ORDER HEMIPTERA: THE TRUE HOST INVOLVED IN MYCOBACTERIUM ULCERAN TRANSMISSION, OR IS IT AN INNOCENT BY-STANDER?

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ABSTRACT

Buruli ulcer disease is an infection of the subcutaneous layer. The causative organism, *Mycobacterium ulcerans* is a slow-growing environmental pathogen often associated with wetland and slow moving streams. One insect order believed to be associated with *M. ulcerans* is the order hemiptera, in which the aquatic bugs (Naucoris sp and Belostoma sp) belong. Some Mycobacteria species are endosymbiont of *Acanthamoeba* and laboratory experiments has confirmed this in *M. ulcerans* in an endemic area in Benin persisting in an amoeba for 14 days. Aquatic insect are believed to feed on amoeba, planktons, snail and fish from which they get infected. Protozoan and planktons may be the true reservoir or host of *M. ulcerans* but little research has been done in this area. Though many studies have found *M. ulcerans* in these insects, the exact mechanism of transmission to humans is still elusive. This study aims to review the available data on aquatic bugs, protozoans and other invertebrates (snail and fish) to ascertain if aquatic insects are themselves victims of the *M. ulcerans* through feeding.

Keywords: Mycobacterium ulcerans Aquatic bugs Naucoridae Belostomatidae Buruli ulcer Arsenic Protozoan Hemiptera

1.0 INTRODUCTION

Buruli ulcer Disease (BUD) is predominantly an infection of the subcutaneous fat, causing serious necrotizing infections on the skin (Portaels *et al.* 1999). The limbs are the most affected part of the body but affect more of the lower limbs than the upper limbs. The disease starts as a painless nodule, papule or an area of edema which if left untreated result in large ulceration covering up to about 15% of the skin (George, 1999).

The causative organism of BUD is *Mycobacterium ulcerans* (MU) with the first detailed description of MU infection given in 1948 by MacCallum *et al.* in Australia (MacCallum 1948). Sir Albert Cook was the first to describe a skin ulcer consistent to BUD in 1897 at Kampala, Uganada. In 1950, the first BUD case was reported in Congo and the bacillus was identified by
Fenner and given the name *Mycobacterium ulcerans*. BUD has being reported in many countries after it was first described in Australia (MacCallum 1948), and later named in Uganda. The Uganda Buruli Group gave it the name “Buruli ulcer” because the cases they describe were first detected in Buruli County in Kyoga (Clancey 1962). The tropical and subtropical regions seem to be the most affected with special foci on West Africa (WHO. 2000). However some cases have also be reported in temperate areas (Johnson 1996). WHO counts at least 33 countries with tropical, subtropical and tropical climates reporting BUD.

Although the disease has a low mortality rate, it morbidity rate cannot be overlooked and it has a huge socioeconomic impact on affected populations. Ulceration often results in scarring, contractual deformities, amputations, and disabilities. In Africa, most cases of the disease occur in children between the ages of 4–15 years. Women and children seem to be the most affected but the reasons for this is known, and warrant further investigation (Wansbrough-Jones 2006; Williamson 2008). BUD is usually reported among the rural poor with little access to health centers for early diagnosis and treatment. This also create difficulties in estimating the exact prevalence since is under reported (Boleira 2010).

One characteristic of BUD is its association with wetlands. Individuals living close to water bodies, like streams, pond, swamps and lakes are the most affected and also where there has been human-linked changes in the aquatic environment, particularly those created as a result of environmental disturbance such as deforestation, dam construction, and agriculture (Duker 2004; Wagner 2008; Walsh 2008). BUD is still known as the mysterious disease because no clear mode of transmission has been established by research (Merritt et al. 2010). A number of vectors have been linked to the disease including infected mosquitoes, biting water bugs belonging to the families Naucoridae (creeping water bugs) and Belostomatidae (giant water bugs) could be considered reservoirs and vectors in the transmission of MU to humans in nature (Marsollier et al. 2005; Mosi 2008). In Australia it was found that possum with clinical BU shed MU in their fecal matter (Fyfe et al. 2010) but when a pilot study was conducted on BU Patients in Ghana, their fecal matter was MU negative by PCR. (Sarfo et al. 2011).

