SPECIAL ARTICLE

IN THE CASE OF TRANSMISSION OF *MYCOBACTERIUM ULCERANS* IN BURULI ULCER DISEASE *ACANTHAMOEBA* SPECIES STAND ACCUSED

M.D. WILSON[1], D.A. BOAKYE[1], L. MOSI[1] and K. ASIEDU[2]
[1]Department Of Parasitology, Noguchi Memorial Institute for Medical Research, University of Ghana, P.O. Box LG 581, Legon, Ghana [2]Department of Neglected Tropical Diseases, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland

Corresponding author: *Professor Michael D. Wilson* ***E-mail:*** *mwilson@noguchi.mimcom.org*

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SUMMARY

Buruli ulcer disease caused by *Mycobacterium ulcerans* results in extensive destruction of skin and soft tissue and long-term functional disabilities that ultimately require surgery and rehabilitation. The disease is associated with aquatic and swampy environments with the mycobacterium occurring in biofilms, soil, aquatic insects, fish and wildlife however, the mode of transmission to humans remains an enigma. Current transmission ideas including bites from predatory water bugs and mosquitoes, do not explain satisfactorily the spasmodic disease distribution in human populations. Here we argue that *Acanthamoeba* species are the natural hosts of *M. ulcerans* and are mainly responsible for disease transmission because; (i) Acanthamoebae are known natural hosts of several microbial pathogens including *M. marinum, M. avium and Legionella pneumophila*, (ii) culture of slow-to-grow microbial pathogens hosted in nature by *Acanthamoeba* spp is enhanced when the media is seeded with the protozoa, (iii) acanthamoebae and *M. ulcerans* share similar bio-ecological and epidemiological settings, (iv) documented evidence that prior growth of *L. pneumophila* and *M. avium* in acanthamoebae influences entry mechanisms, intracellular growth and virulence in human monocytes, (v) *Acanthamoeba* spp also infect humans and cause diseases via routes of openings including broken skin and sites of trauma similar to *M. ulcerans* and (vi) *M. ulcerans* is rather a fastidious intracellular organism as recent analysis of the genome indicate. We argue further that temperature plays a significant role in transmission determining the fate of either the intracellular microbe or the host cells. Also, *Acanthamoeba*-pathogen association has a long evolutionary history because the same set of bacterial genes and gene products e.g. in *L. pneumophila* are required for survival in both mammalian and protozoan host cells. We suggest that the involvement of *Acanthamoeba* in the transmission of *M. ulcerans* to humans better explains the disease's epidemiology.

INTRODUCTION

Buruli ulcer (BU) caused by infection with *Mycobacterium ulcerans* is the third most common human mycobacterial infection after tuberculosis and leprosy. Infections can result in extensive destruction of skin and soft tissue and long-term functional disabilities, requiring plastic surgery and rehabilitation if not treated early. BU occurs near slow flowing rivers, streams, ponds, lakes and swamps in tropical and subtropical countries, and epidemics can occur after floods. This association with water bodies is undisputable but its transmission to humans is not exactly understood.[1]

TRANSMISSION

A classic study published in 2002 implicated predatory water bugs in the disease transmission.[2] In this study water bugs fed on infected grubs successfully transmitted the bacillus from salivary glands to experimental animals which reinforced earlier hypothesis that aquatic insects were possible vectors of the disease.[3] Since then several studies aided mainly by DNA technology, have found the mycobacterium in biofilms, soil, water bugs, insects, fish, amphibians and wildlife, but none offers any biological explanation for the persistence of *M. ulcerans* within these organisms.

The distribution of BU in human populations does not conform to any discernable pattern other than its clear association with aquatic environments, which compounds further the difficulty in delineating the mode of transmission. Interestingly because some BU patients remember antecedent trauma at sites of the

body prior to the development of ulcers, bites from water bugs and mosquitoes therefore lead the field of potential vectors in transmission. Furthermore, most ulcers occur on the lower limbs so some investigators believe that the disease is acquired from contaminated soil. The current situation therefore is a mystifying state of affairs with investigators following 'leads' with attendant hypotheses of modes of transmission.[4]

In this article we posit that *Acanthamoeba* species are the natural hosts of *M. ulcerans* in the environment and that they are primarily responsible for the transmission and persistence of the disease.

M. ulcerans was considered by many as an extracellular organism because it occurs in central parts of lesions that are devoid of host cells although this view is changing with new evidence. Of relevance though, is the close relationship of *M. ulcerans* to *M. marinum*, and *M. tuberculosis* which are known intracellular parasites therefore it is odd that *M. ulcerans* should be an exception.

Additionally, *M. ulcerans* is slow growing, which laboratory cultures are enhanced significantly if the medium is seeded with *Acanthamoeba*, a characteristic also observed with slow-growing intracellular *Francisella tularensis* and *Vibrio cholerae*. These bacteria are known to survive and multiply in *Acanthamoeba* spp in nature, which raises the question; if *Acanthamoeba* enhances *M. ulcerans* growth in cultures why not also in the natural environment? It also hints at intracellular multiplication of *M. ulcerans* within *Acanthamoeba* spp. However a study that investigated *M. ulcerans* in disease-endemic environments did not find infected protozoa.[5] This study and Marsollier *et al.* [2] must have significantly influenced the research direction away from the involvement of *Acanthamoeba* species in transmission.

