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

Michael S. Avumegah[1]; Christian Wejse[2].

[1]" Deakin University. School of Medicine. Geelong Waurn Ponds. Victoria Australia (mavumega@deakin.edu.au)

[2]" Aarhus University Hospital. Dep. of Infectious Diseases, Denmark (wejse@dadlnet.dk)

ABSTRACT

Burul 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 resorvior 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 acertain 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 seems 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 it association with wetlands. Individuals living close to water bodies, like streams, pond, swamps and lakes are the most affected and also where there has being 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).

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

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^{o}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 fumerate 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 IS*2404*, when applied to environmental

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

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.

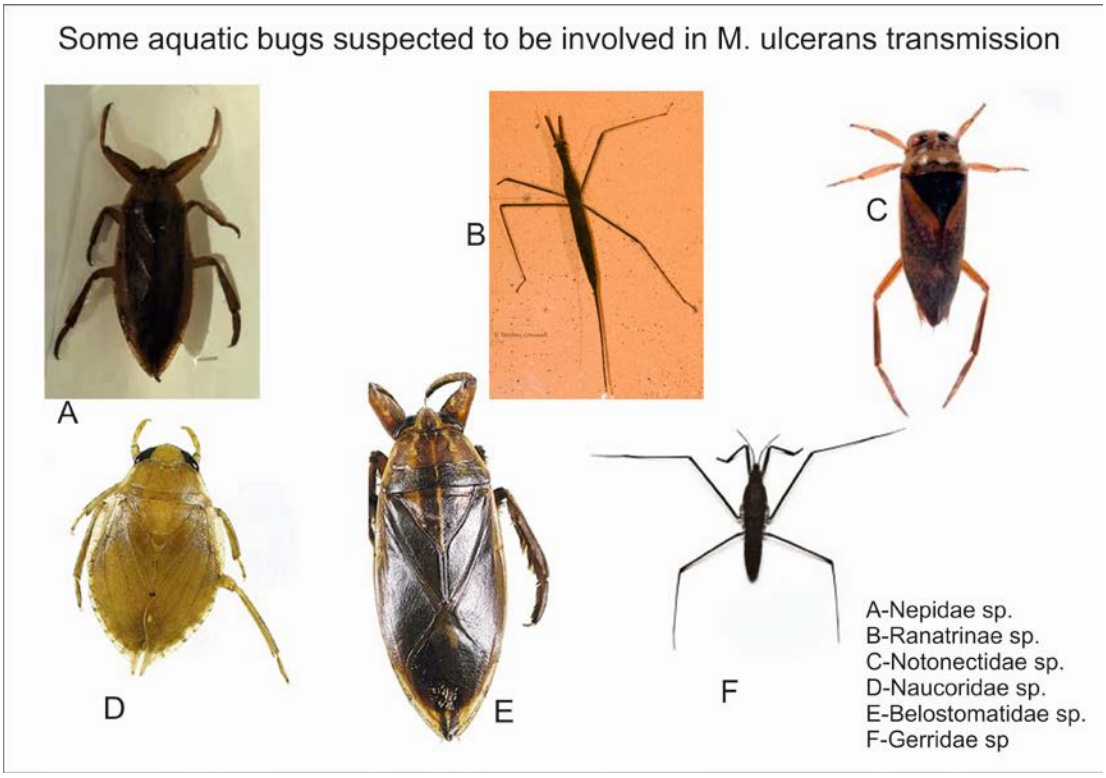
Mycolactone-like metabolites have also been isolated from the frog pathogen *Mycobacterium liflandii* and the fish pathogen *Mycobacterium marinum* (Merritt 2005; Ranger *et al*. 2006). Mycolactone A/B and it similar structures have been isolated from species located in or around freshwater habitats 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. lifllandii* and MU is one gene that codifies monooxygenase p450. (Wansbrough-Jones 2006).

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

3.3 Views on possible host and reservior 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 cordofna*) 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 IS*2404* 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



PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

A-C and - (Ebong *et al*. 2012). D and E – (Wansbrough-Jones 2006). F- (F Portaels et al., 1999).

