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RISK PROFILE FOR TRANSMISSION OF*MYCOBACTERIUM ULCERANS* IN GHANA

BY

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DECLARATION

I hereby declare that with the exception of the references cited to other people's work which has been duly acknowledged, this work is the result of my own research work done under supervision and has neither in part or whole been presented elsewhere for another degree.

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DEDICATION

I dedicate this work to my dear wife Angela and my little angel Selikem.

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ABSTRACT

Buruli ulcer disease (BU) is a skin disease caused by *Mycobacterium ulcerans*. Currently, Buruli ulcer has been reported in over 30 countries, the subtropical regions of Asia, in Latin America, in the Western Pacific region and in Eastern and Central Africa. Even though it has been reported in other continents, West Africa is the region most affected. It is one of the neglected tropical diseases and second commonest disease caused by Mycobacterium in Ghana and third globally. Suhum-Kraboa-Coltar, Akuapem South districts in the Eastern region and Ga West and Ga South districts of Greater Accra region are some of the districts affected by Buruli ulcer. Unfortunately the exact mechanism of transmission is not known. Even though some risk factors associated with contracting the disease have been identified in previous studies, what pertains in Suhum-Kraboa-Coaltar and Akuapem South districts of the Eastern region is not known. In addition, there is lack of detailed understanding of how environmental and social conditions interact to cause the disease. Finally, the spatial distribution of BU within the communities along the course of Densu river in the study area is also not known. Spatial epidemiology has proven to be useful for understanding the geographical distribution of many diseases. Combining the Geographical Information System (GIS) technology for epidemiologic mapping of Buruli ulcer cases alongside with environmental features and a fine detail case-control study to identify risk factors associated with Buruli ulcer will provide current knowledge of the disease in the study area. This information is crucial in developing clear interventional messages for community education on the disease and which communities are most at risk. The absence of these hampers the prevention and control of BU within the municipalities and consequently leads to ineffective use of scarce resources hence the need for the research. This study sought to develop a risk

profile for the transmission of Buruli Ulcer at the individual and at the Community level in Akuapem South and Suhum Kraboa Coaltar Districts and spatial distribution of Buruli ulcer in the communities along the entire course of the Densu river.

A Case-control study with Spatial mapping was carried out. The cases were identified through active community case search. A case of Buruli ulcer was defined as any person aged 2 years or more who resides in the Suhum-Kraboa-Coaltar and Akuapem South districts diagnosed of Buruli ulcer meeting the WHO clinical case definition for *M. ulcerans* disease. A Control was defined as any person who resides in the same community/neighbourhood as the Buruli ulcer patient comes from but does not have the disease. Standardized questionnaires were administered to the cases as well as their controls. Geographic Positioning System (GPS) receiver was used to map the cases as well as environmental characteristics that are of particular interest in contracting BU.

A backward elimination logistic regression analysis of the data indicated that presence of wetland in the neighborhood (OR=3.9, 95% CI=1.9 -8.2), insect bite in water/mud (OR=5.7, 95% CI = 2.5 -13.1), use of adhesive when injured (OR=2.7, 95% CI = 1.1- 6.8), washing in the Densu river (OR= 2.3, 95% CI = 1.1- 4.96) were significantly associated with the development of BU. Rubbing an injured area with alcohol (OR=0.21, 95% CI = 0.008-0.57) and wearing long sleeves to farm (OR=0.29, 95% CI =0.14 - 0.62) protects against BU. The mapping showed that communities upstream along the Densu River where the river is not polluted had no BU cases but BU cases were identified beyond the polluted area of the Densu river. There was clustering of cases of Buruli ulcer in some communities and these occurred in areas where the river was most contaminated .Hence to avoid contamination.

TABLE OF CONTENTS

LIST OF FIGURES/MAPS

LIST OF TABLES

LIST OF ABBREVIATIONS

ACBRIDGE	Academic Bridge
AIDS	Acquired Immune Deficiency Syndrome
BCG	Bacillus Calmette Guérin
BU	Buruli ulcer
DAAD	Deutscher Akademischer Austausch Dienst
DNA	Deoxyribonucleic acid
DHMT	District Health Management Team
DHMTs	District Health Management teams
GIS	Geographical Information System
GPS	Geographical Positioning System
HIV	Human Immunodeficiency Virus
ID	Identification
IRB	Institutional Review Board
LULC	Land Use Land Cover
M. ulcerans	*Mycobacterium ulcerans*
NBCP	National Buruli ulcer Control Programme
NMIMR	Noguchi Memorial Institute for Medical Research
^{o}C	Degree Celcius
OPD	Outpatient Department
OR	Odds Ratio
PCR	Polymerase Chain Reaction
TB	Tuberculosis
W.H.O	World Health Organization

CHAPTER ONE - INTRODUCTION

1.1 Background

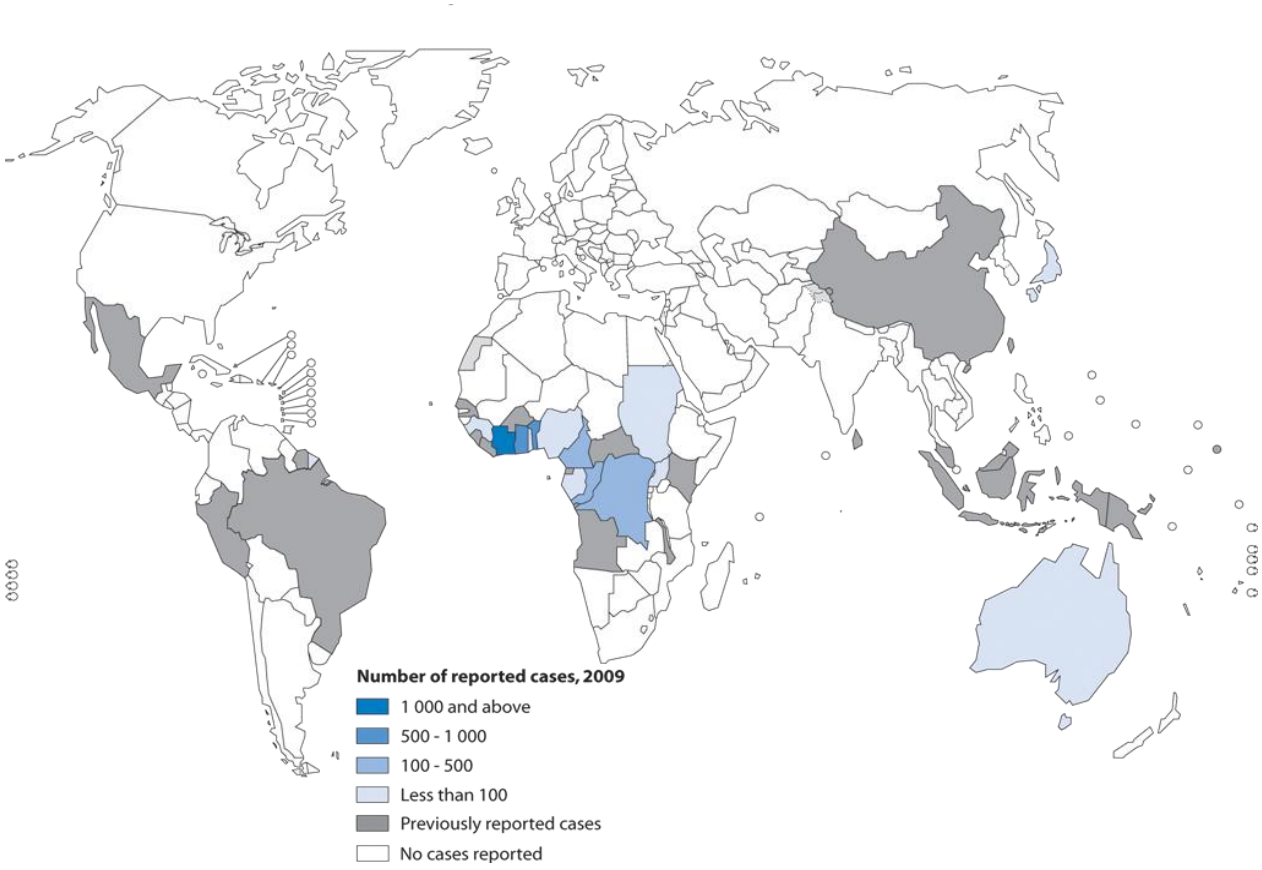
1.1.1 Epidemiology

Buruli ulcer disease (BU) is a chronic debilitating skin disease caused by *Mycobacterium ulcerans* (Walsh et al., 2008;Duker et al., 2006). It is one of the neglected tropical diseases and second commonest disease caused by Mycobacterium in Ghana and third globally (Peeters Grietens et al., 2010; Raghunathan et al., 2005).

In 1897 and 1920s large cutaneous ulcers were described in Uganda and Northeast Congo respectively which were probably caused by *M. Ulcerans* thus dating the disease to early days. However, it was in the twentieth century when the first successful culture of *M. ulcerans* from a leg ulcer in a child from Bairnsdale, Victoria, Australia was done. Cases were identified among refugees from Rwanda who were in an area close to the River Nile in the 1960s and 1970s. Missionaries in Congo who were caring for leprosy patients were also reported to be nursing large ulcers thought to be caused by *M. Ulcerans.* Later on more cases were found in Buruli district near Lake Kyoga, hence the name Buruli Ulcer.

Currently, Buruli ulcer has been reported in over 30 countries, the subtropical regions of Asia, in Latin America, in the Western Pacific region and in Eastern and Central Africa (Merritt et al., 2010; Walsh et al., 2008, 2010; Duker et al., 2006). Even though it has been reported in other continents, West Africa is the region most affected (Merritt et al., 2010; Walsh et al., 2008).

Figure 1: Global distribution of Buruli ulcer, 2009

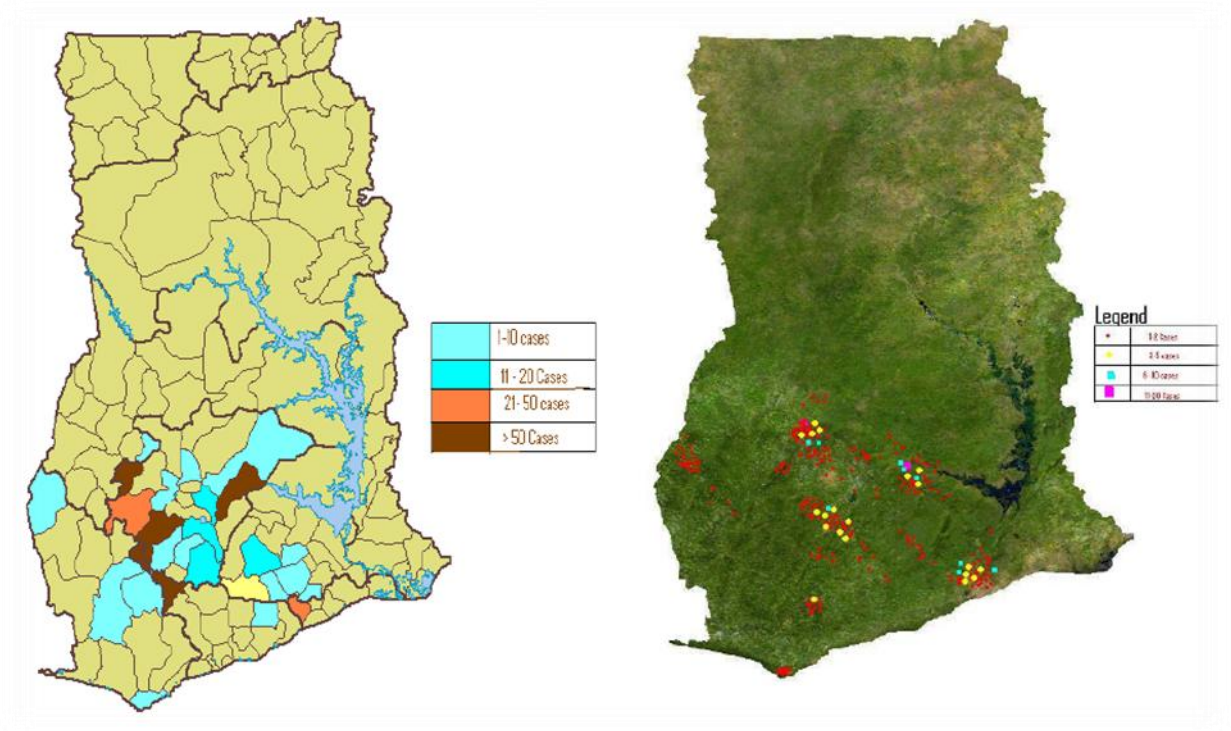


Source: World Health Organization, Buruli Ulcer 2009

Bayley reported the first case of Buruli ulcer in Ghana in 1971 (Aiga et al., 2004a; Bayley, 1971) Ga West District in Ghana had a prevalence rate of 87.7/100 000 and was the district with the highest number of active cases (Amofah et al., 2002). Over 426 communities have reported cases of Buruli ulcer in Ghana. These communities are in Ashanti, Brong Ahafo, Eastern, Greater Accra and the Western regions.