The objective of this study is to 1) review articles that have incriminated insects (especially in the order hemiptera) and other aquatic invertebrate like snails and fish as possible reservoir and transmission agent, 2) Also to review studies on protozoan and their role in MU infection to humans, and 3) to discuss if these aquatic insects and invertebrate are innocent by-standers feeding on the true host which might be protozoan (amoeba and planktons),

2.0 METHODS

2.1 Data sources and search strategy

Selection of the publications cited was based on the following approaches: 1) Direct knowledge from leading experts in Buruli Ulcer research 2) Online search engines for Buruli Ulcer and Mycobacterium ulcerans (predominantly PubMed, Web of Knowledge, Web of Science, Google scholar, Scopus database; 3) Review of the following websites: Buruli ulcer disease maintained by WHO in Geneva, Switzerland (http://www.who.int/buruli/en).
From online search engines, articles were identified by searching for words and phrases like *Mycobacterium ulcerans* or Buruli ulcer and (aquatic bugs; Naucoridae; Belostomatidae; snails; amoeba; protozoan; Gerridae; water strider and hemiptera). A total of about 68 articles were identified from the extensive search with relevance to MU, BUD and Mycobacteria in general with aquatic organisms (including original studies and reviews) out of about 1517 search result items including some few languages (French, Chinese and Arabic). Google translate software was used to translate these languages into English to find their relevance for inclusion in the study. Most of the analysis in this paper is based on information extracted from original articles and systematic reviews since *Mycobacteria* were first described in aquatic organism (1897).

Studies done on aquatic invertebrates and MU for the past 10 years are summarized in Table. The search engines also was used to identify, Buruli Ulcer-Arsenic and Mycobacterium ulcerans and Arsenic relationship, this yielded a dozen of original articles and reviews. Several other studies that provide essential information about agent, host, and environmental characteristics linked to M. ulcerans infection and are also referenced in this paper.

### 3.0 RESULTS AND DISCUSSION

#### 3.1 The Pathogen, *Mycobacterium ulcerans*.

*Mycobacterium ulcerans* belongs to same genus with the organisms that causes Tuberculosis and leprosy. MU is the third most common mycobacterial pathogen of humans after *M. tuberculosis* and *M. leprae* (Portaels et al. 2001). The definitive description was published in 1948, when MacCallum and others cultured the first MU from skin infection when the incubation temperature was set lower than for *M. tuberculosis* in Australia (MacCallum 1948). MU is characterized as an acid-alcohol resistant bacillus (BARR) that does not live freely in the environment. It is believed to occupy a specific niche within or around aquatic environment from where it is transmitted probably by an insect vector or unknown mechanisms to humans. MU is a slow-growing environmental mycobacterium that can be isolated from human lesions on Lowenstein-Jensen medium at 30-32°C (9) with pH of 5.4 -7.4 (Werf et al. 2005) and an incubation period of 5-6 weeks although up to six months may be required (Kishi 2011). It is reported, low oxygen conditions enhances the growth of MU but genomic information says otherwise because MU lacks both nitrate and fumarate reductase enzymes to make it microaerophilic (2.5% oxygen) (Palomino1998). A key gene crtI, in the pathway of carotinoid synthesis seems to offer MU the ability to survive in direct sunlight (Stinear et al. 2004).

The complete genome of MU was obtained in 2004 by Stinear et al., Genomic inferences suggest that MU may have evolved from an *M. marinum* ancestor. It is proposed that the genome has undergone extensive reductive evolution with some mutational event including transposon insertion and accumulation of about 700 pseudogenes. The identification of IS2404 and IS2606, a plasmid borne insertion sequences and also a toxin producing gene Enoly Reductase (ER) and sequence encoding the ketoreductase B domain (KR), set the stage for PCR DNA isolation of MU from soil, fish, biofilms, water filterate, frogs, insects and other invertebrate (Stinear et al. 2007), since culturing from the environment is very difficult (Stinear & Johnson, 2008). The conventional PCR target for MU in human lesions is IS2404, when applied to environmental
samples in African it has proven to target not only MU but other aquatic mycobacterial species in fish and West African clawed frogs. This means that other genes need to be targeted to confirm presence of MU in samples from these regions (Fyfe et al. 2007). Though ISs do not codify any gene, they have the ability to genetically modify gene expression, a total of 13 IS elements have being described in mycobacterial species. This has made multiplex PCR procedures necessary as well as design of probes targeting specifically MU in environmental samples in Africa and this has proven to work perfectly. Application of variable number tandem repeat (VNTR) typing and Single nucleotide polymorphism (SNP) analysis has also been developed to geographical discriminate between MU strains isolates (Kishi, 2011; Qi 2009; Williamson 2008).