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:

Date and country of study	Sampling site	Sampling Size of aquatic bugs	Main aquatic Bugs captured	Major conclusions From study	References
1. Ghana/Benin, 1999	Endemic	5 aquatic bug	Belostomatidae Naucoridae	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.	(Portaels *et al*. 1999)
2. Benin, 2001	Endemic/Non-endemic		Belostomatidae Naucoridae Firefly larvae Aquatic beetle	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	(Portaels *et al.* 2001)

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

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.	(Marsollier *et al*. 2002)
3. MU strain from French Guinea.& France (2004)	Snail were from Cote d'Ivoire	20 snails 10 aquatic bug	Snails Aquatic bug	Water bugs, such as *Naucoris cimicoides*, is able to pick up MU through feeding, and is a potential vector of MU.	(Marsollier *et al*. 2004)
4. France. MU strains from Malayasia/ FrenchGuyana (2005)	Non-endemic	30	*Naucoris cimiciodes*	Mycolactone must play a role in salivary gland colonization. A mutant deficient for toxin was not able to establish a long-term infectious process.	(Marsollier *et al*. 2005)
5. France.	Non-endemic	20	*Naucoris cimiciodes*	MU can colonize and survive in different compartment of insects	(Marsollier *et al*. 2007)

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

MU strain from Malayasia (2007)				body	
6. Benin/Togo, 2007	Endemic	5	*Gerris sp*.	The first isolation of MU from environmental sample (aquatic bug). This confirms the hypothesis that MU has an aquatic niche.	(Portaels et al. 2008)
7. Ghana, 2008	Endemic	12	Belostomatidae	This study suggests that MU can live and colonize Belostomatids and mycolactone does not play a role in in MU colonization of the salivary gland of these insects as the MU count was low.	(Mosi *et al.* 2008)
8. Ghana, 2008	Endemic and Non-endemic	1068	Vertebrate and invertebrate	The results of this study suggested that the distribution of MU is broader than the distribution of human BUD.	(Williamson 2008)
9. Ghana, 2008	Endemic and Non-endemic	22,832	Various invertebrate	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.	(Benbow *et al.* 2008)

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

10. Cameroun, 2010	Endemic	7,407	Aquatic bugs and semi-aquatic bugs. (hemiptera)	This study suggests a possible seasonal variation in MU and BUD in the environmental.	(Marion *et al.*2010)
11. Ghana, 2012	Endemic	65	*Naucoris sp*. (n=47)	This provided the means to study an aquatic hemipteran diet using molecular method for *Naucoris* sp. They feeds on a wide range of prey and body sizes, including rotifers, insects, and Anurans	(Gamboa 2012)
12. Cameroun, 2012	Endemic	728	Belostomatidae Naucoridae Gerridae Nepinae Ranatrinae Notonectidae	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)
13. Benin, 2012	Endemic	9	Vertebrate and invertebrate	The congruence of M. ulcerans in the environment and human infection raises the possibility that humans might play a role in the ecology of M. ulcerans.	(Williamson *et al*. 2012)

Invertebrates- refer to all aquatic insects, tadpoles, fishlings and snails captured during the environmental sampling in the various studies.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

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 finfinshes 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 mycrobacteria 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 orgamsim that causes this disease, known as *Mycobacterium ulcerans* is believed to have evolved from an aquatic *Myacbactrium 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, Scherpbier 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.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

References

Adékambi, T., Ben Salah, S., Khlif, M., Raoult, D., & Drancourt, M. (2006). Survival of environmental mycobacteria in Acanthamoeba polyphaga. *Applied and Environmental Microbiology*, *72*(9), 5974–81. doi:10.1128/AEM.03075-05

Alexander, M. D., Fontaine, S. D., La Clair, J. J., Dipasquale, A. G., Rheingold, A. L., & Burkart, M. D. (2006). Synthesis of the mycolactone core by ring-closing metathesis. *Chemical Communications (Cambridge, England)*, (44), 4602–4. doi:10.1039/b609408b

Asiedu, K., Scherpbier, R., & Raviglione, M. (2000). Buruli ulcer: Mycobacterium ulcerans infection. Retrieved from http://www.cabdirect.org/abstracts/20002013239.html;jsessionid=4CE447A7BF889CA26A5D9A0F6738A2EF?freeview=true

Bataillon, E., Dubard, L., Terre, L., 1897. (1987). Un nouveau type de tuberculose. Comptes rendus des Se´ances de la Socie´te´ de Biologie.