Children less than fifteen years are mostly affected compared to adults, even though any age can be affected (Merritt et al., 2010; Debacker et al., 2006; Phanzu et al., 2006; Aiga et al., 2004).

Figure 2: Distribution of Buruli ulcer cases in Ghana, 2009



Source: National Buruli Ulcer Control Programme, 2009

1.1.2 Clinical Presentation, diagnosis and management

M. ulcerans is a slow-growing mycobacterium and produces a soluble polyketide toxin called mycolactone which has both immunosuppressive and cytotoxic properties (Demangel et al., 2009; Tobias et al., 2009). Buruli ulcer disease evolves in three stages (Raghunathan et al., 2005).

It manifests initially as firm, non tender, subcutaneous nodules 1-2 cm in diameter at the sites of penetrating skin trauma, and sometimes plaques or oedema which is the pre-ulcerative stage. It has been postulated that the *M. Ulcerans* is inoculated through the sites of the skin trauma. The Mycobacterium produces a mycolactone which leads to apoptosis with extensive subcutaneous tissue necrosis and local immunosuppression. In addition, there is poor influx of inflammatory cells and also affect the cutaneous nerves making it painless, explaining the painless nature of the lesions. At this stage the lesion appears clinically as a nodules and sometimes plaques or oedema.

Following days to weeks after the initial lesion, the nodule typically breaks down centrally forming an ulcer with undermined edges. This makes it difficult to actually estimate the true size of the ulcer, thus, the actual size of the ulcer is always underestimated by the external appearance of the ulcer.

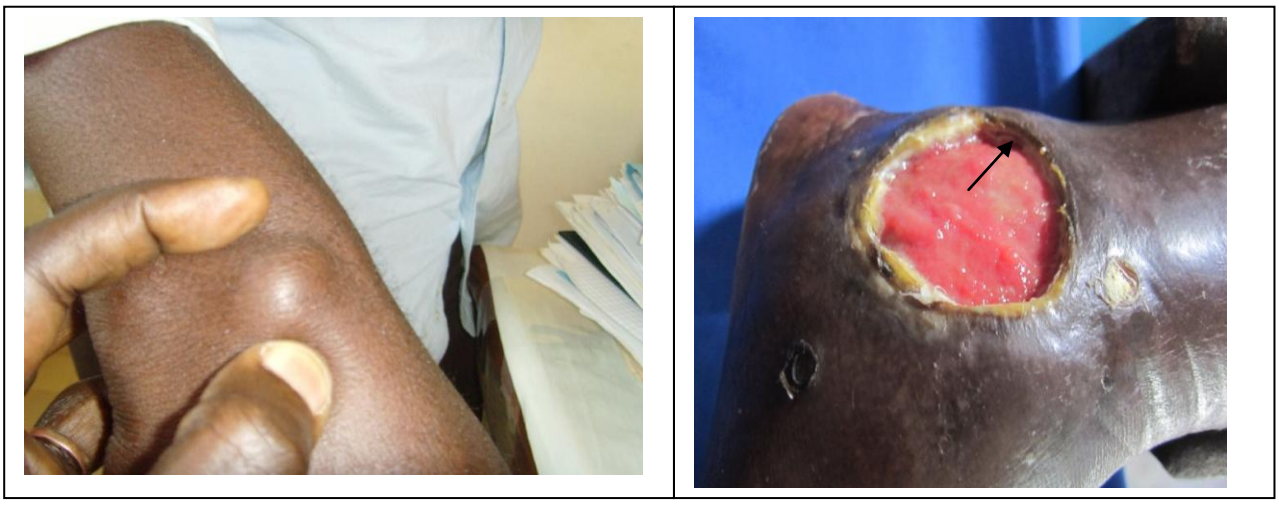


Figure 3: Pictures of typical nodular and ulcer stages of Buruli ulcer disease –arrow showing undermined edges

During the ulcerative phase these painless ulcers may become painful after secondary bacterial infection. Ulcers can be extensive, involving up to 15% of the patient's skin surface. The infection may destroy nerves, appendages, and blood vessels, and it occasionally invades bone if untreated. In the third phase of the disease, these large ulcers may heal spontaneously after the body has been able to mount effective cell-mediated immunity. However they frequently result in chronic lymphedema, significant deformity, disfiguring scarring and contractures (Stienstra et al., 2002, 2001). Despite often extensive host involvement, little mortality occurs with *M. ulcerans* infection. Most of the ulcers are found on the limbs but can present on the trunk, head and in some cases critical areas such as the breast, genitalia and face (Walsh et al., 2008). Such critical areas have resulted in catastrophic consequences where some patients have lost their sight, breast or even genital part.

Diagnosis of buruli ulcer is made by identifying *M. ulcerans* either in the swab or fine needle aspirate specimen. Diagnostic testing such as acid-fast smear, mycobacteria culture and polymerase chain reaction (PCR) for *M. ulcerans* are used to confirm buruli ulcers (Herbinger et al., 2009).

The current WHO recommendations for treatment of buruli ulcer are:

1. A combination of rifampicin and streptomycin/amikacin for eight weeks as a first-line treatment for all forms of the active disease. Nodules or uncomplicated cases can be treated without hospitalization then Streptomycin 15mg/kg intramuscularly daily plus Rifampicin 10mg/g daily for 8 weeks.

2. Surgery to remove necrotic tissue, to cover skin defects, and to correct deformities.

3. Interventions to minimize or prevent disabilities.

1.1.3 Risk factors for Buruli ulcer

Case-control studies have identified some risk factors for contracting BU. These are the use of unprotected water from swamps (Debacker et al., 2006b) and rivers (Aiga et al., 2004b; Raghunathan et al., 2005), and agriculture land use (Wagner et al., 2008). In the Amansie west district of Ghana, researchers demonstrated spatial relationship between BU prevalence and arsenic an immunosuppressant (Duker, 2005).

Even though these and other risk factors have been identified, the exact mechanism by which humans become infected with *M. ulcerans* in or near aquatic habitats is still not known. It has been hypothesized that *M. ulcerans* is transmitted through skin abrasions or skin injuries after contact with water, vegetation or soil. This still remains a hypothesis (Wagner et al., 2008).

1.1.4 Spatial distribution

The mycobacterium, *M. ulcerans* can be detected in both endemic and non-endemic sites although quantitative data is lacking for African countries (Williamson et al., 2008). Buruli ulcer occurs in discrete foci suggesting a spatial correlate with infection. Due to the disparity between hospital and community data, a detailed small-scale study on the location of houses with Buruli Ulcer with respect to specific features will lead to the identification of spatial correlates of infection. For emerging diseases where little is known about vectors or modes of transmission,

the quantification of large-scale patterns and associations with land use and land cover (LULC) types can also provide insight into potential vectors, because vector dynamics are often closely linked to environmental gradients (Wagner et al., 2008; Williamson et al., 2008).

Geographic Information Systems (GIS) technology offers the unique opportunity and the ability to collect vast amount of data over large spatial region. Spatial epidemiology has proven to be useful for understanding the geographical distribution of many diseases (Clements et al., 2009; Daash et al., 2009; Srivastava et al., 2009; Zhou et al., 2009; Blanton et al., 2006).

1.2 Problem Statement

Buruli ulcer has been reported in over 30 countries, from the subtropical regions of Asia, Latin America, the Western Pacific region and in Eastern and Central Africa. Even though it has been reported in other continents, West Africa is the region most affected. It is one of the neglected tropical diseases and second commonest disease caused by Mycobacterium in Ghana and third globally. Suhum-Kraboa-Coaltar, Akuapem South districts in the Eastern region and Ga West and Ga South districts of Greater Accra region are some of the districts affected by Buruli ulcer. The mode of *M. ulcerans* transmission remains to be clarified. The lack of detailed understanding of how environmental and social conditions interact with disease processes to ultimately cause human infections can severely hinder prevention and control programmes. Infectious diseases are often known to be constrained spatially by local and regional environmental factors. Epidemiological knowledge in this respect in the areas of Buruli Ulcer is limited. Therefore, constructing a fine level spatial distribution and risk map for Buruli ulcer within Akuapem South Municipality and Suhum-Kraboa-Coaltar needs to be carried out.

Control efforts have been hampered by poor understanding of the role of ecology. Effective prevention and control activities are based on current and existing knowledge of the risk factors. Lack of data on these risk factors are hampering the prevention and control of BU within the municipalities and consequently leading to ineffective use of scarce resources. An Assessment of environmental, spatial, demographic and biological related factors will help identify modifiable risk factors for appropriate interventions to be implemented for Buruli Ulcer control and aid in development of risk profile for Buruli ulcer

1.3 Justification

In a developing country like Ghana, resources need to be used efficiently to manage emerging diseases such as Buruli ulcer. The use of GIS technology to carry out epidemiologic mapping of Buruli ulcer will provide information on where people are most at risk and can be used to guide prevention, surveillance and to control of Buruli Ulcer. It will also ensure efficient use of resources and ensure that prevention and control interventions for Buruli ulcer especially in endemic communities. Furthermore, understanding the geographical distribution gives important clues regarding the spread of the disease. Geo-referencing saves time in identifying locations when directing different groups of public health workers to return to problem areas to perform control interventions (Chang et al., 2009). Using dengue as an example, risk maps were generated showing the spatial distribution. This was used to facilitate focused operational vector surveillance and control activities targeting high-risk areas (Lozano-Fuentes et al., 2008).

The use of GIS mapping technique may improve our understanding of the potential mode of transmission within the community and this will guide public health intervention (Murray et al., 2009). Combining GIS technology for epidemiologic mapping of Buruli ulcer cases alongside with environmental features and a fine detail case-control study to identify risk factors associated with Buruli ulcer will provide current knowledge of the disease in the study area. This information is crucial in developing clear intervention messages for community education on the disease and which communities are most at risk. From Australia, it has been proven that identifying possible risk factors and modifying them leads to reduction in the cases of buruli ulcer cases reported (Veitch et.al., 1997).

Hypotheses

The null hypotheses tested in this study were:

1. There is no difference in the distribution of Buruli ulcer in the communities along the Densu river

2. There is no difference in the characteristics of people who contract Buruli ulcer and those who do not.

1.4 Objectives

1.4.1 General Objective

To develop a risk profile for the transmission of Buruli Ulcer at the individual and the Community level in Akuapem South and Suhum Kraboa Coaltar Districts

1.4.2 Specific Objectives*:*

1. To determine spatial distribution of Buruli ulcer in Akuapem South, Suhum Kraboa Coaltar, Ga West and Ga South Districts (Along the Course of the Densu River)

2. To determine demographic factors associated with Buruli ulcer in Akuapem South and Suhum Kraboa Coaltar Districts

3. To determine behavioral factors associated with Buruli ulcer in Akuapem South and Suhum Kraboa Coaltar Districts

4. To determine environmental factors associated with Buruli ulcer in Akuapem South and Suhum Kraboa Coaltar Districts

CHAPTER TWO – LITERATURE REVIEW

2.1 Epidemiology of Buruli Ulcer

2.1.1 Burden of Buruli ulcer

Buruli ulcer, a disease caused by *Mycobacterium ulcerans*, is one of the most neglected but treatable tropical diseases. *Mycobacterium* ulcerans is from the family of bacteria

that causes tuberculosis and leprosy, but Buruli ulcer has received less attention than these diseases even though it has been reported in some countries including Ghana as the second most frequent mycobacterial disease in humans after tuberculosis (Merritt et al., 2010; Debacker et al., 2004).

