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The uncommon Mycobaterium ulcerans infection and its public health importance

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Abstract

Mycobacterium Ulcerans infection or Buruli Ulcer is the third most frequent mycobacterial disease in humans .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.

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.

In lab diagnosis ,Mycobacterium ulcerans isolates are sensitive to decontamination methods. All decontamination methods currently used for the isolation of M. ulcerans from clinical specimensMycobacterium 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.

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 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.

Keywords: mycobacteria –human infection –pet animals -diagnosis

Introduction

Mycobacterium Ulcerans infection or Buruli Ulcer is the third most frequent mycobacterial disease in humans often causing serious deformaties and disability the disease is most closely associated with tropical Wetlands especially in west and central Africa.(**Eddyani *et al.*, 2004**).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 (**Portaels, 1989**).

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 enthemic regions and the 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. (**Evans *et al*.,2003**).

In 1998, WHO established the Global Buruli ulcer Initiative, and the importance of Buruli ulcer disease was again recognized by the 57 World Health (**Resolution,2004**).

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 **(Portaels *et al*., 2001 and Eddyani *et al*., 2004**) 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 (**Portaels *et al*., 1999**).

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 (**Marsollier *et al*.,2004**). In a laboratory experiment, M. ulcerans-infected water bugs were able to transmit M. ulcerans disease in the tail of mice after a bite (**Marsollier *et al*.,2002**).

An alternative mode of transmission may involve penetrating skin injuries during fishing or farming activities that seed the micro-organism into subcutaneous tissues (**Meyers *et al*.,1974**). Only two cases have been reported of human-to-human transmission (**Debacker *et al*., 2002**). Physico-chemical data, and reports of Buruli ulcer disease to health authorities, has implicated arsenic acid exposure as a confounding immunosuppressant in some cases (**Ducker *et al*., 2004**). In recent times, BU has emerged as an increasingly important cause of morbidity world-wide, partly related to environmental changes. (**WHO,2000**).

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 (**Hayman,1993**). 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 (**Meyers *et al.*, 1974**).

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. (**Elsner *et al*., 2008**).

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, may be indurated by oedema.(**Palomino and Portaels,1998**).

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 (**Geroge *et al.*, 1998**). 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 (**Van der Werf *et al*.,2003**). 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 (**Stinear *et al*.,2004**). 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 (**Trott *et al*.,2004**) .

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 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.**(Portaels, 1995)**

Decontamination methods and culture conditions

Mycobacterium ulcerans isolates are 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) (**Porteals *et al*.,1988**) have a detrimental impact on the viability of M. ulcerans 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 (**Portaels,1995**). 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 medium, compared to agar media or liquid media such as Middlebrook media **. (F. Portaels, unpublished data**). An antibiotic mixture such as PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) may be used to control.(**Barker, 1973; Portaels, 1989; Portaels,1995 and Ross *et al*.,1997**).

Smear positive tissues fragments from patients with BU are generally positive after seven to ten weeks incubation and the generation time of mycobacteria can vary from 2.3 h in M. phlei (a rapidly growing mycobacterial species) to 15h in M. tuberculosis **(David, 1973).** 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 (**Portaels, 1995 and Ross *et al*., 1997**) and the contamination as for M. tuberculosis, since M. ulcerans is resistant to the antibiotic complex of PANTA , while,

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 (**Pattyn and Portaels,1972**), 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 (**Cirillo *et al*., 1997).**

On the other hand ,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.

Mycobacterium ulcerans is a slow-growing mycobacterium that may be cultured in vitro at 32˚C on the usual media for mycobacterial culture . 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 (**Palomino and Portaels,1998**).

Molecular detection

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, (**Ross *et al*.,1997; Guimares *et al*.,1999 and Stienstra *et al*.,2003**).

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 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 (**Raghunathan *et al*.,2005**).

2) Histopathological examination of tissue obtained during surgery (**Hayman and McQueen,1985 and Guarner *et al*., 2003**).

3) Culture of smears, or of tissue; the diagnostic sensitivity used to be very low but laboratories that use special transport media have acceptable diagnostic yield (**Guimaraes *et al*., 1999**).

4) PCR from biopsy material; most groups now use the high copy insertion sequence IS2404 (**Van der Werf *et al*., 2003**).

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.**(Guimares *et al*.,1999 and Stienstra *et al*.,2003**). Knowledge of M. ulcerans infection in humans has been enhanced by research efforts, especially in several developing countries where the disease is endemie 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 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 endemie countries.(**Portaels,2000**).

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 (**Westenbrink *et al*.,2005**). 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 . In a case control study in Ghana, BCG scars were no more common in control subjects than in Buruli ulcer patients (**Raghunathan *et al*.,2005**) but in a study in Benin, BCG was shown to be protective against more severe M. ulcerans disease notably, osteomyelitis (**Portaels *et al*.,2004**).

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 (**Meyers *et al*., 1974**). This is an important area of research as an unknown proportion of disease progression or spontaneous healing may be due to genetic polymorphisms.

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