Benbow, M. E., Williamson, H., Kimbirauskas, R., McIntosh, M. D., Kolar, R., Quaye, C., … Merritt, R. W. (2008). Aquatic invertebrates as unlikely vectors of Buruli ulcer disease. *Emerging Infectious Diseases*, *14*(8), 1247–54. doi:10.3201/eid1408.071503

Boleira, M., Lupi, O., Lehman, L., Asiedu, K. B., & Kiszewski, A. E. (2010). Úlcera de Buruli. *Anais Brasileiros de Dermatologia*, *85*(3), 281–301. doi:10.1590/S0365-05962010000300002

Clancey, J., Dodge, R., & Lunn, H. (1962). Study of a mycobacterium causing skin ulceration in Uganda. *Ann Soc Belg Méd Trop*. Retrieved from http://lib.itg.be/open/ASBMT/1962/1962asbm0585.pdf

Duker, A. A., Carranza, E. J., & Hale, M. (2004). Spatial dependency of Buruli ulcer prevalence on arsenic-enriched domains in Amansie West District, Ghana: implications for arsenic mediation in Mycobacterium ulcerans infection. *International Journal of Health Geographics*, *3*(1), 19. doi:10.1186/1476-072X-3-19

Duker, A. A., Portaels, F., & Hale, M. (2006). Pathways of Mycobacterium ulcerans infection: a review. *Environment International*, *32*(4), 567–73. doi:10.1016/j.envint.2006.01.002

Ebong, S. M. a, Eyangoh, S., Marion, E., Landier, J., Marsollier, L., Guégan, J.-F., & Legall, P. (2012). Survey of water bugs in bankim, a new buruli ulcer endemic area in cameroon. *Journal of Tropical Medicine*, *2012*, 123843. doi:10.1155/2012/123843

Eddyani, M., De Jonckheere, J. F., Durnez, L., Suykerbuyk, P., Leirs, H., & Portaels, F. (2008). Occurrence of free-living amoebae in communities of low and high endemicity for Buruli ulcer in southern Benin. *Applied and Environmental Microbiology*, *74*(21), 6547–53. doi:10.1128/AEM.01066-08

297 Eddyani, M., Ofori-adjei, D., Teugels, G., De, D., Boakye, D., Meyers, W. M., & Weirdt, D. De.
298 (2004). Potential Role for Fish in Transmission of Mycobacterium ulcerans Disease (Buruli
299 Ulcer): an Environmental Study Potential Role for Fish in Transmission of Mycobacterium
300 ulcerans Disease (Buruli Ulcer): an Environmental Study. doi:10.1128/AEM.70.9.5679

301 Fyfe, J. a M., Lavender, C. J., Handasyde, K. a, Legione, A. R., O'Brien, C. R., Stinear, T. P., …
302 Johnson, P. D. R. (2010). A major role for mammals in the ecology of Mycobacterium ulcerans.
303 *PLoS Neglected Tropical Diseases*, *4*(8), e791. doi:10.1371/journal.pntd.0000791

304 Fyfe, J. A. M., Lavender, C. J., Johnson, P. D. R., Globan, M., Sievers, A., Azuolas, J., &
305 Stinear, T. P. (2007). Development and application of two multiplex real-time PCR assays for
306 the detection of Mycobacterium ulcerans in clinical and environmental samples. *Applied and*
307 *Environmental Microbiology*, *73*(15), 4733–40. doi:10.1128/AEM.02971-06

308 Gamboa, M., Kimbirauskas, R. K., Merritt, R. W., & Monaghan, M. T. (2012). A molecular
309 approach to identifying the natural prey of the African creeping water bug Naucoris, a potential
310 reservoir of Mycobacterium ulcerans. *Journal of Insect Science (Online)*, *12*, 2.
311 doi:10.1673/031.012.0201