Buruli ulcer has been reported in more than 30 countries mainly with tropical and subtropical climates. They include Africa (mainly west), Australia, the Americas, Asia and the Western Pacific, however it may also occur in some countries where it has not yet been recognized (Merritt et al. 2010; Walsh et al. 2008). Non-endemic areas such as North America and Europe have reported cases of Buruli ulcer as a result of international travel (Semret et al., 1999; Ezzedine et al., 2009). The true magnitude of the burden of Buruli ulcer is not known, but the epidemiologic pattern of the disease is determined by the presence or absence of Buruli ulcer foci (Pouillot et al., 2007). However, the incidence of Buruli ulcer has increased over the years but true incidence data is difficult to determine as a result of poor surveillance measures and case confirmation (Williamson et al., 2008). In areas where prevalence studies have been carried out, it ranges from few cases to up to 22% in some communities (Pouillot et al., 2007; Amofah et al., 1993). Ghana reports an average of 1000 cases each year. In 1998 a national case search revealed

a national prevalence of 20.7/100,000 and a prevalence of 87.7/100,000 for Ga district (now Ga West and Ga South municipalities).

Though Buruli ulcer disease is not usually fatal, it leads to profound morbidity especially in areas where treatment options are limited (Williamson et al., 2008; Pouillot et al., 2007). The large ulcers often lead to scarring, contractual deformities, amputations and irreversible disabilities (Merritt et al. 2010; Walsh et al. 2008; Duker et al. 2006; van der Werf et al. 1989). Estimates of Disability Adjusted Life Years (DALYs) unfortunately has not been determined for Buruli ulcer as well as other neglected tropical diseases like guinea worm, endemic syphilis and food borne trematode infections (Williamson et al., 2008b; Pouillot et al., 2007). Socio-economic implications of Buruli ulcer to the individual, family and cost of management to the health system is enormous (Ackumey et al., 2012; Grietens et al., 2008). Ackumey et al. (2012) found social burden enormous, besides the physical pain, disability and anxiety about the progression of the disease. Buruli ulcer affects livelihoods, interrupts education and immensely affects the family as well. The affected patients are generally excluded and stigmatized within their community for several reasons ranging from the smell generated by the lesions and ulceration, disability and a sense of shame.

2.2 Characteristics of *Mycobacterium ulcerans*: The pathogen

M. ulcerans is a slow-growing environmental mycobacterium at optimum temperature of 30 – 33^{o}C. The pathogen can be isolated from a primary lesions such as nodule, plaque, oedema and ulcers after 5 to 8 weeks incubation period, whereas in some cases it may take up to 6 months

(Converse et al. 2011; Merritt et al. 2010; Yeboah-Manu et al. 2004). It belongs to the *M. marinum* complex of mycobacterial pathogens. The *M. marinum* complex is made up of several species example *M. marinum, M. pseudoschottsii* which are pathogenic for fish, and *M. liflandii* which is pathogenic for frogs (Merritt et al. 2010; Kaser et al. 2009; Rhodes et al. 2005).

Genomics are of the opinion that *M. marinum* complex can be considered as one species based on their identity. They share over 97% identity in the 16sRNA gene sequence (Merritt et al. 2010; Demangel et al. 2009; T. P. Stinear et al. 2007). The establishment of separate names was as a result of practical consideration on the differences in the host tropism and pathogenesis. Elaborate genomic analysis has established that *M. ulcerans* evolved from *M. marinum*-like ancestor (Stinear et al., 2007; Demangel et al., 2009). *M. ulcerans* acquired large virulence plasmid and accumulated multiple copies of the insertion sequence, IS*2404* and IS*2606.* The genome has undergone several phases of reductive evolution. A number of mutations occurred including transposon insertion. The net effect of all these mutations and insertions produced over 700 pseudogenes (Demangel et al., 2009).

M. ulcerans genome strain does not have nitrate and fumerate reductase systems, this explains the narrow temperature range for its optimal growth since it cannot grow in low oxygen condition. The restricted growth temperature range for *M. ulcerans* perhaps determines its affinity for the skin. So far the pathogen has not been isolated in any internal organ of human patients (Merritt et al. 2010; Demangel et al. 2009). The unique ability of *M. ulcerans* is to produce a secondary metabolite called mycolactone (George et al., 1999; Demangel et al., 2009; Tobias et al., 2009). Mycolactone is an immunosuppressive and cytotoxic macrocyclic

polyketide characterized by a 12 – membered macrolactone core appended to highly unsaturated acyl side chain. A polyketide belong to a class of naturally occurring compounds. Some of them have potent pharmaceutical activities such as immune suppressor rapamycin, antibiotic erythromycin A and antiparasitic agent avermectin (Tobias et al., 2009; McGuire et al., 1952). The toxin-producing colonies have a yellowish colour and it is encoded by the *pksA* gene, located on a giant plasmid (Converse et al., 2011; Tobias et al., 2009; Stinear et al., 2004, 2005). The toxin destroys subcutaneous fat cells by both apoptotic and necrotic mechanisms resulting in the large undermined ulcers.

2.3 Ecological Distribution of Pathogen and Disease

2.3.1 Detection of M. ulcerans in the environment

M. ulcerans have been detected in the environment even though it has a slow growth rate. Direct culture of environmental sample does not yield results due to the presence of many faster growing bacteria and fungi in the environment. However, culture on artificial media for *M. ulcerans* from the environment yield results.

Following the successful development of the first Polymerase Chain Reaction (PCR) probes for *M. ulcerans* based on the detection of IS*2404* by Ross et al. (1997), it has changed the phase of Buruli ulcer diagnosis and the detection of *M. ulcerans* in the environment. This process has been facilitated by the rapid adoption of the technique leading to identification *M. ulcerans* DNA in environmental samples. Some of the environmental samples include soil, detritus, biofilms, water filtrates, fish, frogs, snails, aquatic insects, leeches, crustaceans, mollusks, rodents and

mosquitoes (Durnez et al., 2010; Portaels et al., 2008; Williamson et al., 2008; Johnson et al., 2007; Marsollier et al., 2002, 2004).

The use of IS*2404* PCR has been accepted as the gold standard in diagnosis and the confirmation of Buruli ulcer. This procedure however has some flaws when applied to environmental samples. In confirming the presence of *M.ulcerans,* the PCR method detects deoxyribonucleic acid (DNA) and not the organism. DNA of *M. ulcerans* can be released into the environment when an infected organism dies. It is possible for the DNA to be attached to several substrates in the environment. Different researchers have found varying levels of the *M. ulcerans* in water filtrates (Vandelannoote et al., 2010; Williamson et al., 2008). In Ghana, Vandelannoote et al. (2010) found 7.7% water samples positive for *M. ulcerans*. The challenge with most of these findings is whether the DNA is from an infected source or as a result of death of the infected organism and subsequent release of the DNA into the environment. Interpretation of such results may be complex.

Fyfe et al. (2007) reported 30% of environmental samples selected from plant materials, suspended solids, detritus and soil from highly endemic area were weakly positive by quantitative PCR. Even though in low endemicity area PCR positive samples were also identified but the percentage was low 3% (4/156).

The definitive proof that *M. ulcerans* is present in the environment is supported by the successful culture of the pathogen in an aquatic invertebrate (Portaels et al., 2008)

2.3.2 M. ulcerans association with disturbed water bodies

Nearly all epidemiological studies have associated increased number of cases of Buruli ulcer with communities in close proximity to human disturbed aquatic habits (Merritt et al. 2010). Both stagnant and moving water bodies have been clearly documented to be associated (Raghunathan et al., 2005; Duker, 2005; Johnson et al., 2005; Aiga et al., 2004; Thangaraj et al., 1999). The observed increased in the number of Buruli ulcer cases have been reported with flooding and during heavy rainfall (Wagner et al., 2008; Merritt et al., 2005; Johnson et al., 1996). Damming of streams and rivers to create impoundment and wetland (Duker et al., 2006; Merritt et al., 2005). Areas where resorts were modified to wetlands (Merritt et al., 2005), and in addition, deforestation practices leading to increased in flooding, migration of population closer to water bodies, rice cultivation, agricultural irrigation and sand winning operations were all associated with increased incidence of Buruli ulcer (Kibadi et al., 2008; Wagner et al., 2008 Duker et al., 2006; Merritt et al., 2005).

Water bodies that are associated with increased sedimentation and euthrophication are known to have low dissolved oxygen concentration that may enhance the growth of *M. ulcerans* (Merritt et al., 2010; Palomino and Portaels, 1998). Hayman postulated that in Australia *M. ulcerans* gets into the surface water through deforestation erosion and run off contamination. The environmental conditions present in the surface water with a lot of sediments create the optimum medium for *M.ulcerans* growth. Once there is deforestation, there is loss of riparian cover resulting in increased water temperature and subsequently creating the optimal temperature 30 – 33^{o}C for *M. ulcerans* growth (Horsburgh Jr and Meyers, 1997). Ultraviolet (UV) light has been documented to affect the viability of *M.ulcerans* (Stinear et al., 2004). Sedimentation reduces the

level of penetration of UV light and hence offers protection for *M. ulcerans* biofilms near the bottom substrates and on submerged plant surfaces as discussed by Merritt et al. (2005). In view of this, deforestation and high-impact agriculture may promote increased nutrients, higher temperatures, UV attenuation and lower dissolved oxygen. These may create the perfect environmental conditions that promote the growth of *M. ulcerans.*

2.3.3 Landscape ecology of the disease

Buruli ulcer is rare in savanna regions of West Africa and drier areas in Australia but has been widely reported and associated with riverine environment. Currently there is evidence showing the relationship between landscape features and land use that are related to Buruli ulcer disease. Wagner et al. (2008) quantified Buruli ulcer cases in communities surrounded by deforestation, abundant wetlands and other habitats that experience frequent flooding. Together with his team and the use of GIS they were able to show that areas with increase cases of Buruli ulcer were in low-lying areas and their topography far removed from urban settings (Wagner et al., 2008).In Amansie West district of Ghana, Duker et al. (2004) found out that arsenic levels in the soil and gold mining were significant covariates related to increased risk of developing Buruli ulcer. In both Ghana and Benin, Buruli ulcer has been reported among residents along several major rivers (Sopoh et al., 2011, 2007; Johnson et al., 2005;Amofah et al., 2002,1993). However, it is non-existent in communities within few kilometers of Lake Volta in Ghana and the Mono river in Benin.

2.4 Environmental reservoirs and transmission

The mode of transmission of tuberculosis and leprosy are well known as from person-to-person. So far, evidence for human to human transmission of *M. ulcerans* is extremely rare even though the pathogen is acquired through the environment. Debacker et al. 2003 reported one case of Buruli ulcer as consequence of human bite. It was hypothesized that the patient had contamination of the body surface with *M. ulcerans* from the environment and the bite drove the pathogen into the skin. Acquatic bugs (Hemiptera) were first suggested by Portaels and colleagues as possible reservoirs of *M. ulcerans* in nature. The team later confirmed this by isolating *M.ulcerans* from pure culture of water strider (*Hemiptera: Gerridae, Gerris sp*) from Benin (Portaels et al., 2008)

Other studies based on detection of *M. ulcerans* DNA in aquatic insects (Hemiptera, water bugs; Odonata, dragonfly larvae; Coleoptera, beetle larvae) collected from Buruli ulcer endemic swamps confirmed their earlier findings and also that small fish might also contain *M. ulcerans* (Marsollier et al., 2005, 2003, 2002). Marsollier and team carried out several laboratory studies and were able to demonstrate that *M. ulcerans* could survive and show limited replication within the salivary glands of biting aquatic bugs (*Naucoridae: Naucoris cimicoides*). The team concluded that biting water bugs belonging to the families Naucoridae (creeping water bugs) and Belostomatidae (giant water bugs) could be considered reservoirs, and most importantly could serve as vectors in the transmission of *M.ulcerans* to humans in nature.