3.2 Mycolatone – A unique feature of MU and necessary for survival in host.

The isolation and characterization of mycolactone was done by Small and coworkers in 1999, when they described two polyketide-derived macrolides from MU (Kishi 2011). MU bears it virulence to a plasmid encoded toxic macrolides (Hayman 1985). It is believed that acquisition of this plasmid is an evolution from a M. marinum-like ancestor (Stinear et al. 2007). However, there has been no evidence thus far to suggest toxin production by M. tuberculosis and M. leprae. Mycolactone is a heat –stable exotoxin active in extremely low concentration and not present in laboratory cultures (Hayman1985). These macrolides were designated mycolactones A and B. Inoculation of mycolactone A/B into guinea pigs produced lesions similar to that of Buruli ulcer in humans indicating a direct correlation of this macrolide with the ulcer. (George 1999). Similar structures of Mycolactone A/B have been confirmed from clinical isolates of M. ulcerans from Africa, Asia, Australia, and America. The other variants of the toxin are designated as mycolactones C, D, and E with mycolactone A/B being the most powerful macrolide found mostly in strains from Africa and Malaysia. In Australia mycolactone C appears to be more.

Mycolactone-like metabolites have also been isolated from the frog pathogen Mycobacterium liflandii and the fish pathogen Mycobacterium marinum (Merritt 2005; Ranger et al. 2006). Mycolactone A/B and it similar structures have been isolated from species located in or around freshwater habitats which has attracted considerable attention not only for their biological activity, but also for being the first examples of polyketide macrolides isolated from a human pathogen (Alexander et al. 2006; van Summeren 2005; Yin 2006). The genes encoding mycolactone are three large multi-enzyme complexes-polyketide synthases by the names; mlsA1 (51 kb), mlsA2 (7 kb), and mlsB (42 kb). These genes are located on the M. ulcerans virulence plasmid known as pMUM001 (Stinear et al. 2004). This makes MU the only mycobacterial species whose virulence is attributed to a plasmid borne insertion sequence (Stinear et al. 2007). Molecular findings shows 98 to 99.8 % genetic similarity between MU and M. marinum expect that M. marinum does not produce mycolactone (Stinear et al. 2007; Werf et al.2005). On the other hand research has found out that M. liflandii also present IS2404 and possesses all the genes that code for mycolactone and produces similar ulcer close to BU (Stinear et al. 2007). The major difference between M. liflandii and MU is one gene that codifies monooxygenase p450. (Wansbrough-Jones 2006).
3.3 Views on possible host and reservoir of MU

Many researchers have suggested plausible evidence that implicate protozoan as possible host for MU in nature (Adékambi 2006; Greub 2004). Protozoans offer a safe place for MU to find nutrients and protection form extreme environmental conditions when they form cysts during feeding on MU biofilms (Thomas 2007; Thomas et al. 2004). The role of insects in the transmission of MU to humans is not yet known but it is believed, aquatic insects feeding on infected MU protozoa like amoeba, might carry MU in their body and infect humans upon bite (Eddyani et al. 2008; Stinear 2008). This has been proven experimentally with animal models, but as to whether this actually occurs in nature is yet to be ascertained (Marsollier et al. 2004; Mosi et al. 2008). Although we are aware that, MU and BUD are associated with water bodies, the relationship between aquatic insects, protozoan, snails and fishes associated with these wetland and their role in transmission to humans is poorly understood (Duker 2006; Wagner et al. 2008; Walsh et al. 2008). One insect order of importance though is the order Hemiptera from which Naucoridae (Naucoris cimicoides) and Belostomatidae (Belostoma cordofana) belongs and these have being associated with MU as possible hosts (Marsollier et al. 2005; Mosi et al. 2008). All attempts to culture MU from environmental samples (insects, water and soil) have proven futile, and PCR detection of the IS2404 is inadequate in the characterization of MU since other mycobacterial species possess that insertion (Fyfe et al. 2007). In 2008, Portael et al. in a study from Benin were for the first time able to culture MU strain 00-1441 from a water strider (Gerris sp) a hemipteran (Stinear 2008). This indicates that MU can exist as intact organisms in insects and not only as DNA fragments in the environment. Figure 1 shows some insects that have been studied for the presence of MU.