312 Gauthier, D. T., & Rhodes, M. W. (2009). Mycobacteriosis in fishes: a review. *Veterinary*
313 *Journal (London, England : 1997)*, *180*(1), 33–47. doi:10.1016/j.tvjl.2008.05.012

314 George, K. M. (1999). Mycolactone: A Polyketide Toxin from Mycobacterium ulcerans
315 Required for Virulence. *Science*, *283*(5403), 854–857. doi:10.1126/science.283.5403.854

316 Greub, G., & Raoult, D. (2004). Microorganisms Resistant to Free-Living Amoebae. *Clinical*
317 *Microbiology Reviews*, *17*(2), 413–433. doi:10.1128/CMR.17.2.413-433.2004

318 Gryseels, S., Amissah, D., Durnez, L., Vandelannoote, K., Leirs, H., De Jonckheere, J., …
319 Eddyani, M. (2012). Amoebae as potential environmental hosts for Mycobacterium ulcerans and
320 other mycobacteria, but doubtful actors in Buruli ulcer epidemiology. *PLoS Neglected Tropical*
321 *Diseases*, *6*(8), e1764. doi:10.1371/journal.pntd.0001764

322 Hayman, J., & McQueen, A. (1985). The Pathology of Mycobacterium Ulcerans Infection.
323 Retrieved from http://informahealthcare.com/doi/abs/10.3109/00313028509084759

324 Johnson, P. D., Veitch, M. G., Leslie, D. E., Flood, P. E., & Hayman, J. A. (1996). The
325 emergence of Mycobacterium ulcerans infection near Melbourne. *The Medical Journal of*
326 *Australia*, *164*(2), 76–8. Retrieved from http://europepmc.org/abstract/MED/8569576

327 Kishi, Y. (2011). Chemistry of mycolactones, the causative toxins of Buruli ulcer. *Proceedings*
328 *of the National Academy of Sciences of the United States of America*, *108*(17), 6703–8.
329 doi:10.1073/pnas.1015252108

330 MacCallum P, Tolhurst JC, Buckle G, S. H. (1948). A new mycobacterial infection in man. *J*
331 *Pathol Bacteriol*, *60:93-122*.

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.656v2 | CC-BY 4.0 Open Access | rec: 8 Dec 2014, publ: 8 Dec 2014

Marion, E., Eyangoh, S., Yeramian, E., Doannio, J., Landier, J., Aubry, J., … Marsollier, L. (2010). Seasonal and regional dynamics of M. ulcerans transmission in environmental context: deciphering the role of water bugs as hosts and vectors. *PLoS Neglected Tropical Diseases*, *4*(7), e731. doi:10.1371/journal.pntd.0000731

Marsollier, L., André, J.-P. S., Frigui, W., Reysset, G., Milon, G., Carbonnelle, B., … Cole, S. T. (2007). Early trafficking events of Mycobacterium ulcerans within Naucoris cimicoides. *Cellular Microbiology*, *9*(2), 347–55. doi:10.1111/j.1462-5822.2006.00790.x

Marsollier, L., Aubry, J., Coutanceau, E., André, J.-P. Saint, Small, P. L., Milon, G., … Cole, S. T. (2005). Colonization of the salivary glands of Naucoris cimicoides by Mycobacterium ulcerans requires host plasmatocytes and a macrolide toxin, mycolactone. *Cellular Microbiology*, *7*(7), 935–43. doi:10.1111/j.1462-5822.2005.00521.x

Marsollier, L., Robert, R., Aubry, J., André, J. Saint, Kouakou, H., Legras, P., … Carbonnelle, B. (2002). Aquatic Insects as a Vector for Mycobacterium ulcerans Aquatic Insects as a Vector for Mycobacterium ulcerans. doi:10.1128/AEM.68.9.4623

Marsollier, L., Sévérin, T., Aubry, J., Merritt, R. W., André, J. Saint, Legras, P., … Andre, J. Saint. (2004). Aquatic Snails , Passive Hosts of Mycobacterium ulcerans Aquatic Snails , Passive Hosts of Mycobacterium ulcerans. doi:10.1128/AEM.70.10.6296

Merritt, R. W., Benbow, M. E., & Small, P. L. C. (2005). Unraveling an emerging disease associated with disturbed aquatic environments : the case of Buruli ulcer " T, (Figure 2).