Recently, Mosi et al. (2008) also investigated the ability of *M Ulcerans* to colonize aquatic bugs (Belostomatidae) collected from Africa. Both teams, Mosi et al. (2008) and Marsollier et al.

(2005, 2003, and 2002) affirm that Belostomatid bugs could become infected with *M. ulcerans* through feeding.

Several authors have suggested the role of other non-insect aquatic invertebrates as intermediate hosts or environmental reservoirs for *M. ulcerans* (Merritt et al., 2005; Kotlowski et al., 2004; Marsollier et al., 2003, 2002) and Williamson et al. (2008) and Benbow et al. (2008) confirmed the earlier suggestions (Benbow et al., 2008; Williamson et al., 2008)

In endemic fields of Ghana and Benin, Kotlowski et al. (2004) reported *M. ulcerans* in aquatic snails from endemic regions. Stinear found out that the average estimates of *M. ulcerans* in detritus increased by two orders of magnitude when compared to water (Stinear et al., 2000). An elaborate environmental study by Williamson et al. (2008) is showed that *M. ulcerans* DNA can be detected within biofilm on the plant surface and detritus both of which can serve as food for certain aquatic invertebrates and fish, suggesting reservoirs and movement throughout the aquatic food web (Marion et al., 2010; Williamson et al., 2008; Merritt et al., 2005).

Even though several studies have clearly demonstrated different aquatic invertebrates in Africa to serve as environmental reservoirs for *M. ulcerans*, direct transmission by biting water bugs has not been proven.

In Australia, more than 80% of Buruli ulcers in the past 15 years have been in the temperate southeastern state of Victoria (Merritt et al., 2010; Johnson et al., 2007). A comparison between Buruli ulcer cases from Africa and Australia shows that people in Southeastern state of Victoria

have less direct contact with the environment. The two well described outbreaks in Australia, 1.2 – 6.0% of the entire resident population in the outbreak areas developed Buruli ulcer (Johnson, Azuolas, et al., 2007; Johnson, Hayman, et al., 2007; Veitch et al., 1997). Johnson et al. (2007) described that visitors may also be at risk, and in one case, contact with an endemic town for just one day appeared to be sufficient to develop Buruli ulcer up to 7 months later. Following the outbreak of Buruli ulcer in Australia, where the disease occurred in individuals who had limited contact with the environment, in such cases of brief exposure and high attack rates, Hayman postulated that transmission by aerosol could partially explain the outbreaks (HAYMAN, 1991).

This hypothesis was tested during a three year period when a large cluster of Buruli ulcer cases occurred in East Cowes, Phillip Island. Only part of the town was affected, particularly the newly created wetland and golf course at the center of the affected area. Interestingly, the golf course used a mixture of ground water and recycled water for irrigation. The excess run-off water from the golf course was likely to have drained towards the new wetland linking the ground water and the recycle water thus the two systems. Most of the Buruli ulcer cases lived closed to the wetland or the golf course which was partly supporting the postulated mode of transmission by drifting aerosols from contaminated irrigation water (Veitch et al., 1997; Johnson et al., 1996; Flood et al., 1994).

IS2404 PCR was applied to environmental samples, and positive results were obtained from the wetland and golf course irrigation system-the first direct evidence that *M. ulcerans* DNA is present in environmental samples. This supported the hypothesis that the golf course and the Phillip Island wetlands were contaminated with *M. ulcerans.* There is no evidence from Australia

of the presence of IS2404 in any other environmental mycobacterium up to date. In contrast, samples in Africa such as aquatic mycobacteria associated with disease in fish and West African clawed frogs (*Xenopus tropicalis*) also contain IS2404 for which reason IS2404 lacks high level of specificity for use as a sole criteria for *M. ulcerans* in Africa.

Interventions such as drainage of the wetland, reduction in recycled water use, cleaning of the irrigation equipment at the golf course, and subsequent separation of ground water from recycled water yielded good results by reducing the number of cases in the following years. Currently, Buruli ulcer is rare in Phillip Island.

Several possums (Australian native tree-dwelling marsupials) with Buruli ulcer were identified at Phillip Island around the same time. Specifically, 38% of ringtail possums (*Pseudocheirus peregrinus* (Boddaert)) and 24% of brushtail possums (*Trichosurus vulpecula Flannery*) captured at Point Lonsdale had laboratory-confirmed *M. ulcerans* on the skin lesions and/or *M. ulcerans* PCR positive feces (Fyfe et al., 2010)

A mother suspected a mosquito bite initiated the event that led to her child develop Buruli Ulcer on the ear. Prior to this case, in 2004 there was a reported increase in local mosquito activity which was observed to be associated in time with new cases of Buruli ulcer. The observed Buruli lesions were on ankles and elbows, and on the back where gaps in clothing could allow access for mosquitoes (Johnson, Azuolas, et al., 2007). This led to series of investigation to assess the role of mosquito in the transmission of *M. ulcerans.* Fyfe et al. (2007) used an improved real-time quantitative IS2404 PCR environmental screening method, captured over 11,000 adult

mosquitoes and identified *M. ulcerans* DNA in or on an estimated 4.3/1,000 mosquitoes in Point Lonsdale. The most common PCR positive mosquito pools was *Aedes camptorhynchus* as the most common species on the Bellarine peninsula (Johnson, Azuolas, et al., 2007). A PCR amplification and sequence analysis of one variable number tandem repeat (VNTR) locus confirmed that mosquitoes were carrying *M. ulcerans* DNA which was indistinguishable from that of the human outbreak strain (Lavender et al., 2008; Fyfe et al., 2007)

A 7 year (2002- 2008) period review of notifiable diseases in Victoria demonstrated a statistically significant correlation between notifications of Buruli ulcer and Ross River Virus/Barmah Forest Virus infections (RRV/BFV). Ross River Virus and Barmah Forest Virus infections are both transmitted by mosquitoes, however, there was no correlation with any other nonmosquito borne notifiable disease (Johnson et al., 2009). Quek et al. (2007) in their case-control study conducted on the Bellarine Peninsula showed that use of insect repellent and being bitten by mosquitoes on the lower legs were found to be independently associated with Buruli ulcer in the multivariate model (Quek et al., 2007)

Transmission research conducted in southeastern Australia lends support to mosquitoes as being a possible vector of the pathogen for Buruli Ulcer disease in southeastern state of Victoria (Wallace et al., 2010; Merritt, Craig, et al., 1992; Merritt, Dadd, et al., 1992).

So far the exact sequence of events linking mosquitoes, humans, contaminated possum excreta and infected possums has not yet been determined. However, either direct or indirect mosquito transmission from a possum reservoir presents different possible route from the aerosol

transmission from contaminated environmental water sources (Merritt et al., 2010). The probability and plausibility of neither aerosol nor mosquito transmission hypothesis by direct contact with the environment or by other vectors need to be examined.

2.5 Risk factors for Buruli ulcer

2.5.1 Demographic factors

With host related risk factors, Buruli ulcer is known to affect children less than 15 years predominantly(Debacker et al., 2006; Raghunathan et al., 2005; Amofah et al., 1993). In several cross-sectional studies and case series, children and adolescents have higher rates of infection of *M. ulcerans* than adults (Sopoh et al., 2007 Phanzu et al., 2006; Hospers et al., 2005; Johnson, Sopoh, et al., 2005; Noeske et al., 2004; Asiedu and Etuaful, 1998; van der Werf et al., 1989). A number of these studies based their observation on patient count rather than rates. Some researchers have argued that there is the need to study the behavior of children in endemic region to ascertain the possible mode of transmission of BU.

Studies from Australia and one from Togo have reported higher number of Buruli ulcer infection among adults than in children (Johnson, Azuolas, et al., 2007; Quek et al., 2007 James et al., 2003; Veitch et al., 1997).Other researchers found no statistically significant differences by age group (Pouillot et al. 2007; Marston et al. 1995).

Comparative studies that evaluated sex as a risk factor in all cases reported no association between sex and Buruli ulcer disease (Pouillot et al., 2007; Quek et al., 2007; Debacker et al.,

2006; Raghunathan et al., 2005; Stienstra et al., 2004; Marston et al., 1995). Asiedu et al. (2000) and Raghunathan et al. (2005) found that women are mostly affected, there was no significant association between sex and *M. ulcerans* infection. The large numbers of women and children with Buruli ulcer may simply be a reflection of the population structure.

The sites of the Buruli ulcer lesion varies, studies done in West Africa have observed lesions on the trunk, head, neck and upper limbs for children whereas in adults the lesion tend to occur on the lower limbs (Johnson, Sopoh, et al., 2005; Stoffel et al., 2005; Debacker et al., 2004; van der Werf et al., 1989). According to Van der Werf et al. (1989) there is asymmetry in the presentations of the lesions on the legs. They reported more lesions on the left leg than the right leg in adults. However, this asymmetry was not confirmed by recent studies in Ghana and Cameroon (Hospers et al., 2005; Noeske et al., 2004)

2.5.2 Socio-economic factors

Socioeconomic (SES) status in a rural African setting may be related to land ownership, household size, livestock ownership and duration of residence. Socioeconomic risk factors were not evaluated by most studies but the few that did had different findings. Transmission has been linked with low socio-economic status, poverty and living in remote areas (Asiedu and Etuaful, 1998). Fabienne Nackers and his team as part of the large scale case-control study from August 2002 to August 2003 in sounthern Benin to estimate the protective effectiveness of Bacillus Calmette Guerin (BCG) identified that there was an association between socioeconomic status and the risk of Buruli ulcer disease (Nackers et al., 2007) whereas Pouillot et al. (2007) found no association (Pouillot et al., 2007). No association was found between *M. ulcerans* infection and

other indices used to measure socioeconomic status such as household size and livestock ownership (Raghunathan, Whitney, Asamoa, Stienstra, Taylor Jr, et al., 2005; Pouillot et al., 2007).

On education, adults with only primary education or less were found to be at a higher risk of *M. ulcerans* infection (Pouillot et al., 2007), however Stienstra et al. (2002) observed no association (Stienstra et al., 2002). Most of the comparative studies showed no association between participation in agricultural activities and the risk of *M. ulcerans* infection (Nackers et al., 2007; Pouillot et al., 2007; Aiga et al., 2004) but Debacker and the team proved that there was an increased risk of infection with agricultural activities (Debacker et al., 2006).

In assessing the relationship between household expenditure and the risk of *M. ulcerans* infection, Pouillot and the team in the Cameroon study found no association (Pouillot et al., 2007).

2.5.3 Health related factors

Bacillus Calmette Guerin (BCG) was suggested to provide protection against *M. ulcerans* infection in an earlier study in 1971 by the Uganda Buruli Group (The, 1971). Subsequent evaluations of BCG never reported the vaccine to be effective in conferring protection against Buruli ulcer (Pouillot et al., 2007; Quek et al., 2007; Debacker et al., 2006; Nackers et al., 2006; Raghunathan et al., 2005; Amofah et al., 1993). Portaels and colleagues in their *Mycobacterium bovis* BCG vaccination as prophylaxis against *Mycobacterium ulcerans* osteomyelitis in Buruli ulcer disease study reported some protection against osteomyelitis (Portaels et al., 2004, 2002).

When two Buruli ulcer treatment centers were compared in terms of differences in treatment outcome reported that BCG may be associated with improved healing of ulcers following surgery (Teelken et al., 2003). HIV/AIDS has been reported to increase the risk of *M. ulcerans* infection (Johnson et al., 2008; Raghunathan et al.,2005) but personal health factors such as cancer or use of immunosuppressive medications within the previous year were not associated with risk of *M. ulcerans* infection (Quek et al., 2007).

Individuals with family members with Buruli ulcer and have been exposed do not have increased risk of developing the disease (Nackers et al., 2007; Raghunathan et al., 2005; Aiga et al., 2004; Marston et al., 1995). However, Sopoh and the team in their study "Family Relationship, Water Contact and Occurrence of Buruli Ulcer in Benin" were the first to show the existence of family association with Buruli ulcer (Sopoh et al., 2010).