Figure 1

Some aquatic bugs suspected to be involved in M. ulcerans transmission

A-Nepidae sp.  
B-Ranatrinae sp.  
C-Notonectidae sp.  
D-Naucoridae sp.  
E-Belostomatidae sp.  
F-Gerriidae sp.
3.4 Protozoan as possible reservoir of MU

The findings and conclusion of Mosi et al., (Table1-No 8), warrant further research. In their study Belostomatidae were fed with MU infected mosquito lavae and the colonization of MU in the salivary gland was monitored, this was a build-up on a previous study by Marsolier et al. 2005 (Table-No5). One of the aims of both studies was to find out if aquatic bugs can be infected through feeding and which of them is the true host and reservoir of MU. Several studies done on insects and some vertebrate has yielded unsatisfactory results regarding the exact prey these insects or other organisms are feeding on. The argument here is that, if insects in endemic communities test positive for MU DNA and in insects in non-endemic areas test negative, it presupposes that these insects are not natural carriers of MU. MU might be acquired through feeding and they are themselves victims of MU infection. This also has been suggested by many investigators (Portaels et al. 1999). Study has shown that Mycobacteria can live as endosymbiont in Acanthamoeba and be protected from adverse condition in the environment (Yu et al. 2007) and are capable of multiplying also in zooplanktons (Portaels et al.1999; Thomas 2007).

A study in Benin on amoeba, have shown that M. ulcerans in a laboratory experiment was phagocytized by Acanthamoeba polyphaga and persisted inside the amoebae for up to 14 days without disturbing the growth of the amoebae (Eddyani et al. 2008). Gryseel et al., also found out that, amoeba are potential natural host of MU, although they found other mycobacteria species in amoeba. This was the first report on protozoan in the MU and BUD research and needs further investigation (Gryseels et al. 2012; Merritt et al. 2010). Aquatic bugs are known to feed on a wide range of aquatic eukaryotic microorganisms as indicated above which might unfortunately implicate them in the MU conundrum but as a matter of fact not the true host of MU.

3.5 Results of some major studies on aquatic invertebrates

Table 1, below also shows major research on various aquatic invertebrate, mainly insect belonging to the order hemiptera. Some of the results obtained in Table 1, like Nos.3, 4, and 5 were done in the laboratory settings and not an in situ study. The table shows the country of the study and some major outcomes of the study. Although these studies has broaden our understanding on MU and BUD ecology, none has being able to elucidate the exact mode of transmission of MU to humans in the environment. The results also show some few contradictions in some outcome of the result, typical is Nos 4 & 7 another can be seen in Nos 3&8. As much as some researchers are of the view that previous methodology might have posed some challenges in securing accurate data to define the ecology of MU and BUD (H. Williamson 2008) in both endemic and non-endemic communities’ typical case being a study by Williamson in Nos 8 &13, care must be taken in putting down any method and outcome since a lot about MU and BUD is still unknown, especially, the mode of transmission, the choice of host, the exact niche, etc.
### Table 1-TABLE OF RESULTS OF SOME MAJOR STUDIES ON AQUATIC INVERTEBRATES:

<table>
<thead>
<tr>
<th>Date and country of study</th>
<th>Sampling site</th>
<th>Sampling Size of aquatic bugs</th>
<th>Main aquatic Bugs captured</th>
<th>Major conclusions</th>
<th>From study</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghana/Benin, 1999</td>
<td>Endemic</td>
<td>5 aquatic bug</td>
<td>Belostomatidae</td>
<td>The insect studied in this research are believed to transmit MU to humans but this cannot be confirmed since they do not directly bite humans in nature.</td>
<td>(Portaels et al. 1999)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Naucoridae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Firefly larvae</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Aquatic beetle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Belostomatidae</td>
<td>The insects captured are all aggressive predators of smaller aquatic invertebrates and protozoan and are water-filtering organisms capable of concentrating M. ulcerans from water or mud in swamps and ponds to infect them through feeding</td>
<td>(Portaels et al. 2001)</td>
<td></td>
</tr>
<tr>
<td>Benin, 2001</td>
<td>Endemic/Non-endemic</td>
<td></td>
<td>Naucoridae</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: MU = Melioidosis Ulcerans; M. ulcerans is the bacteria that causes Melioidosis, also known as WaterUlcer disease.
3.
Cote d’Ivoire, 2002

**Endemic**

| Various invertebrate including 80 Naucoridae | *Naucoris sp.* | This was the first strong evidence implicating *Naucoris sp.*, because it was able to transmit MU and caused BUD in an experimental mouse. |

| Snail were from Cote d’Ivoire | 20 snails | Snails | Water bugs, such as *Naucoris cimicoides*, is able to pick up MU through feeding, and is a potential vector of MU. |

| Aquatic bug | 10 aquatic bug | Aquatic bug | (Marsollier *et al.* 2004) |

3.
MU strain from French Guinea.& France (2004)

4.
France.

**Non-endemic**

| 30 | *Naucoris cimicoides* | Mycolactone must play a role in salivary gland colonization. A mutant deficient for toxin was not able to establish a long-term infectious process. |

| 20 | *Naucoris cimicoides* | MU can colonize and survive in different compartment of insects |

(Marsollier *et al.* 2005)
<table>
<thead>
<tr>
<th>Region</th>
<th>Year</th>
<th>Endemic Status</th>
<th>Sample Type</th>
<th>Count</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MU strain from</td>
<td>2007</td>
<td>Malayasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benin/Togo, 2007</td>
<td>2007</td>
<td>Endemic</td>
<td>5</td>
<td>Gerris sp.</td>
<td>The first isolation of MU from environmental sample (aquatic bug). This confirms the hypothesis that MU has an aquatic niche.</td>
<td>(Portaels et al. 2008)</td>
</tr>
<tr>
<td>Ghana, 2008</td>
<td>2008</td>
<td>Endemic</td>
<td>12</td>
<td>Belostomatidae</td>
<td>This study suggests that MU can live and colonize Belostomatids and mycolactone does not play a role in MU colonization of the salivary gland of these insects as the MU count was low.</td>
<td>(Mosi et al. 2008)</td>
</tr>
<tr>
<td>Ghana, 2008</td>
<td>2008</td>
<td>Endemic and Non-endemic</td>
<td>1068</td>
<td>Various invertebrate</td>
<td>The results of this study suggested that the distribution of MU is broader than the distribution of human BUD.</td>
<td>(Williamson 2008)</td>
</tr>
<tr>
<td>Ghana, 2008</td>
<td>2008</td>
<td>Endemic and Non-endemic</td>
<td>22,832</td>
<td>Various invertebrate</td>
<td>There was no significant difference between invertebrate abundance in BUD endemic and non-endemic areas. This rule out the evidence that hemiptera or other invertebrates are primary vectors of MU.</td>
<td>(Benbow et al. 2008)</td>
</tr>
<tr>
<td>10.</td>
<td>Cameroun, 2010</td>
<td>Endemic</td>
<td>7,407</td>
<td>Aquatic bugs and semi-aquatic bugs. (hemiptera)</td>
<td>This study suggests a possible seasonal variation in MU and BUD in the environmental. (Marion et al. 2010)</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Ghana, 2012</td>
<td>Endemic</td>
<td>65</td>
<td><em>Naucoris</em> sp. (n=47)</td>
<td>This provided the means to study an aquatic hemipteran diet using molecular method for <em>Naucoris</em> sp. They feeds on a wide range of prey and body sizes, including rotifers, insects, and Anurans (Gamboa 2012)</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Cameroun, 2012</td>
<td>Endemic</td>
<td>728</td>
<td>Belostomatidae, Naucoirdae, Gerridae, Nepinae, Ranatriniae, Notonectidae</td>
<td>Diversity of water bugs depends partly on the types of water bodies in the same endemic area, with streams and ponds as selective habitats offering best life conditions. Light attraction and the moon phases appeared to be influencing factor for aquatic bug’s distribution. (Ebong et al. 2012)</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Benin, 2012</td>
<td>Endemic</td>
<td>9</td>
<td>Vertebrate and invertebrate</td>
<td>The congruence of <em>M. ulcerans</em> in the environment and human infection raises the possibility that humans might play a role in the ecology of <em>M. ulcerans</em>. (Williamson et al. 2012)</td>
<td></td>
</tr>
</tbody>
</table>

