

The Uncommon *Mycobacterium ulcerans* Infection and Its Public Health Importance

Emad Mokhtar Riad*

Animal Health Research Institute, Egypt

*Corresponding Author: Emad Mokhtar Riad, Animal Health Research Institute, Egypt.

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Abstract

Mycobacterium ulcerans belongs to a group of mycobacteria that are potentially pathogenic for humans and animals. *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 and The disease may occur in temperate climates; most investigators believe that the aetiological agent proliferates in mud beneath stagnant waters, buruli ulcer is now recognized as a distinct disease that places a major burden affected health.

Treatment of advanced disease is often difficult and complicated by persistence and relapse. Surgery is still considered the main treatment option despite its poor acceptability, high costs.

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 but these animals do not appear to develop overt disease.

In a laboratory experiment, *M. ulcerans*-infected water bugs were able to transmit *M. ulcerans* disease in the tail of mice after a bite while an alternative mode of transmission may involve penetrating skin injuries during fishing or farming activities that seed the micro-organism into subcutaneous tissues and some cases have been reported of human-to-human transmission, on the other hand 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 Trauma is probably the most frequent means by which *M. ulcerans* is introduced deep into the skin.

The disease in pet animals is considered of great importance in public health issue, some records of the mycobacterium *ulcerans* in cats were reported and discussed the epidemiological relationship with the buruli ulcer infection in contact women in some countries.

Clinically, BU is primarily a disease of the skin. Two broad forms are recognized, namely: non-ulcerative and ulcerative disease. *M. ulcerans* proliferates and produces a toxin (mycolactone) which is responsible for the cytotoxic effects observed in BU lesions.

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 24h kills more than 90 % of the bacilli.

Mycobacterium ulcerans is a slow-growing mycobacterium that may be cultured in vitro at 32°C on the usual media for mycobacterial culture.

Keywords: *M. Ulcerans*; Animals; Human-diagnosis-treatment

Introduction

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.

The disease may occur in temperate climates; most investigators believe that the aetiological agent proliferates in mud beneath stagnant waters; buruli ulcer is now recognized as a distinct disease that places a major burden affected health special in entemic regions.

Treatment of advanced disease is often difficult and complicated by persistence and replace. Surgery is still considered the main treatment option despite its poor acceptability, high costs, and failure to prevent recurrence [1].

In 1998, WHO established the Global Buruli ulcer Initiative, and the importance of Buruli ulcer disease was again recognized by the 57th World Health. Assembly in 2004 [2].

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.

Epidemiology

Infection in human mainly aged between 5-51 years. 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 [3,4] but these animals do not appear to develop overt disease. Koalas, possums, brush tail possums 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 [5].

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* [6].

In a laboratory experiment, *M. ulcerans*-infected water bugs were able to transmit *M. ulcerans* disease in the tail of mice after a bite [7].

An alternative mode of transmission may involve penetrating skin injuries during fishing or farming activities that seed the micro-organism into subcutaneous tissues [8]. Only two cases have been reported of human-to-human transmission [9]. Physico-chemical data, and reports of Buruli ulcer disease to health authorities, has implicated arsenic acid exposure as a confounding immunosuppressant in some cases [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) recognized BU as an important public health problem, and established the Global BU Initiative [11].

Mode of Transmission

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 [12]. 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 [8].

The disease in pet animals is considered of great importance in public health issue, some records of the mycobacterium *ulcerans* in cats were reported and discussed the epidemiological relationship with the buruli ulcer infection in contact women in some countries.

The cases studied as a subcutaneous mass on its nasal bridge, the cytological examination of an aspirate demonstrated specific pyogranulomatous inflammation and at the surgery the lesion consisted of an encapsulated mass containing viscid fluid a stained section with Ziel-neelsen method revealed numerous acid fast bacilli and the molecular studies established the infection was caused by *Mycobacterium ulcerans* [].

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The epidemiology of BU is poorly understood. Some evidence exists for an environmental reservoir associated with slow-flowing or stagnant water. However, culture of *M. ulcerans* from the environment has never been successful [13].

Clinical Manifestations

Clinically, BU is primarily a disease of the skin. Two broad forms are recognized, namely: non-ulcerative (papules, nodules, plaques and oedematous forms) and ulcerative disease and the Lesions are usually single and initially appear as firm, painless, non-tender, movable, subcutaneous nodules of 1 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.

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 have established that mycolactone (apolyketide derived macrolide) is responsible for the cytotoxic effects observed in BU lesions [14].

Samples 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 24h 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 and culture conditions

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) [15] have a detrimental impact on the viability of *M. ulcerans* [16]. 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.

Environmental mycobacteria are abundant in nature [17]. 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.

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 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. [13,17-19].

Smear positive tissues 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 2.3h in *M. phlei* (a rapidly growing mycobacterial species) to 15h in *M. tuberculosis*. Using the radiometric BACTEC 460 system, a generation time of 23h 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 [13,17-19] contamination as for *M. tuberculosis*, since *M. ulcerans* is resistant to the antibiotic complex of PANTA [16].

M. ulcerans is able to multiply over a wide range of pH values (between 5.4 and 7.4). 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 [20], 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 [21].

Furthermore, such hosts could protect *M. ulcerans* against the effects of naturally-occurring anti mycobacterial agents. 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 authors have proposed a new hypothesis for a source of *M. ulcerans* and a mode of transmission to animals and humans.

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.

Isolation and Identification

Mycobacterium ulcerans is a slow-growing mycobacterium that may be cultured in vitro at 32°C on the usual media for mycobacterial culture [22]. 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 [16].

The development of PCR for quick identification of *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 *M. ulcerans* genome, IS2404 [19,23,24].

M. ulcerans resembles *M. marinum* in many aspects but there is a major difference in that *M. ulcerans* appears to produce a secreted toxin, or class of toxins, chemically identified as ketolide usually referred to as mycolactone [25]. 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 *M. ulcerans*. Three of the polyketide synthases involved in the biosynthesis of mycolactones appear to be coded by genes located on a giant plasmid [26]. Strains of *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 *M. ulcerans* disease. Another mycobacterium, referred to as *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 *M. ulcerans* disease in humans [27] which tested positive for the IS2404 that was previously considered species-specific for *M. ulcerans*, and appears able to produce mycolactones [28].

Public Health Reviews

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 [29]. 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.

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 [30]. 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.

Immune protection by *M. bovis* BCG lasting six months has been found in an earlier study in Uganda [31]. In a case control study in Ghana, BCG scars were no more common in control subjects than in Buruli ulcer patients [32] but in a study in Benin, BCG was shown to be protective against more severe *M. ulcerans* disease notably, osteomyelitis [33].

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 [8]. 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 diagnosis 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. 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 onchocercoma, keratin (sebaceous retention) cyst, lipoma, and lymphadenitis or lymphadenopathy. 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 [32]; in pre-ulcerative lesions, a smear may be taken from a biopsy.
2. Histopathological examination of tissue obtained during surgery [34-37].
3. Culture of smears, or of tissue; the diagnostic sensitivity used to be very low [22] but laboratories that use special transport media have acceptable diagnostic yield [23].
4. PCR from biopsy material; most groups now use the high copy insertion sequence IS2404 [24,25].

Conclusion

Mycobacterium ulcerans belongs to a group of mycobacteria that are potentially pathogenic for humans and animals and 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.

Knowledge of *M. ulcerans* infection in humans has been enhanced by research efforts, important area of research is an unknown proportion of disease progression or spontaneous healing may be due to genetic polymorphisms.

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