 460 system (F. Portaels, unpublished data). An antibiotic mixture such as PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) may be used to control contamination as for *M. tuberculosis*, since *M. ulcerans* is resistant to the antibiotic complex of PANTA (31).

Detection of *Mycobacterium ulcerans* in the aquatic environment by polymerase chain reaction

Given that culture of *M. ulcerans* from the environment has not yet been successful, several investigators have resorted to the use of molecular techniques, such as PCR using primers specific for *M. ulcerans*, to detect the organism in nature.

Stinear *et al.* have successfully applied these techniques to directly detect *M. ulcerans* in aquatic environments (water samples, detritus and vegetation) (46), and Portaels *et al.*, to demonstrate *M. ulcerans* in aquatic insects (38) and fish (F. Portaels, unpublished data). These findings strongly support the hypothesis that *M. ulcerans* is present in some aquatic foci in BU endemic regions, and that water-filtering aquatic vertebrates (e.g. microphagous fish) and invertebrates (e.g. mosquito larvae) and the predators of these organisms in endemic zones could mechanically filter and concentrate environmental mycobacteria, including *M. ulcerans*.

The narrow temperature tolerance and sensitivity to oxygen of *M. ulcerans* (30) suggest that the bacterium could persist saprophytically, but with greater difficulty compared to other environmental mycobacteria. In common with most of the environmental mycobacteria, *M. ulcerans* is able to multiply over a wide range of pH values (between 5.4 and 7.4) (36). Given these considerations and comparisons of the different biological properties of other known environmental mycobacteria, such as the ability to grow at temperatures above 40°C (31), the authors propose that *M. ulcerans* may be maintained in some hosts that protect the bacilli against changes in the physical parameters of the environment, such as temperature and oxygen concentration. This does not necessarily imply that *M. ulcerans* is pathogenic for such hosts, but the two could interact, for example, in a manner somewhat analogous to *M. avium* in water-borne amoebae (8). Furthermore, such hosts could protect *M. ulcerans* against the effects of naturally-occurring antimycobacterial agents (27). The water flea, *Daphnia*, can serve as a host for *M. marinum*, and in the laboratory, *Daphnia* take up *M. ulcerans* when artificially seeded with *M. ulcerans*. However, the mycobacteria do not appear to be pathogenic for this host (W.M. Meyers, unpublished observations).

Based on the previously described temperature requirements, microaerophilic growth dynamics and survival at wide pH ranges, the conditions that prevail in the mud beneath swamps, the authors have proposed a new hypothesis for a source of *M. ulcerans* and a mode of transmission to animals and humans (Fig. 3).

Environmental mycobacteria (probably including *M. ulcerans*) are present in water or mud at the bottom of swamps. These mycobacteria may be mechanically concentrated by small water-filtering organisms such as