Merritt, R. W., Walker, E. D., Small, P. L. C., Wallace, J. R., Johnson, P. D. R., Benbow, M. E., & Boakye, D. A. (2010). Ecology and transmission of Buruli ulcer disease: a systematic review. *PLoS Neglected Tropical Diseases*, *4*(12), e911. doi:10.1371/journal.pntd.0000911

Mosi, L., Williamson, H., Wallace, J. R., Merritt, R. W., & Small, P. L. C. (2008). Persistent association of Mycobacterium ulcerans with West African predaceous insects of the family belostomatidae. *Applied and Environmental Microbiology*, *74*(22), 7036–42. doi:10.1128/AEM.01234-08

Palomino, J. C., Obiang, A. M., Realini, L., Meyers, W. M., & Portaels, F. (1998). Effect of Oxygen on Growth of Mycobacterium ulcerans in the BACTEC System. *J. Clin. Microbiol.*, *36*(11), 3420–3422. Retrieved from http://jcm.asm.org/content/36/11/3420.short

Portaels, F., Chemlal, K., Elsen, P., Johnson, P. D., Hayman, J. A., Hibble, J., … Meyers, W. M. (2001). Mycobacterium ulcerans in wild animals. *Revue Scientifique et Technique (International Office of Epizootics)*, *20*(1), 252–64. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11288515

Portaels, F., Elsen, P., Guimaraes-Peres, a, Fonteyne, P. a, & Meyers, W. M. (1999). Insects in the transmission of Mycobacterium ulcerans infection. *Lancet*, *353*(9157), 986. doi:10.1016/S0140-6736(98)05177-0

Portaels, F., Meyers, W. M., Ablordey, A., Castro, A. G., Chemlal, K., de Rijk, P., … Pedrosa, J. (2008). First cultivation and characterization of Mycobacterium ulcerans from the environment. *PLoS Neglected Tropical Diseases*, *2*(3), e178. doi:10.1371/journal.pntd.0000178

Qi, W., Käser, M., Röltgen, K., Yeboah-Manu, D., & Pluschke, G. (2009). Genomic diversity and evolution of Mycobacterium ulcerans revealed by next-generation sequencing. *PLoS Pathogens*, *5*(9), e1000580. doi:10.1371/journal.ppat.1000580

Radford, A. J. (1975). Mycobacterium Ulcerans in Australia. *Australian and New Zealand Journal of Medicine*, *5*(2), 162–169. doi:10.1111/j.1445-5994.1975.tb03649.x

Ranger, B. S., Mahrous, E. a, Mosi, L., Adusumilli, S., Lee, R. E., Colorni, A., … Small, P. L. C. (2006). Globally distributed mycobacterial fish pathogens produce a novel plasmid-encoded toxic macrolide, mycolactone F. *Infection and Immunity*, *74*(11), 6037–45. doi:10.1128/IAI.00970-06

Sarfo, F. S., Lavender, C. J., Fyfe, J. A. M., Johnson, P. D. R., Stinear, T. P., & Phillips, R. O. (2011). Mycobacterium ulcerans DNA not detected in faecal samples from Buruli ulcer patients: results of a pilot study. *PloS One*, *6*(5), e19611. doi:10.1371/journal.pone.0019611

Stinear, T., & Johnson, P. D. R. (2008). First isolation of Mycobacterium ulcerans from an aquatic environment: the end of a 60-year search? *PLoS Neglected Tropical Diseases*, *2*(3), e216. doi:10.1371/journal.pntd.0000216

Stinear, T. P., Mve-Obiang, A., Small, P. L. C., Frigui, W., Pryor, M. J., Brosch, R., … Cole, S. T. (2004). Giant plasmid-encoded polyketide synthases produce the macrolide toxin of Mycobacterium ulcerans. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(5), 1345–9. doi:10.1073/pnas.0305877101