**Invertebrates**— refer to all aquatic insects, tadpoles, fishlings and snails captured during the environmental sampling in the various studies.
3.6 Fish and snails as possible host and reservoir of MU

Mycobacteriosis in fish has long been identified since 1897; they affect many aquatic invertebrates and vertebrates, and cause many kinds of infections (Bataillon 1987). A review by Gauthier et al., (2009), could count about 20 different mycobacteria species associated with finfishes including *Mycobacterium marinum*, (Gauthier 2009) the reported ancestor of MU (Stinear et al. 2007). It is intriguing to find that no studies have come out yet to report any pathogenesis of MU in aquatic bugs, snails, fishes, frogs but only in animal models and in humans. *Mycobacterium liflandii* is known to cause some infection in West African crow frog and some fish because it posses the mycolactone *IS2404* while other environmental mycobacteria species do not (Merritt et al. 2010).

It will be an interesting finding to infect frogs and some fishes with MU and monitor if they develop any disease of any sort. The fact that MU and BUD are associated with wetlands and water bodies has led to many aquatic sampling to investigate the presence of MU DNAs in these environments (F Portaels et al., 1999). Many researchers have suggested various arthropods as possible host and reservoirs including; mosquitoes, flies and scorpions for environmental mycobacteria (Radford, 1975). But none has been able to come out conclusively with the exact mode of transmission of MU to humans.

A study on the potential role of fish in transmission by Eddyani et al., in Ga district of Ghana and Benin found some fishes positive for MU and is possible animals that prey on fish, like some amphibians and birds may be involved in MU distribution in the environment. How this directly affects humans is yet to be determined. (Eddyani et al. 2004). A recent study by Wilson et al, on tadpole and amphibians in Ghana also reported the same (Willson et al. 2013). All these confirms earlier hypothesis that aquatic bugs might be involved in the BUD since some members of the hemiptera feed on small fish, snails and protozoan (Portaels et al. 2001). If this is so, then another area that needs further research is among the fish and snail fauna in both endemic and non-endemic since these are food delicacies for inhabitants in the regions. To validate the hypothesis, all parameters listed need to be thoroughly investigated. And one question that still need to be answered is, what is/are the exact prey of aquatic bugs? And can that affect the distribution of BUD in endemic and non-endemic areas?

4.0 CONCLUSION

Buruli ulcer disease (BUD) is one of the Neglected Tropical Diseases (NTDs). The organism that causes this disease, known as *Mycobacterium ulcerans* is believed to have evolved from an aquatic *Mycobacterium marinum*-like ancestor by the acquisition of a virulent plasmid (Stinear et al. 2007). BUD is usually associated with wetland and highly disturbed environment (Duker et al. 2006; Wagner et al. 2008; Walsh et al. 2008). It has been reported in over 30 countries, in the tropic, sub-tropic, Asia, some part of the temperate regions but high incidence is in West Africa (Asiedu, Scherbier 2000). The exact mode of transmission as at now is unknown; research speculates that some aquatic insects and small mammals might serve as vectors of MU but this is yet to be ascertained (Portaels et al. 1999). There is the need for more research in this field especially in the protozoan and plankton communities, to establish a clear mode of transmission of MU to humans from the environment.
References