2.5.4 Environmental factors

Epidemiologic studies of BU have implicated stagnant water bodies in *M. ulcerans* transmission (Raghunathan et al., 2005). An outbreak of Buruli ulcer disease in a suburban community in Australia was attributed to dammed water used to irrigate the community golf course. There were no additional cases after the irrigation was halted (Veitch et al., 1997). In Africa, BU primarily afflicts rural farmers in swampy environments and riverine areas (Asiedu and Etuaful, 1998; Marston et al., 1995), especially those enriched with arsenic (Duker et al. 2004). An outbreak of Buruli Ulcer Disease in Nigeria and another in Australia were associated with environmental changes, such as flooding or damming (Ross et al., 1997; Veitch et al., 1997;

HAYMAN, 1991). Cases of Buruli ulcer were more likely to drink from unprotected water sources like swamp water, ponds than from protected water like wells or free-flowing rivers (Nackers et al., 2007; Debacker et al., 2006; Barker and Carswell, 1973;) but Aiga et al. (2004) found an increased risk with the use of river water (Aiga et al., 2004).

In the Amansie West study the source of water used for drinking or cooking was not found to be a risk factor (Pouillot et al., 2007; Raghunathan et al., 2005; Aiga et al., 2004; Amofah et al., 1993) but Debacker et al. (2006) and Wagner et. al.(2008) suggested that the availability of pumped water is an important factor in reducing the risk for BU (Wagner et al., 2008; Debacker et al., 2006). Fetching, drawing and domestic water uses were not associated with BU (Raghunathan et al., 2005; Aiga et al., 2004; Barker and Ninkibigaya, 1972). Some researchers hypothesized that if there is something common to mothers and their children, then it may likely be water fetching and its domestic use but there was no association.

With exception of Amofah et al.(1993), several studies have found an association between proximity of the home or farm site to water source and increased risk of Buruli ulcer (Nackers et al., 2007; Pouillot et al., 2007; Quek et al., 2007; Raghunathan et al., 2005; Marston et al., 1995).Wading in water was found to be a risk factor for Buruli ulcer (Pouillot et al., 2007; Raghunathan et al., 2005) however hunting (Raghunathan et al., 2005), fishing (Pouillot et al., 2007; Raghunathan et al., 2005; Marston et al., 1995) and swimming (Marston et al., 1995)were not found to be associated Buruli ulcer. However, Aiga et al. (2004) found swimming to be a risk factor in a case control study carried out in Ghana.

Living near forest or plantation and walking through the bush and forest have not been shown to be associated with Buruli ulcer (Raghunathan, Whitney, Asamoa, Stienstra, Taylor Jr, et al., 2005; Pouillot et al., 2007).

Sharing indoor living space with livestock, compared with handling or owning livestock, appeared to protect against BU.

2.5.5 Behavioural factors and insect bites

Daily washing with soap reduces the risk of BU, as bacterial contamination of skin surfaces may facilitate *M. ulcerans* infection. The regular use of toilet soap (i.e., wrapped bar soap) while bathing may remove bacteria deposited on the skin, which is a plausible explanation (Raghunathan et al., 2005). The use of soap and water for washing was found to be associated with a decreased risk of *M. ulcerans* infection (Nackers et al., 2007; Raghunathan, et al., 2005) and washing of clothes was also found to protect against *M. ulcerans* infection (Pouillot et al., 2007; Marston et al., 1995). However, no protective association was observed among those who used less costly mass-produced Key soap or homemade amonkye soap (Raghunathan et. al., 2005).

Even though some studies found no relationship between swimming and Buruli ulcer others did. Swimming in a river or pond was found to be associated with BU (Aiga et al., 2004; The, 1971). A case–control study in the Amansie West District of Ghana showed that the only significant risk factor for BU was swimming in rivers on habitual basis.

Several studies have been carried out to evaluate the role of insect bite in the transmission of *Mycobacterium ulcerans*, however the findings were inconsistent. Case-patients were more likely than controls to recall any incidence of insect bite either by mosquito or others while wading in water (Pouillot et al., 2007; Quek et al., 2007). Raghunathan et al. (2005) in a case-control study in Ghana on risk factors for Buruli ulcer disease found no association between insect bite and Buruli ulcer.

The use of mosquito nets and repellants were found to reduce the risk of Buruli ulcer(Nackers et al., 2007; Pouillot et al., 2007; Quek et al., 2007) where as a study by Raghunathan and team found no decreased risk of infection with the use of bed nets. Mosquito coil use was assessed by other researchers but found no association with BU (Pouillot et al., 2007; Raghunathan et al., 2005).

Wearing of certain types of clothing have been evaluated extensively. Wearing long legged trousers (long pants) has been found to reduce the risk of Buruli ulcer (Pouillot et al., 2007; Raghunathan et al., 2005) but not wearing protective clothing increases the risk of infection (Pouillot et al., 2007; Quek et al., 2007; Raghunathan et al., 2005; Marston et al., 1995). Interestingly, the use of gardening gloves and shoes were not protective against *M. ulcerans* infection.

2.5.6 Spatial distribution of Buruli ulcer

Geographic information system is used for input, storage, manipulation, and output of geographic information. It provides a powerful tool to combat diseases especially those of vector-borne (Eisen and Lozano-Fuentes, 2009). GIS-based approaches have been used to visualize or model spatial patterns of risk for exposure to malaria parasites in Africa, leishmaniasis, influenza cases and dengue in various parts of the world (Chang et al., 2009; Lozano-Fuentes et al., 2008). This provides crucial information facilitating allocation of resources to areas most in need of vector and disease control (Lozano-Fuentes et al., 2008). GIS maps can be developed for any number of vector-borne, zoonotic, waterborne, or food borne diseases where case locations are available. In the UK GIS is an important tool with which to 'join up' government and geographical data between agencies in tackling health issues. Also in Hungary one study was conducted which used the National Public Health Service to establish monitoring in primary care facilities on selected chronic diseases (Moss et al., 2006).

There is evidence that alteration of the landscape that affects the aquatic environment affects the presence of Buruli ulcer. With alteration in the landscape, the structure and functions of the ecosystem is affected including habitat loss, species extinction, introduction of new species colonization and other processes in the ecosystem (Ceballos et al., 2010; Linderman et al., 2006; King et al., 2005; Moloney and Levin, 1996; Forman, 1995). Changes in the spatial pattern of land use/ land cover (LULC) create favorable conditions for vectors and hosts resulting in high incidence of diseases (Jackson et al., 2006). Documentation on LULC shows the role it plays in the dynamics of infectious diseases in both wildlife and human populations (Farnsworth et al.,

2005; Smith et al., 2005). Residential development or agricultural land use results in higher contact rates between humans and disease vectors or may even decrease the natural species causing disease in the environment.

A lot of research has been done on the distribution of *M. ulcerans* but much attention has not been paid to the ecological aspect of it (Wagner et al., 2008). However, most epidemiological research finds some relation between BU and the alteration of fresh water habitat including marshes, impoundments, wetlands, and slow moving riverine environments (Duker, 2005; Johnson, Sopoh, et al., 2005; Merritt et al., 2005; Raghunathan, et al., 2005; Aiga et al., 2004; Thangaraj et al., 1999; Horsburgh Jr and Meyers, 1997; HAYMAN, 1991 Barker and Carswell, 1973). The probability of BU presence increased with increasing longitude and percent agricultural land use in a 20-km buffer surrounding a village, and decreased as latitude and the percent urban land use in a 50-km buffer surrounding a village increased (Wagner et al., 2008). Smaller spatial scale researches done showed an association between BU and water (Debacker et al., 2006; Aiga et al., 2004). However, Wagner et al. (2008) using the Land Sat images suggest that proportion of water land cover surrounding villages and the distance to the nearest river are not associated with an increased probability of BU presence at the village level.

2.6 Criteria for establishing transmission

In biomedical research, some criteria exist for identifying living agents as biologically significant reservoirs and/or vectors of pathogens. The role of aquatic insects, adult mosquitoes and other biting arthropods as reservoirs and as vectors of *M. ulcerans* transmission vary. It is

important to discuss what makes a vector to be identified and be linked with transmission of *M.ulcerans.*

For any vector to be incriminated it means it satisfies Koch's postulates, thus;

(1) The vector must be shown to acquire the pathogen from an identified source such as an infected vertebrate host or other reservoir, and thereafter become infected with the pathogen;

(2) The vector must be shown convincingly to have close associations with infected hosts, including humans, in time and space;

(3) Individual vectors collected in endemic settings must repeatedly be found infected with the pathogen; and

(4) Efficient transmission to competent vertebrate hosts must be demonstrated experimentally. Thus under well controlled conditions, by individual vectors, such as by bite or other means of direct contact (Barnett, 1960).

Aquatic and semi-aquatic Hemiptera and other insects have been identified to harbor *M. ulcerans* but it does not provide ample evidence for them as obligatory insects (Marsollier et al., 2007). It has become evident that further research is required to confirm the association between mosquito bites, adult mosquito infection, and incidence of Buruli ulcer in humans. The pathway proposed by Fyfe et al. (2007) require further exploration to establish the link between mosquito feeding on infected possums and transmission of the agent via the same species of mosquitoes (Fyfe et al., 2010)

Secondly, Bradford Hill provided a guideline to incriminate a vector for establishing causation of infection and disease in epidemiological/ecological context (Hill, 1965). Bradford Hill guidelines focuses on epidemiological/ecological association and use of logical inference to deduce support, provide convincing evidence for an undisputable conclusion of cause and effect. In which case A is the ‘‘cause’’ and B the ‘‘effect’’ in the relationships being studied (Howick et al., 2009; Plowright et al., 2008)

In applying Hill's criteria to Buruli ulcer disease causation, ‘‘A’’ would be contact between an insect vector infected with *M. ulcerans*, and ‘‘B’’ would be human infection with M. ulcerans.

(1) Plausibility. The cause and effect association of A and B must be plausible. Simply put, what is being observed is rational and that the association between A and B reflect common understanding of the normal behavior and consequence attributed to it. Following up on Buruli ulcer and vector transmission, the available evidence is not sufficient to conclude that biting hemipterans are a critical vector of *M. ulcerans.* With the available information it is known to act as an environmental reservoir.

(2) Temporality. Assuming that the cause "A" results in an effect "B", in that scenario then A must consistently precede B in temporal sequence. Unfortunately for Buruli ulcer, there is not much evidence that bites of a specific insect or insects consistently precede development of *M. ulcerans* infection in humans. However mosquito bites have been reported to be associated with increased risk of Buruli ulcer (Quek et al., 2007). Certain bites may go unnoticed especially if they are painless, nevertheless painful bites preceding the disease will be more likely remembered e.g. a naucorid or belostomatid bite.

(3) Strength. There should be statistically significant association between the cause "A" and the effect "B". Almost all epidemiological studies have displayed strong association between contact with water or wetland and *M. ulcerans* infection in man. What has not been established is the link between the bite and the infection e.g. bite from hemipterans and *M. ulcerans* infection. In addition, association between mosquito bite and development of *M. ulcerans* in Africa has not been established.

(4) Biological gradient or dose-response relationship. In simple mathematical terms, there is proportionate increase in infection as the cause also increases thus infection in effect "B" should increase proportionately as cause "A" increases. Mosquitoes caught in highly endemic area in Southeastern Australia are more likely to be PCR positive than those caught in low endemicity areas (Johnson, Azuolas, et al., 2007). On the other hand, there is no evidence that higher infection rate of *M. ulcerans* of aquatic insects' results in higher incidence of infection of in humans.

(5) Consistency. With the aspect of consistency, review of the existing epidemiological studies show some support for each other however there is no clear consistency among epidemiological scenarios to support the postulate that insects are the predominant vector in most regions. It provides an overall lack of consistent data on the role of vectors in *M. ulcerans* transmission.