Stinear, T. P., Seemann, T., Pidot, S., Frigui, W., Reysset, G., Garnier, T., … Cole, S. T. (2007). Reductive evolution and niche adaptation inferred from the genome of Mycobacterium ulcerans , the causative agent of Buruli ulcer, 192–200. doi:10.1101/gr.5942807.192

Thomas, V., Bouchez, T., Nicolas, V., Robert, S., Loret, J. F., & Lévi, Y. (2004). Amoebae in domestic water systems: resistance to disinfection treatments and implication in Legionella persistence. *Journal of Applied Microbiology*, *97*(5), 950–63. doi:10.1111/j.1365-2672.2004.02391.x

Thomas, V., & McDonnell, G. (2007). Relationship between mycobacteria and amoebae: ecological and epidemiological concerns. *Letters in Applied Microbiology*, *45*(4), 349–57. doi:10.1111/j.1472-765X.2007.02206.x

Van Summeren, R. P., Feringa, B. L., & Minnaard, A. J. (2005). New approaches towards the synthesis of the side-chain of mycolactones A and B. *Organic & Biomolecular Chemistry*, *3*(14), 2524–33. doi:10.1039/b505980a

403 Wagner, T., Benbow, M. E., Brenden, T. O., Qi, J., & Johnson, R. C. (2008). Buruli ulcer disease
404 prevalence in Benin, West Africa: associations with land use/cover and the identification of
405 disease clusters. *International Journal of Health Geographics*, *7*, 25. doi:10.1186/1476-072X-7-
406 25

407 Walsh, D. S., Portaels, F., & Meyers, W. M. (2008). Buruli ulcer (Mycobacterium ulcerans
408 infection). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, *102*(10), 969–
409 78. doi:10.1016/j.trstmh.2008.06.006

410 Wansbrough-Jones, M., & Phillips, R. (2006). Buruli ulcer: emerging from obscurity. *Lancet*,
411 *367*(9525), 1849–58. doi:10.1016/S0140-6736(06)68807-7

412 Werf, T. S. Van Der, Stienstra, Y., Johnson, R. C., Phillips, R., Adjei, O., Fleischer, B., …
413 Asiedu, K. (2005). Public Health Reviews Mycobacterium ulcerans disease, *020099*(04), 785–
414 791.

415 Williamson, H., & Benbow, M. (2008). Distribution of Mycobacterium ulcerans in Buruli ulcer
416 endemic and non-endemic aquatic sites in Ghana. *PLoS Neglected Tropical* …. Retrieved from
417 http://dx.plos.org/10.1371/journal.pntd.0000205

418 Williamson, H. R., Benbow, M. E., Campbell, L. P., Johnson, C. R., Sopoh, G., Barogui, Y., …
419 Small, P. L. C. (2012). Detection of Mycobacterium ulcerans in the environment predicts
420 prevalence of Buruli ulcer in Benin. *PLoS Neglected Tropical Diseases*, *6*(1), e1506.
421 doi:10.1371/journal.pntd.0001506

422 Willson, S. J., Kaufman, M. G., Merritt, R. W., Williamson, H. R., Malakauskas, D. M., &
423 Benbow, M. E. (2013). disease, *1*, 1–13.

424 Yin, N., Wang, G., Qian, M., & Negishi, E. (2006). Stereoselective Synthesis of the Side Chains
425 of Mycolactones A and B Featuring Stepwise Double Substitutions of 1,1-Dibromo-1-alkenes.
426 *Angewandte Chemie*, *118*(18), 2982–2986. doi:10.1002/ange.200600012

427 Yu, H. S., Jeong, H. J., Hong, Y.-C., Seol, S.-Y., Chung, D.-I., & Kong, H.-H. (2007). Natural
428 occurrence of Mycobacterium as an endosymbiont of Acanthamoeba isolated from a contact lens
429 storage case. *The Korean Journal of Parasitology*, *45*(1), 11. doi:10.3347/kjp.2007.45.1.11

430