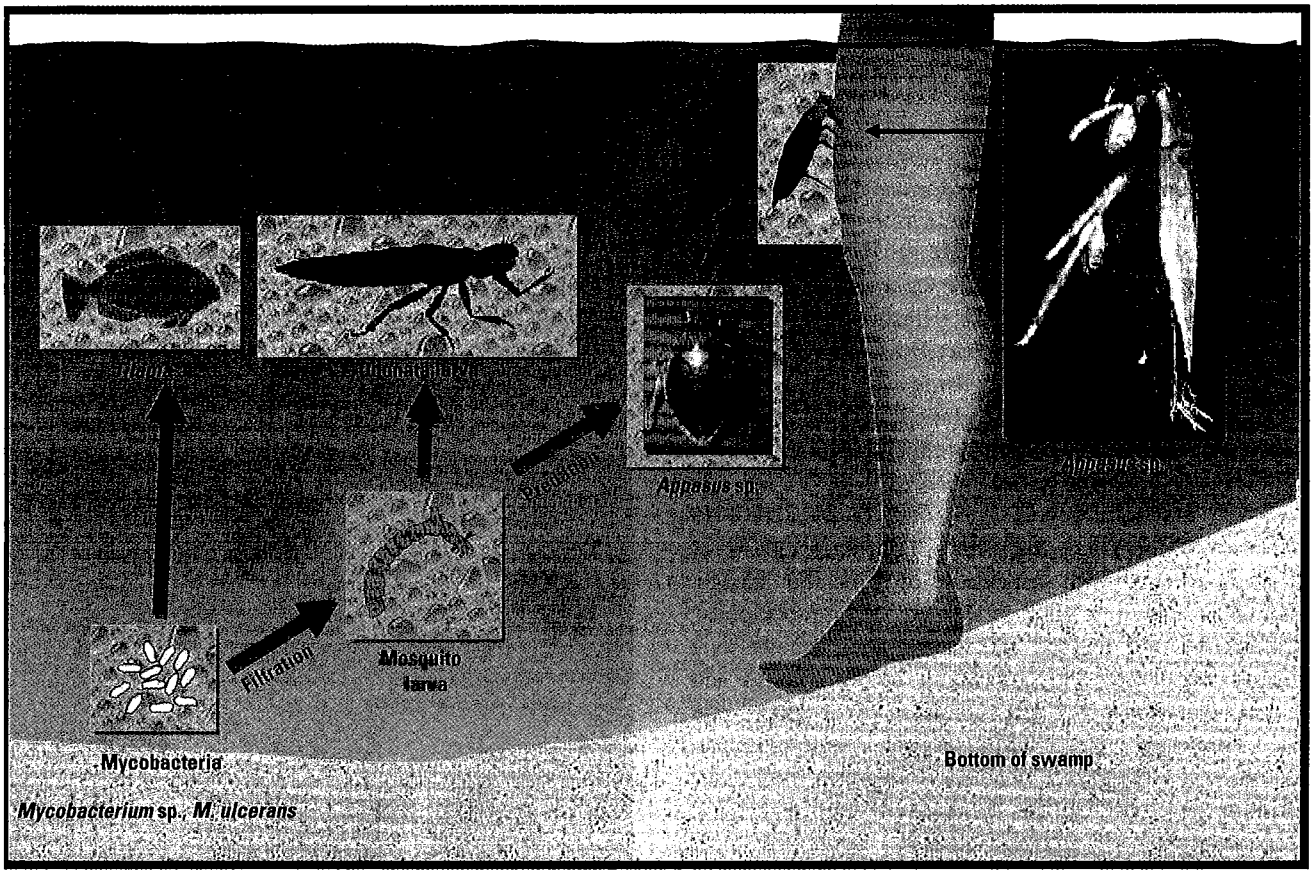

Fig. 3
Environmental sources of *Mycobacterium ulcerans* and possible mode of transmission to animals and humans

microphagous fish, mosquito larvae, small crustacea or molluscs, or even some protozoa such as amoebae.

These water-filtering organisms could concentrate environmental mycobacteria, including *M. ulcerans*, and subsequently be ingested by water-dwelling predators such as firefly larvae, water bugs such as Naucoridae and Belostomatidae, or even by some non-microphagous fish. These water-dwelling predators thereby become passive reservoirs of environmental mycobacteria. Some of these aquatic predators, especially water bugs, may bite animals or humans and mechanically introduce environmental mycobacteria (including *M. ulcerans*) into the skin or deposit the mycobacteria on the surface of the skin.

Some aquatic insects such as beetles and water bugs leave their aquatic biotope and fly several kilometres to neighbouring areas or other ponds or marshes. This could explain how humans living near water who have no history of direct contact with the natural source of the water, are infected with *M. ulcerans*. In Uganda, most of the cases occurred within ten miles of the River Nile (5).

Trauma seems essential for the introduction of *M. ulcerans* into the skin. The aetiological agent may be introduced directly by bites of these insects, or by trauma at skin sites contaminated by water, detritus, vegetation or insect products containing *M. ulcerans*. If the hypothesis of the authors regarding the transmission of *M. ulcerans* to animals or humans is confirmed, this would appear to be the first implication of insects in the transmission of a mycobacterial disease.

Parallel modes of transmission of *M. ulcerans* may also exist, such as transmission by aerosols from the surface of swamps.

Since the presence of *M. ulcerans* in the aquatic environment has now been definitely confirmed by several studies which have used PCR, efforts should be made to culture *M. ulcerans* from the environment. Despite the positive PCR results, the ultimate reservoirs of BU have not been definitely identified, nor has the mode of transmission been completely elucidated.

Mycobacterium ulcerans infection in wild animals

Experimental infection

In the paper that first described *M. ulcerans*, MacCallum *et al.* reported that the organism was initially cultivated in mice and that experimental lesions could be produced by intraperitoneal and hind-limb inoculation (22). Following this historic paper, Meleney and Johnson, in 1950, were successful in culturing the mycobacterium from the leg of a six-year-old boy who had been living with his missionary parents in what was then the Belgian Congo (24). However, injections of tissue digest into the groin and intraperitoneally in guinea-pigs did not cause lesions.