Intriguingly, it has been known for some time that a number of *Mycobacterium* species including *M. marinum*, *M. smegmatis*, *M. avium* and *M. simiae* live intracellularly in amoeba and recently Adekambi *et al.* [6] demonstrated the growth of 26 environmental *Mycobacterium* species from a variety of sources within *A. polyphaga* and survival inside cysts. Moreover, several reports of outbreaks of environmental mycobacterium infections have implicated acanthamoebae which should have drawn attention to their possible involvement in the transmission of *M. ulcerans*. What is more fascinating is the variety of attributes of various species and strains of *Mycobacteria* that are associated with *Acanthamoeba* spp; slow and rapid growing, extremes of high and low temperature resistant, chlorine tolerant, survival in low oxygen tension water, antibiotic resistant, survival in deionised water etc.[7]

Acanthamoeba spp. are recognized environmental hosts of several intracellular microbial pathogens including viruses, bacteria, yeast and protozoa e.g. *Cryptosporidium parvus and Chlamydia*, particularly as biological hosts for pathogen multiplication. They are among the most prevalent free-living protozoa in the environment; from soil, dust, air, natural and treated water, seawater, swimming pools, sewage, sediments, air-conditioning units, domestic tap water, to treatment plants, hospitals and dialysis units, eyewash stations to mammalian tissues, reptiles, amphibian, fish and vegetation. They occur predominantly at the water-air interface in biofilms, feeding on bacteria which support their growth. *Acanthamoeba* spp enter the human host by contact through ulcerated or broken skin or by aerosolization through lower respiratory tract and enter macrophages.[7] In humans it causes *Acanthamoeba* dermatitis, acanthamoeba keratitis after minimal trauma to the corneal epithelium, granulomatous amoebic encephalitis and *Acanthamoeba* pneumonitis in immune compromised individuals. The trophozoites acquire the bacteria in complex interactions which results in uptake by phagocytosis. Intriguingly some genera of bacteria are digested by *Acanthamoeba* spp while others are not.[7] Undigested bacteria are confined in the phagosome of the amoeba within which they multiply and avoid killing by inhibiting the acidification of phagosome and subsequent lysosome fusion by processes that are still not well understood.

Legionella pneumophila and *Francisella tularensis* are two intracellular organisms hosted by Acanthamoebae that are particularly interesting, sharing several epidemiologic characteristics with *M. ulcerans*. *Legionella pneumophila* is commonly isolated from natural and man-made aquatic systems and infects and replicates within free-living amoebae in these environments. *Francisella tularensis* is also associated with natural water systems and also infects wildlife. These two bacteria are also slow-replicating organisms and we subscribe to the views of Stinear *et al* [8] that *M. ulcerans* has recently evolved from the generalist, more rapid-growing environmental *M. marinum* to become a niche-adapted specialist - a more fastidious intracellular organism. In brief, it is known that *M. ulcerans* survives and grows in both *Acanthamoeba* and macrophages, and that following proliferation phases within macrophages it lyses the infected host cell.

Studies have shown temperature to be the key determinant of the fate of either the bacteria or the host

cells in the host-microbial pathogen relationships. The uptake and growth of *Legionella* within acanthamoeba is temperature sensitive.[9]

Acanthamoeba castellani would encyst at 15°C, up to 20°C digests *L. pneumophila*, at 25°C-35°C the bacteria replicate freely [10] and at 37°C and above lyses the protozoa.[11] A stable relationship exists between *Odyssella thessalonicensis* and amoebae at 20°C but at 30°C-37°C it lyses the amoeba.[12] *Parachlamydia acanthamoeba* also survives within *Acanthamoebae* between 25°C - 30°C but lysis the host at 32°C - 37°C.[13]

The growth of bacteria within macrophages is also temperature-dependent, for example, temperature restriction applies to persistence of *M. marinum* in cultured mammalian cells. *M. marinum* would grow optimally at 25°C -33°C but poorly or not at all at 37°C and would cause only local lesions of cooler body surfaces usually of the extremities, but not the disseminated disease. However a strain adapted to optimal growth at 37°C causes disseminated systemic disease when injected in mouse [14] which is suggestive of lyses of host cells and release of the mycobacterium.

The intracellular survival of killing by phagosome-lysosome fusion, growth and multiplication bacteria (*dit Mycobacterium* spp) in two different host cells *Acanthamoeba* and mammalian host cells are indicative of a long evolutionary history of association. We support this by the fact that *Legionella* spp. use of similar mechanisms, the same set of genes including *mip*, aspartate-β-semialdehyde (asd) and Dot/*icm* genes and gene products to parasitize both mammalian and protozoan host cells.[9] Furthermore growth in *A. castellanii* enhances both *Legionella* spp. capacity to invade macrophages and intracellular replication similarly observed with *M. avium.*[15] By inference the passage through *Acanthamoeba* primes bacteria for intracellular growth within mammalian cells, thus the natural selection of organisms that are better adapted to surviving in the hostile environment of phagocytic cells.

On the basis of the above we postulate that *M. ulcerans* is primarily an intracellular bacterium of both *Acanthamoeba* and human host cells and that it is transmitted to human principally by infected trophozoites through broken skins [Box 1]. In the environment and at ambient temperatures below 37°C (we suspect 29-33°C the temperature range for laboratory culture) it survives normally within *Acanthamoeba* and on human contact, and at normal body temperatures and above *M. ulcerans* lyses the protozoa.

We postulate further that M. ulcerans persists in unfavourable and harsh environment inside the cyst of Acanthamoeba.

We support this with the fact that Acanthamoeba cyst can remain viable for more than 20 years of desiccation [16] and that just as other bacteria M. ulcerans can survive within cysts. This long term viability within cysts may even explain why outbreaks of BU occur in endemic areas with floods, a return to favourable conditions for Acanthamoeba. Furthermore, activities near water bodies are risk factors which can be reduced by wearing protective clothing.[1] Acanthamoeba spp are known natural hosts and vectors of microbial pathogens. Their aquatic habitat and ability to infect humans through broken skin, makes it a perfect intermediate host. In conclusion, we argue that Acanthamoeba spp should be considered prime suspect as a vector in BU transmission until proven 'not guitly'.

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