(7) Experimentation. In experimentation, the criteria use experimental manipulations which are feasible and can be structured realistically. It is expected that treatment conferred on the effect

"B" must show a positive association. Experimental data on insects and *M. ulcerans* shows that a lot of work has been done but it is difficult to generalize conclusions on the association due to variation in the outcomes. Lastly, it is difficult to carryout large scale experiments to evaluate and replicate an earlier experiment.

(8) Specificity. Bradford Hill's criteria explain that effect "B" should follow the cause "A". in situations where you have multiple causes of B, this criterion becomes difficult to satisfy. With the available literature, there is paucity of data to prove specificity between insect bites and Buruli ulcer cases. In addition, since the current data suggests multiple transmission model for Buruli ulcer, it indicate that the disease lack specificity with regard to insect vectors.

(9) Coherence. The overall coherence is not strong. It looks at the association of the effect "B" with the cause "A" that it must cohere to knowledge of similar relationships in other similar associations. *M. marinum* group does not depend on invertebrate vectors for their transmission and infection causation in fish. It becomes more difficult in comparing that to the proposed mechanism of disease causation due to *M. ulcerans.*

In summary, from the available information regarding cause and effect, there is not enough epidemiological evidence for undisputable conclusion on the mode of transmission of Buruli ulcer.

2.7 Case Management

Until recently, surgery was the only available treatment for BU. This procedure often involved extensive excision, with or without skin grafting. This belief developed because of the massive destructive nature of the disease and early reports which suggested that wide surgical excision was the only effective treatment and even after introduction of antibiotics, early trials with clofazimine (Revill et al., 1973) and rifampicin/clofazimine (Epsey et al., 2002) demonstrated only marginal benefits. On the other hand, access to surgery has been very limited because of inadequate surgical capacities in most affected areas of endemic developing countries. Besides, where such facilities are available, the cost of surgery is far beyond the means of most of those severely affected (Ross et. al., 1997). Other additional problems that further compound the difficulty in surgery is the need for prolonged hospitalization – averaging at least three months and hospitals where surgical treatment is possible, have limited bed capacities. This further reduces the number of patients who can be admitted and treated.

Moreover, recurrence rates after surgical treatment have been recorded. In a one-year follow-up after excision of small early lesions in the Amansie West district of Ghana (Amofah et al., 1993). Barogui et al., (2009) estimated a16% recurrence rate. Other researchers in some countries have reported recurrence rates of 28%, mainly among late severe cases (Sizaire *et al.,2003*). John Travis (1999) listed the additional human suffering, inflated treatment costs and often frustrations that occur with successful management of the disease.

In Australia, where patients are usually treated with conservative surgery combined with oral anti-mycobacterial drugs, relapses at distant sites during and after antibiotic therapy have been observed in a few patients (Johnson PD *et al.*, 2007).

Due to evidence that a combined antibiotic treatment with rifampicin and streptomycin has the potential to inhibit growth of *M. Ulcerans* the World Health Organisation issued provisional guidelines recommending standard antimycobacterial therapy in 2004 (WHO 2004; Etuaful et al. 2005). Presently, prospective drug trials are being conducted comparing the efficacy of different dosages and durations of anti-mycobacterial treatment in different stages of BU disease. According to the lesion category different treatment schemes are applied (antibiotic treatment alone or in combination with surgery). Based on the results of several experimental studies performed in the mouse footpad model (Dega H et al., 2002; Morsollier et al., 2003; Etuaful et al., 2005) and a pilot clinical trial conducted in Ghana under the auspices of WHO demonstrated that after daily treatment with rifampin and streptomycin for at least 4 weeks, *M. ulcerans* could no longer be cultured from the lesions. Furthermore, the antibiotic treatment reduced the surface area of most lesions by more than 50%, thus allowing for less-extensive surgical excision.

WHO introduced new provisional antibiotic treatment guidelines for BU following a successful pilot study from Ghana and Benin which confirmed that human lesions can be sterilized with antibiotics rifampicin and streptomycin (Etuaful *et a.l*, 2005; Chauty *et al*, 2007). This WHO recommended drug treatment is a Directly Observed therapy (DOTs) of Streptomycin, 15mg/kg

body weight by intramuscular injection daily plus Rifampicin, 10mg/kg body weight by mouth daily for 8 weeks.

Table 1: SUMMARY OF TREATMENT METHODS FOR DIFFERENT STAGES OF ACTIVE BURULI ULCER

STAGE OF DISEASE	RECOMMENDED ANTIBIOTIC TREATMENT	STANDARD SURGICAL TREATMENT
1. **Early disease** : (I) Nodules, papules, small ulcers (< 2 cm diameter) (ii) Plaques, oedematous lesions, medium size ulcers (2-5 cm diameter)	(I) Antibiotic treatment with Rifampicin- Streptomycin for 4 weeks only) (ii) Rifampicin-Streptomycin for 4 Weeks before surgery and then Rifampicin-Streptomycin for 4 weeks	(I) Surgical excision and primary closure (ii) Surgical excision, wound care and skin grafting
2**. Late disease** : Large ulcers (> 5 cm diameter)	Rifampicin-Streptomycin for 8 weeks	Surgical excision, skin grafting and wound care after 4 or 8 weeks of antibiotic treatment

Source: WHO: GUIDELINES FOR CONTROLLING BURULI ULCER IN THE AFRICAN REGION

This protocol has led to a new approach to treatment with the potential to reduce cost, allow delivery of care closer to the homes of patients and encourage patients to report at health facilities earlier as the fear of surgery is lessened.

Most cases of BU occur in resource-limited settings and therefore there is an urgent need to simplify treatment and improve outcomes (WHO 2004). Due to the characteristic clinical presentation of ulcerative lesions, experienced clinicians are generally able to establish the diagnosis on clinical grounds, with the analysis of diagnostic swabs providing laboratory confirmation in the majority of cases.

In contrast, pre-ulcerative lesions have uncharacteristic clinical features and, consequently, a variety of possible differential diagnoses exists. An accurate clinical diagnosis is therefore more difficult at early stages, and priority should be given to the laboratory confirmation or differential diagnosis of these cases based on an analysis of tissue specimens (Siegmund et al., 2007, Chauty et al., 2007).

Recurrence and treatment failures have been attributed to factors such as host factors like malnutrition, HIV Co-infection, pathogen factors which looks at the virulence of the pathogen, strain and health system factors. Some of the Health system factors linked to the recurrence/treatment failure are incorrect diagnosis and incomplete adherence.

2.8 Health Seeking Behaviour

People's attitude towards medical care is not only determined by availability and access to relevant health care (Aujoulat et al.,2003) but depends on personal experiences, perceptions, explanations, attitudes of the social network and health beliefs interact and influence health-seeking behaviour (Liefooghe et al., 1997).

Therefore it is important to understand the people's interpretation of the causes of their sickness (Stienstra et al., 2002). Especially diseases whose etiology could not be readily explained are often given various explanations among the people. Social stigma or exclusion may prevent BUD affected people from seeking medical treatment. Reasons for stigma and exclusion were fear of acquiring BUD, the mysterious nature of the disease, a lack of knowledge on the mode of transmission and a lack of proper treatment (Stienstra et al., 2002; Asiedu et al., 1998). Stienstra et al., (2002) found that patients were also hindered in functioning as a leader and were avoided by others. But also marital problems and sexual functioning were mentioned. It is interesting to note that unaffected respondents and people in less endemic locations mentioned stronger stigmas (Renzaho et al., 2007). Similar findings were found in Kenya and Vietnam for tuberculosis – a disease which is also associated with high negligence (Johansson et al., 2000; Liefooghe et al., 1997).

CHAPTER THREE - METHODS

3.1 Study Design

It was a Case - Control study with spatial mapping. The cases were identified through community active case search. All identified cases were mapped in their various residents and the entire course of the Densu River from the Etiwa Mountains to the Weija Lake was as well outlined.

3.2 Study Area

The case-control part of the study was carried out in Suhum-Kraboa-Coltar and Akuapem South districts of the Eastern Region of Ghana. The Spatial mapping of Buruli Ulcer cases involved both the Eastern and Greater Accra Regions following the entire course of the Densu River.

3.2. 1 Background of Densu River

The Densu River is 116 km long which takes its source from the Atewa – Atwiredu Mountains near Kibi in the East Akyem District of the Eastern Region. The Densu spans an area of 2,490 km^2, the main tributaries are Adeiso, Nsakyi, Dodro, and Kuda. The Densu River flows into the Weija Reservoir before entering the Gulf of Guinea. There are over 200 settlements in the basin and the total population is almost 600,000 with about 40% of the population engaged in agriculture. The mean annual runoff is 500x106 m^3. The Densu River is of specific importance

since it includes the Weija Reservoir which supplies water for approximately half of the Accra Metropolitan Area.

The Densu Basin is located at the South Eastern part of Ghana and lies within longitudes 10 30'W -10 45'W and latitudes 50 45'N - 60 15'N. It shares its catchment boundary with the Odaw and Volta Basins to the east and north, the Birim in the northwest and the Ayensu and Okrudu in the west. The map below gives a clearer picture of the location of the Basin