In Uganda, Clancey inoculated mice, both intraperitoneally and in the footpad, with suspensions of tissue and cultures. Male white rats were also inoculated by intraperitoneal, intratesticular and footpad routes (9). Male rabbits were similarly inoculated, and inoculation by intravenous and intramuscular routes was also attempted. Male guinea-pigs were subjected to intraperitoneal, intramuscular, intratesticular and footpad inoculation. Infection from tissue inoculum developed in the footpads of rats and mice; intraperitoneal and scrotal infection developed after intraperitoneal injection of a culture suspension, and intratesticular, scrotal and hind-limb infection occurred after the intratesticular inoculation of rats with a culture suspension. Neither rabbits nor guinea-pigs developed lesions, regardless of the route of inoculation.

An editorial in the *Medical Journal of Australia* in 1975 reported that numerous animals, including rodents, cows, possums, chick embryos and amphibians, had been infected experimentally, but no natural animal reservoir had been detected at that time (3). However, in 1950, Bolliger *et al.* in Sydney, had successfully inoculated *M. ulcerans* into brushtail possums (*Trichosurus vulpecula*) and had found that an uninoculated possum in the same room, kept in a separate cage, also developed infection (6). Marcus *et al.* successfully inoculated anole lizards (*Anolis carolinensis*) with *M. ulcerans* by the subcutaneous route, producing both necrotising and granulomatous lesions (23).

More recently, in 1998 and 1999, George *et al.* purified a polyketide-derived macrolide, mycolactone, from *M. ulcerans* and demonstrated that mycolactone was responsible for the cytotoxic phenotype of *M. ulcerans* (13, 14). Guinea-pigs were used to study the effects of the toxin produced by *M. ulcerans*, and intradermal injection of purified mycolactone was demonstrated to produce histopathological changes similar to those found in patients with BU. Injection of mycolactone provokes similar pathological changes to those caused by viable *M. ulcerans*.

Intracutaneous inoculation of nine-banded armadillos (*Dasypus novemcinctus*) produces cutaneous lesions that are typical of BU, both clinically and histopathologically (50).

Natural infection

The first naturally-acquired *M. ulcerans* infections of free-living animals other than humans were reported by Mitchell *et al.* in 1984. Ulcers were described in seven koalas (*Phascolarctos cinereus*) living on Raymond Island in Gippsland lakes, near Bairnsdale in south-eastern Australia (28). Figure 4 shows an infected koala with a small ulcer above the left eye.

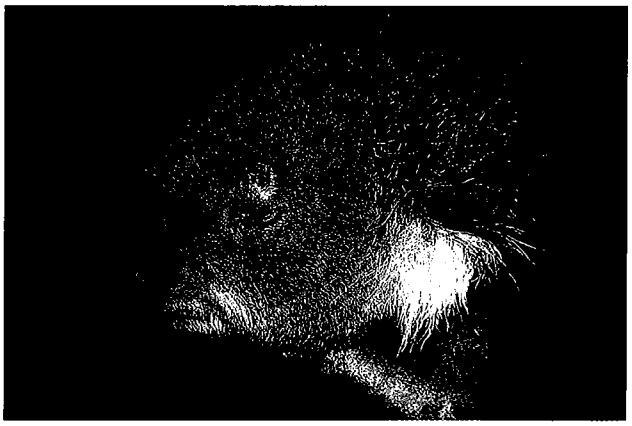

Fig. 4
Koala (*Phascolarctos cinereus*) from Phillip Island (Australia) with an ulcerative lesion above the left eye
Photo: courtesy of P. Mitchell

Between 1993 and 1995, an outbreak of *M. ulcerans* infection occurred amongst residents of east Cowes, Phillip Island (near Melbourne, Australia) (49). Epidemiological and laboratory investigations suggested that the source of this outbreak was a golf course irrigation system and a nearby swamp (43, 46). Human exposure was presumed to occur through direct or indirect exposure to aerosols arising from water contaminated with *M. ulcerans*. A large koala sanctuary is located within 5 km of the outbreak region, but no *M. ulcerans* infections were reported in koalas from Phillip Island during this period. Isolated human cases continued to appear in east Cowes until October 1998.

In July 1996, an unwell brushtail possum was captured in east Cowes. The animal was destroyed for humane reasons. A presumptive diagnosis of *M. ulcerans* infection was made based on the presence of skin ulcers. Histopathological findings suggested *M. ulcerans* infection and revealed numerous acid-fast bacilli. No fresh material was available for culture, but multiple formalin-fixed samples were subjected to IS*2404* PCR (44). All samples tested negative, suggesting an alternative mycobaterial infection. Therefore, although brushtail possums are experimentally susceptible, no proven

natural *M. ulcerans* infection has been documented in this species.

In January 1998, an adult ringtail possum (*Pseudocheirus peregrinus*) from east Cowes was found to have lesions clinically consistent with *M. ulcerans* infection, but no material from this animal was subjected to PCR or culture to confirm the diagnosis. In May 1998, another ringtail possum from east Cowes was found with ulcers on the nose and hind feet (Fig. 5). On this occasion, IS*2404* PCR confirmed that the infection was *M. ulcerans*. Two further ringtail possums with IS*2404* PCR-confirmed *M. ulcerans* infection were identified in east Cowes in January 2000. No new infections have occurred in humans in east Cowes since 1998, and the human epidemic in this region peaked in 1994. Recent environmental testing suggests that the water sources originally implicated are no longer contaminated (46). These data suggest that the incubation period for *M. ulcerans* infection may be longer in ringtail possums than in humans. An alternative hypothesis is that ringtail possums have been recently exposed to a new, unidentified source of *M. ulcerans* in east Cowes, that has not yet led to human infections.

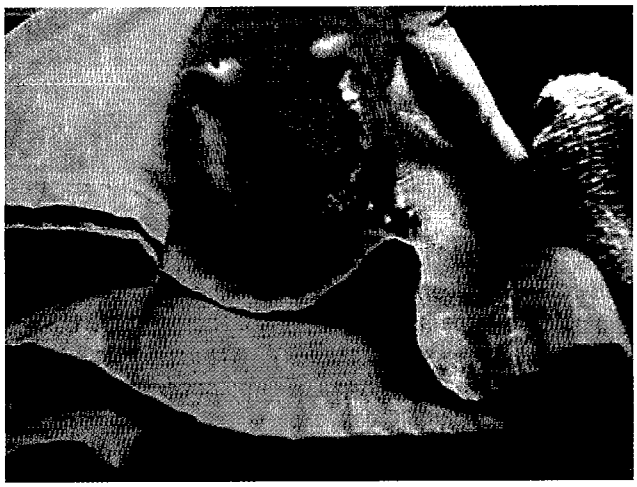

Fig. 5
Ringtail possum (*Pseudocheirus peregrinus*) from east Cowes (Australia) with *Mycobacterium ulcerans* infection on the nose
Photo: courtesy of P. Flood

In November 1997, an alpaca (*Lama pacos*) from an alpaca farm near Lakes Entrance in east Gippsland (near Bairnsdale, south-eastern Australia) was noted to have a large ulcer on one leg. The alpaca is a hoofed mammal from South America, which is related to camels. These animals are not native to Australia and have recently been imported for the production of high quality wool. Mycobacteria were observed on a swab from the lesion and *M. ulcerans* was confirmed by culture and IS*2404* PCR. To the knowledge of the authors, no other natural infections in alpacas, other ruminants or any other animal species have been reported in Australia or elsewhere.

General discussion and conclusion

Mycobacterium ulcerans belongs to a group of mycobacteria that are potentially pathogenic for humans and animals. These are sometimes called 'opportunistic mycobacteria' or 'occasional pathogens'. Most species belonging to this group are ubiquitous in nature, and may become pathogenic under special circumstances. These mycobacteria generally cause mycobacterial diseases that are not contagious.

Knowledge of *M. ulcerans* infection in humans has been enhanced by research efforts, especially in several developing countries where the disease is endemic and the incidence sometimes can be high. In some areas of Benin and other countries of West Africa, the number of cases may exceed those of tuberculosis or leprosy (35). Although the disease has never been observed in wild animals in these countries, animals that have been mechanically colonised by *M. ulcerans* (fish and some aquatic insects) have been discovered in BU endemic countries.

The cases of BU reported in wild and domesticated animals (koalas, possums and an alpaca) in Australia, confirm that BU can attack some animals.