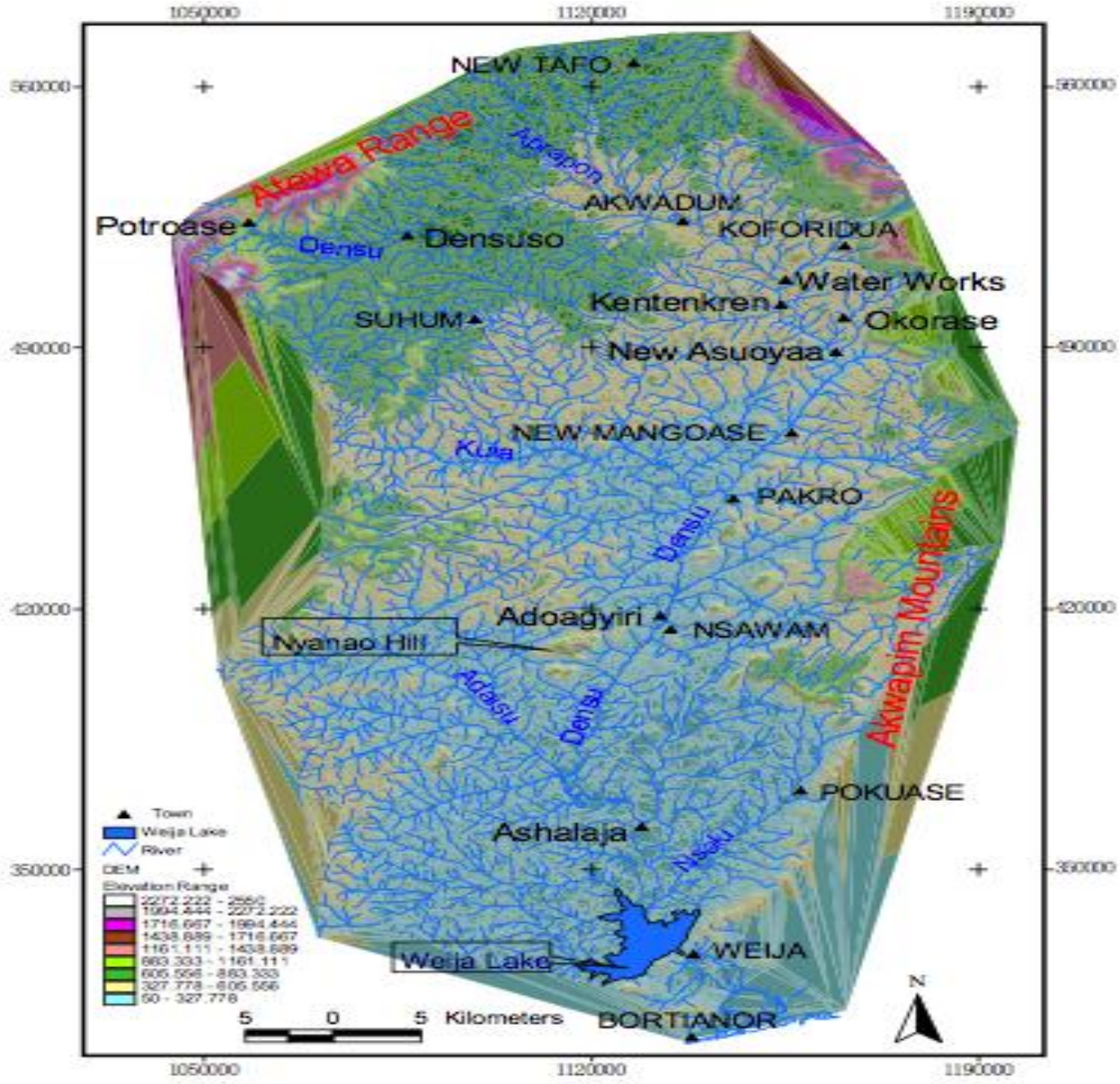


Figure 4: The Basin of the Densu river

Profile of Study Area

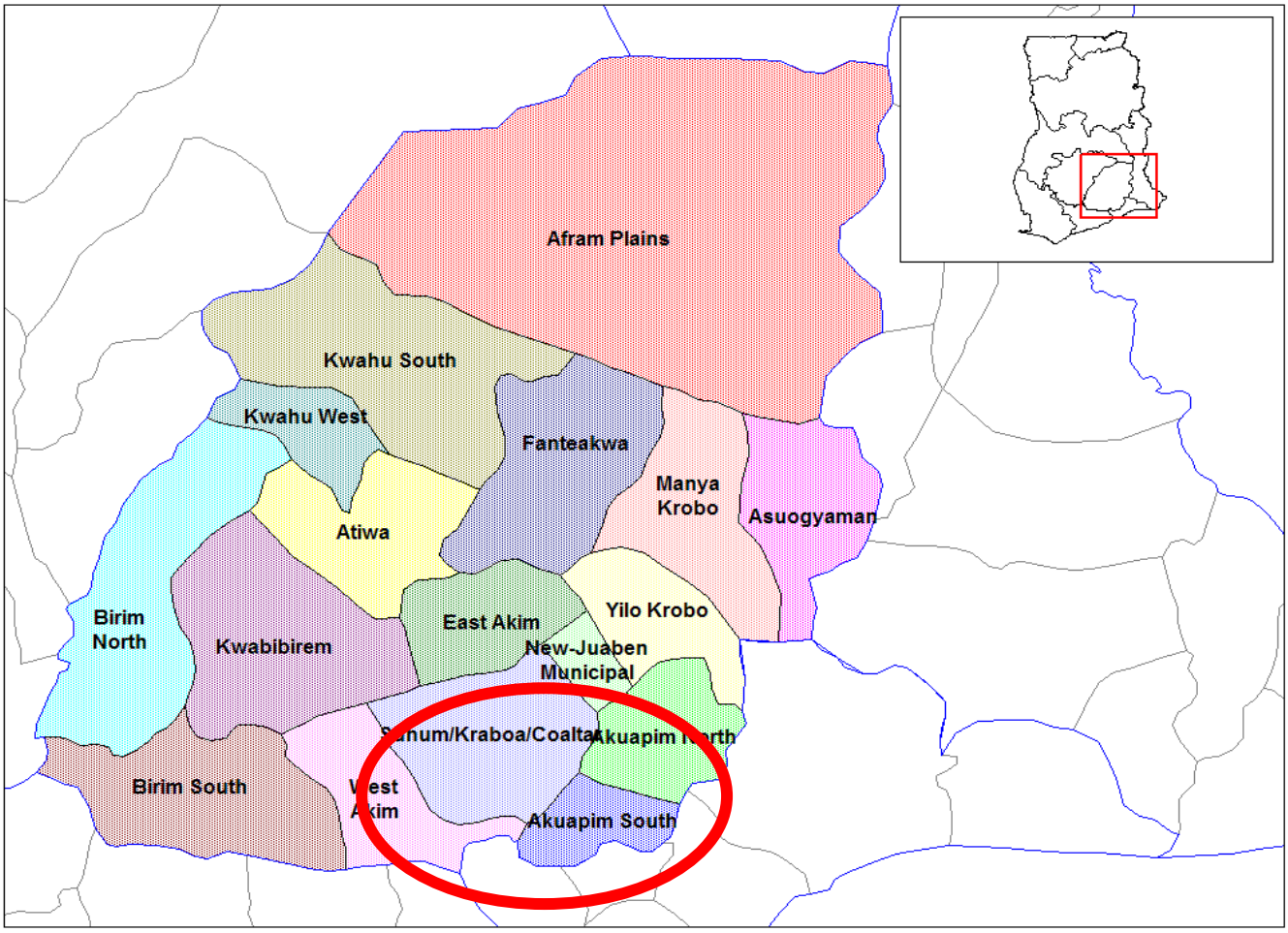


Figure 5: Showing Suhum-Kraboa-Coaltar and Akuapem South Districts, the case control study area.

The Akwapim South Municipality is one of the 21 Districts in the Eastern Region. It has a population of 130,031 with a growth rate of 1.4% (Akuapim South Municipality, Annual report 2009). It is located in the South-Eastern part of the Eastern Region and covers an area of 440km^2 with hilly topography. The district has two (2) main ecological zones namely the moist semi-deciduous forest and coastal savannah grassland. The district is bounded to the North West by Suhum Kraboa Coaltar District.

The Municipality consists of six sub-districts namely Nsawam, Adoagyiri, Pokrom, Pakro/Dago, Pampanso and Aburi with Nsawam as the district capital. Residents of the Akwapim South district depend on the River Densu for their source of water supply, 30% of the population depends on pipe-borne water treated by the Ghana Water Company at the municipal water treatment plant.

Suhum Kraboa Coaltar District shares boundary with Akuapem South Municipality. It covers an area of 1018 km^2 with a population of 188,661. It is located in the Southern part of the Eastern Region (Suhum Kraboa Coaltar, Annual report 2009). The district has 6 Area councils with 48 electoral areas. Agriculture is the predominant occupation of the people in the district absorbing about 70% of the total population. Currently, sand winning has become a lucrative job in the area. The common diseases in the district are malaria, acute respiratory tract infection, diarrhoeal diseases, enteric fever, yaws, schistosomiasis, onchocerciasis and of late hypertension and diabetes.

Ga West and Ga South districts are neighboring districts in the Greater Accra Region. These two districts were originally the Ga West district which was later divided into Ga West and Ga South in 2008 with total population of 502,179. The districts are made up of both rural (40%) and urban (60%) communities. The rural communities are made up of more than 400 scattered settlements. Many of the rural communities in the district are more than 10 kilometers from the existing government and NGO/quasi-government health facilities and thus have limited access

geographically to any health service delivery point. There are 15 government health facilities, private clinics and maternity homes in the district. There is no access to pipe borne water in most part of the districts, the rural communities depend on boreholes, wells, ponds and the Densu River for their water supply, and the urban communities buy water from tankers. There is active sand winning activities in both districts and this has lead to the destruction of the environment with the creation of pools of stagnant water all over the districts.

3.3 Study Population

The target population for the study was made up of all people living in the Akuapem South, Suhum Kraboa Coaltar, Ga West and South districts at the time of study. Clinically diagnosed Buruli ulcer patients were recruited into the study following an active case search in the communities by trained Community Based Surveillance Volunteers, Community Health Workers and principal investigator in all the four districts.

3.4 Sample size

A total of 113 cases and 113 community controls were used in the study. However, from the sample size that was calculated using the following parameters, for case-control study where the exposure of interest was water (unprotected). About 50% of the controls had been exposed to unprotected water as well as 75% of the cases. With a confidence interval (1-α) of 95% and a power (1-β) of 80%, detectable odds ratio of 3.0, a minimum of 65 cases and 65 controls was required from Epi Info software version 3.5.1. However, all the cases identified were included in

the study thereby increasing the power of the study. A higher odds ratio of 3 was used in the sample size calculation because the disease in question was rare and this as well under powers the study to detect any risk factor with an odds ratio less than 3.0.

3.5 Definitions

Case*:* Any person aged 2 years or more who resided in the Suhum-Kraboa-Coaltar and Akuapem South districts diagnosed of Buruli ulcer meeting the WHO clinical case definition for *M. ulcerans* disease. WHO clinical case definition for Buruli ulcer divides the disease into two stages: active and inactive. The active form is characterized by non-ulcerative (papules, nodules, plaques, and edema) and ulcerative disease. The distinctive features of a Buruli ulcer include undermining edges, white cotton wool-like appearance, and thickening and darkening of the skin surrounding the lesion.

Control: Any person who resided in the community/neighbourhood where the case came from but did not have Buruli ulcer. The controls were matched by 5 year age range and sex to the cases.

3.6 Data Collection Method/Technique and Tools

This research was conducted in the Eastern and Greater Accra Regions of Ghana. Districts that were involved were Suhum-Kraboa Coaltar district, Akuapem South Municipal of the Eastern Region, Ga west and Ga South Municipalities of Greater Accra region. Field work started from

May 2010 to December 2011. All stakeholders were engaged during the initial planning and proposal development phase to solicit their buy-in and sort their input for better working relation and collaboration. The National Buruli Control Programme was involved and gave the necessary support to the study.

Using an existing map of the Densu River, all the communities along the river were demarcated from its source Etiwa mountains in the eastern region to the Weija Lake in Ga South Municipal. Figure 6 below is the map of the Densu river and the districts where the active case search was carried out.

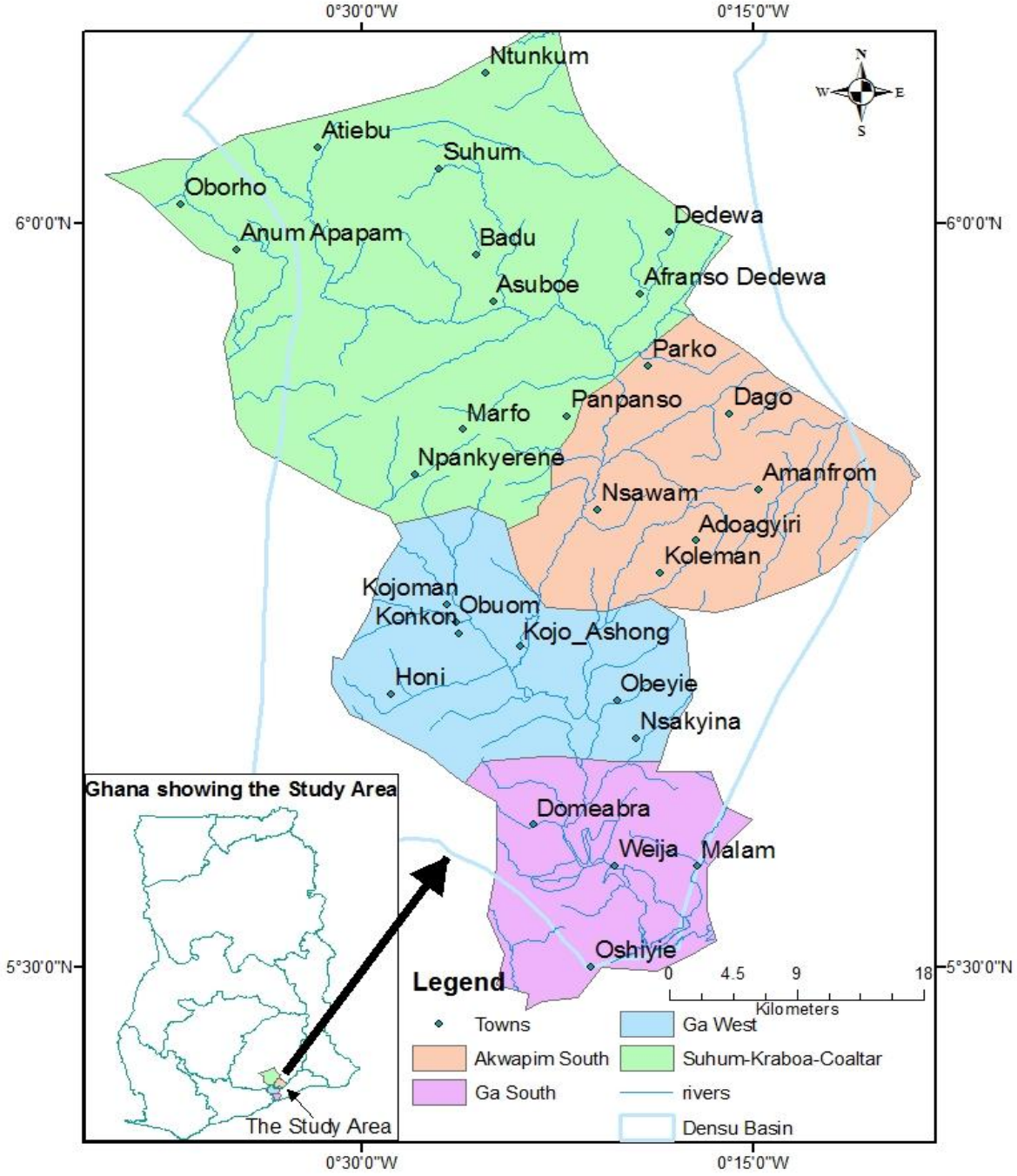


Figure 6: Map of the Study Area showing the Basin of the Densu River

The study area was divided into geographic study units to make numbering of the communities, active case search, detailed data collection and case tracing more manageable. Communities within the study area and 10 kilometers radius from the Densu River were included in the study.