Both humans and animals are probably infected through contact with environmental sources of *M. ulcerans*. The precise mode of transmission of BU in humans and animals is not known. Aerosols may carry *M. ulcerans* and infect hosts via the respiratory tract (19). Recent findings of the authors regarding aquatic insects suggest that some insects may be involved in the transmission of the disease to humans and possibly to animals (38). The mechanism may be direct transmission in water, through bites of aquatic bugs, or an indirect infection through *M. ulcerans* carried by insects that occasionally leave the aquatic habitat. These insects may thus play an important role in the spread of *M. ulcerans* beyond the favourable aquatic environment in which the bacterium is protected against high temperatures and ultraviolet light. Trauma is probably the most frequent means by which *M. ulcerans* is introduced into the skin from contaminated surfaces, and direct inoculation seems the most frequent mode of infection (26). Direct or indirect exposure through aerosols contaminated by *M. ulcerans* is a further possible mode of transmission, as suggested by the extensive outbreak among residents of Phillip Island (43, 46).

The existence of *M. ulcerans* in various wild animals in endemic areas in which incidence of BU is high is a critical area requiring further research. The discovery of BU in wild animals should alert observers to monitor re-emergent or new foci of the disease, especially in countries such as Australia where the public have infrequent contact with potentially contaminated water.

Further detection of mechanical colonisation (e.g. in fish) by *M. ulcerans*, or of *M. ulcerans* infection in wild animals would certainly provide important information to improve the understanding of the pathogenesis and epidemiology of this disease. Buruli ulcer is not a zoonosis since the disease is not considered contagious. Nevertheless, humans and animals can be infected from a common environmental source.

Eradication of an infectious disease that is contracted from the environment is problematic. Among the various potential methods, perhaps the most important is to limit contact with the suspected environmental source of the aetiological agent. Several studies have shown that in endemic areas of BU, the disease is more frequent among inhabitants who obtain water for domestic use from swamps instead of wells (4). The disease in humans is probably less frequent in Australia than in West Africa because contact with water containing *M. ulcerans* is infrequent in Australia.

Although these preventive measures can diminish the incidence of BU in humans, no reduction in the incidence in wild animals would be obtained. Surveys of wild animals for *M. ulcerans* infection in endemic areas could provide invaluable data concerning the general control of BU.

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Mycobacterium ulcerans chez les animaux sauvages

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Résumé

L'infection due à *Mycobacterium ulcerans*, ou ulcère de Buruli, est par sa fréquence, la troisième maladie mycobactérienne affectant l'homme chez qui elle entraîne souvent séquelles invalidantes et incapacités physiques. La maladie est très étroitement associée aux zones marécageuses des tropiques, notamment en Afrique centrale et de l'Ouest. La plupart des experts estiment que l'agent étiologique prolifère dans la boue au fond des eaux stagnantes. Les modes de transmission possibles sont le contact direct avec l'environnement contaminé, les aérosols en provenance des eaux de surface et la faune aquatique (notamment certains insectes). La transmission entre êtres humains est exceptionnelle. La contamination des plaies par *M. ulcerans* semble jouer un rôle important dans le déclenchement de la maladie. Une fois introduit sous la peau ou dans les tissus sous-cutanés, *M. ulcerans* se multiplie et produit une toxine nécrosante. Cependant, les lésions consécutives à l'infection varient du nodule localisé ou de l'ulcère à une maladie ulcérative ou non ulcérative étendue, ou à une ostéomyélite.

Si la première mise en culture de *M. ulcerans* réalisée sur des prélèvements humains remonte à 1948, toutes les tentatives de mise en culture de la mycobactérie à partir de l'environnement ont échoué. Ces échecs peuvent s'expliquer par des erreurs d'échantillonnage, de transport ou de décontamination, par la difficulté de mettre en culture ce micro-organisme exigeant et thermosensible, ou par la relative lenteur de sa croissance par rapport à celle d'autres mycobactéries présentes dans l'environnement. Néanmoins, des études moléculaires récentes utilisant des amorces spécifiques ont révélé la présence de *M. ulcerans* dans l'eau, dans la boue, chez des poissons et des

insectes aquatiques. Aucun réservoir naturel n'a été identifié, mais la possibilité que *M. ulcerans* colonise certaines espèces de la microfaune, par exemple les amibes sauvages, reste à étudier.
Des hôtes de diverses espèces, dont des lézards, des amphibiens, des embryons de poussin, des phalangers-renards, des tatous, des rats, des souris et des bovins ont été infectés expérimentalement avec *M. ulcerans*. Les seules infections naturelles observées ont eu lieu en Australie, chez des koalas, des phalangers-renards et un alpaca vivant en captivité. Les lésions étaient cliniquement identiques à celles observées chez l'homme. L'infection à *M. ulcerans* est une maladie émergente dans certains pays tropicaux en voie de développement. Sa réémergence rapide peut être due à des facteurs environnementaux ou socio-économiques, par exemple les inondations résultant de la déforestation ou la forte croissance démographique sans amélioration technique des pratiques agricoles, entraînant un risque accru pour un nombre croissant de personnes. L'éradication des maladies associées à ces facteurs paraît difficile. La question cruciale reste de savoir si les animaux sauvages jouent un rôle dans la transmission de la maladie. Pour y répondre, il conviendrait d'effectuer rapidement des enquêtes sur les animaux sauvages dans les régions où l'ulcère de Buruli est endémique.

Mots-clés
Alpacas – Eau – Environnement – Insectes aquatiques – Koalas – Mycobactéries – Mycobacterium ulcerans – Phalangers-renards – Poissons – Ulcère de Buruli.

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Mycobacterium ulcerans en animales salvajes

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Resumen
La infección por *Mycobacterium ulcerans*, conocida como úlcera de Buruli, es la tercera micobacteriosis más frecuente en el hombre, causante a menudo de graves deformidades y minusvalías. La enfermedad se manifiesta en estrecha asociación con medios pantanosos tropicales, sobre todo en África Central y Occidental. Según la mayoría de los investigadores, el agente etiológico prolifera en el lodo que se forma debajo de las aguas estancadas. El contagio puede producirse por contacto directo con elementos contaminados presentes en el medio, aerosoles procedentes de superficies acuáticas o animales acuáticos (por ejemplo insectos). La transmisión de persona a persona es rara. La contaminación de heridas cutáneas por *M. ulcerans* parece ser importante a la hora de desencadenar la enfermedad. Una vez dentro de la piel o el tejido subcutáneo, *M. ulcerans* se multiplica y genera una toxina que provoca necrosis. No obstante, la enfermedad puede manifestarse de varias formas distintas, desde una úlcera o nódulo localizado hasta una osteomielitis o una enfermedad ulcerosa o no ulcerosa generalizada.
Aunque en 1948 se describió por vez primera el cultivo de *M. ulcerans* a partir de muestras de un paciente, los reiterados intentos de cultivar esta micobacteria a partir de muestras de flora y fauna naturales han resultado infructuosos. Ello puede atribuirse quizá a defectos en el muestreo, el transporte, la descontaminación o el cultivo de este microorganismo exigente y sensible al calor, o tal vez a su largo periodo de crecimiento en comparación con el de otras micobacterias presentes en el medio natural. Con todo, gracias al uso de técnicas moleculares con secuencias cebadoras específicas, recientemente ha podido

detectarse la presencia de *M. ulcerans* en agua, lodo, peces e insectos. Aunque por ahora no se ha descubierto ningún reservorio natural, queda por investigar la posibilidad de que *M. ulcerans* colonice a organismos de la microfauna, por ejemplo amebas que vivan en medio natural.
Entre los huéspedes que se han demostrado sensibles a la infección experimental por *M. ulcerans* figuran lagartos, anfibios, embriones de pollo, zarigüeyas, armadillos, ratas, ratones y bovinos. La infección natural de animales se ha observado únicamente en Australia (en koalas, zarigüeyas australianas y una alpaca en cautividad). En todos los casos las lesiones fueron clínicamente idénticas a las que se observan en el hombre. La infección por *M. ulcerans* es una enfermedad rápidamente emergente en algunos países tropicales en desarrollo. La reemergencia puede estar relacionada con factores ambientales o socioeconómicos, por ejemplo, desforestación conducente a inundaciones, y expansión poblacional sin técnicas mejoradas de agricultura, poniendo de esta manera más personas en riesgo. La erradicación de enfermedades relacionadas con estos factores es difícil. La importante cuestión de saber si los animales salvajes desempeñan alguna función en la transmisión de la enfermedad ha sido hasta la fecha apenas explorada. Para darle respuesta, es preciso estudiar sin más tardanza la fauna salvaje de las zonas en que la úlcera de Buruli es endémica.

Palabras clave
Agua – Alpacas – Entorno natural – Insectos acuáticos – Koalas – Micobacterias – Mycobacterium ulcerans – Peces – Úlcera de Buruli – Zarigüeyas australianas.

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