Most of the communities had community registers which were obtained from the disease control officer of the DHMT of the various districts/municipalities and each community assigned unique identification (ID) numbers. Ghana Health Service has a directory of all community based volunteers (CBV) who they constantly work with in delivering public health services and as well surveillance activities in those communities. With the help of the various District Directors and disease control officers, the community based volunteers who were involved in buruli ulcer activities from all the communities within the study area were selected and invited for training.

One day training was organized for the volunteers. Items used included pictures of buruli ulcer cases, skin conditions that could likely be buruli ulcer and the case definition for Buruli ulcer. The volunteers were trained in batches and then immediately sent out to carry out the active case search in their respective communities together with the research team. This was done to minimize the possibility of forgetting the knowledge and skills acquired. CBV were given laminated pictures of buruli ulcer lesions and WHO posters on buruli ulcer to post in their various communities for awareness creation, easy identification of buruli ulcer cases and self referral.

Figure 7: Picture show community sensitization with Buruli ulcer pictures

The volunteers were equipped to undertake active case search, giving identification (ID) numbers to BU cases, registering the cases, linking cases with national buruli ulcer control programme registers in the community, seeking informed consent from the BU suspected cases and scheduling interviews.

The community based volunteers in each community served as the liaison between the community and the research team. They introduced the team initially to the head of the community and the opinion leaders and solicited their cooperation on the research being carried out. In some communities this information was passed on to the members of the community in the night by using the town crier (beating "gongon") or using the community's own means of

passing on information to each other. The team comprising of the principal investigator, research assistants and the community based volunteers was then given a period within which the community was visited and active case search conducted. During the active case search the research team moved from village to village in search of possible Buruli ulcer cases. In a village, the team on entering a household displayed the pictures of the lesions to household members. Individuals who had any of those lesions were selected, examined and enrolled. For those who were not present, posters and pictures on buruli ulcer were left in the house for self examination on their return. The community based volunteers returned to those houses for a follow-up to identify possible cases to be enrolled into the study. In some cases some of the inhabitants that were not available after viewing the posters self referred themselves to be enrolled for the research.

With consent from parents, children were made to undress for thorough skin examination since most of them will not report of any skin lesion to their parents. All suspected buruli ulcer patients identified in the community were enrolled and a written consent form was administered to them. The consent form was translated to participants who could not read or write. Participants who consented to the study were made to sign or thumbprint the consent form in the presence of a witness who also signed the same form. Due to superstition and some cultural beliefs some of the participants refused to thumbprint, however they were willing to participate in the study. These participants only gave verbal consent and this was documented on the consent form.

Suspected cases of Buruli ulcer that consented to participate in the study were interviewed using a standard structured questionnaire. Each interview took at least 30minutes. Two trained field workers (research assistants) conducted interviews for the buruli ulcer cases in the community.

Questions administered covered issues on demography (age, sex, place of residence, marital status, occupation, and educational status), behavioural activities like swimming, wading, fishing, wearing of protective clothing and personal hygiene. Environmental issues like presence of wetland/swamp, Vegetations, Cocoa Plantations, Coffee plantations, type of house they were staying in, their sources of drinking water and sharing of living space with animals/pets were assessed and peculiar characteristics of their locations were also noted.

Taking Samples from lesions

For all the suspected cases, samples were taken from their various lesions. The type of sample taken depended on the type of lesion. Ulcerative lesions were cleaned and exudates collected with two cotton swabs from the base of the circumferential periphery of the undermined edges of the ulcer. A fine-gauge needle (23G by 25 mm) attached to 10 ml syringe was inserted through the centre of non-ulcerated lesions or into viable inflamed skin immediately adjacent to ulcers and moved back and forth within the subcutaneous tissue to obtain the aspirate. The aspiration was terminated when the fluid first appeared at the needle hub. The needle was then withdrawn from the lesion, the needle detached from the syringe and the specimen released into a sterile receptacle.

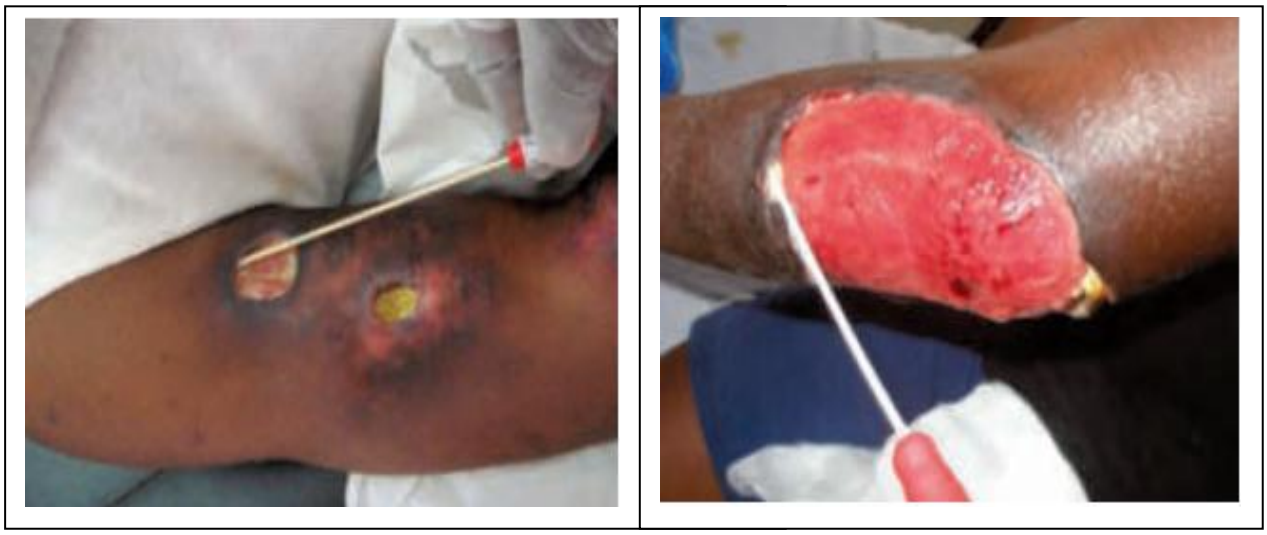


Figure 8: Taking swab from suspected buruli ulcer lesions

Specimen collection, Storage and Transportation of samples

All specimens collected were stored in and transported at 4˚C in a sterile specimen container with transport medium to the bacteriology laboratory of Noguchi Memorial Institute of Medical Research for confirmation.

For each suspected case, a community based control was randomly selected based on spinning a bottle in the middle of the community, following the direction of the tip of the bottle, and choosing the first house in that direction. Within the first house entered any person of the same sex and within five years age range who was without buruli ulcer was selected as a control. If the person refused to participate in the study, the second person in the house was approached until a suitable control was identified and interviewed. Where no suitable control was identified in that house the next house was visited and the same procedure was repeated until the appropriate

control was selected, informed consent was administered and the control interviewed with the same standard structured questionnaire.

Swabs taken from the undermined edges of ulcers and fine needle aspirates (FNAs) from nodules and oedematous lesions were taken for direct smear microscopy using the Zeihl-Neelsen staining method. IS2404-PCR test was also done to detect *M. ulcerans* DNA (Ross et al., 1997).Two swabs were taken from each suspected case. These cotton swabs were put in plastic containers and sealed. They were labeled with information that included the name of patient, identification number, age, sex, and date of specimen collection. Specimens were stored on ice and transported to the laboratory for analysis.

Detection of *Mycobacterium ulcerans* in Swab or FNA Specimen

M. ulcerans in this study was detected by the use of three confirmatory diagnostic tests. These were direct smear microscopy, culture and polymerase chain reaction.

Direct Smear Microscopy

The direct observation of acid-fast bacilli (AFBs) under the microscope was done using the Ziehl-Neelsen (ZN) staining technique.Two drops of sample suspension was put on each labeled slide. Each slide was allowed to air-dry and the smear fixed by heat-drying without boiling. It was then flooded with ZN carbolfuchsin. The slide was heated slowly until steaming for 3-5 minutes without letting it boil dry. Afterwards the steam was rinsed gently with running water until the slide was free of stains. The slide was then decolorized with 20% Sulphuric acid for 2-

5minutes. This was then again rinsed thoroughly and excess water drained. Counter-staining was then done with 3% methylene-blue chloride solution for a maximum of 120 seconds. Final rinsing was then done, excess water drained and slide allowed to dry without blotting. It was then examined under the microscope for acid-fast bacilli (AFBs). The quantification used for reporting the results is as shown in the table 2 below

Interpretation of Results

Table 2: Reporting of AFBs

No. of AFB seen on average	No. of fields seen	Report
No AFB / 100 immersion fields	100	No AFB observed
1-9 AFB / 100immersion fields	100	Scanty
10-99 AFB / 100 immersion fields	100	+
1-10 AFB / 1 immersion field	50	++
>10AFB / 1 immersion field	20	+++

Note: + -Small numbers of AFBs seen in smear

++ -Moderate numbers of AFBs

+++ -Numerous numbers of AFBs

Culturing of *M. Ulcerans*

Samples were decontaminated using the oxalic acid method. Briefly, 2mls of homogenized tissue sample was added to 2mls of 5% oxalic acid. This was mixed by vortexing and then left standing at room temperature for 30mins with intermittent mixing. This mixture was neutralized with

40ml of sterile phosphate buffered saline (PBS). It was then centrifuged at 3000g for 30 minutes and then pellets re-suspended in 1ml of PBS. Two (2) drops of this final mixture were inoculated in duplicate Lowenstein-Jenssen (L-J) tubes. These were incubated at 32^0C. They were examined daily for contamination for the first week and then subsequently weekly for episodes of growth for 8-12 weeks.

Polymerase Chain Reaction (PCR)

IS2404 Specific PCR for the Detection of *M.ulcerans* in BU Samples

This method amplifies minute quantities of DNA to levels that can be detected in the laboratory. This sequence of the DNA that is amplified is determined by the sequence of the PCR "primers" that initiate PCR amplification. The diagnostic protocol consists of heat and alkali lysis, extraction of total DNA from sample and then PCR reaction to detect *M. ulcerans*-specific DNA in extracted total DNA.

Mycobacterial DNA Lysis and Extraction

Mycobacterial DNA was extracted from 500ul of BU sample by the QIAamp DNA extraction minikit (Qiagen, Hilden, Germany) as per instructions of manufacturer. Briefly 20ul of proteinase K was added to a mixture of 400ul aliquot of BU sample and 400ul of lysis buffer. This was mixed by vortexing and incubated at 56˚ C for 30 minutes. It was allowed to cool, after which 400ul of absolute ethanol was added to facilitate mycobacterial DNA precipitation. 700ul of the final mixture was transferred to a collection tube attached to the spin column and

centrifuged at 6000g. The collection tube was then discarded with the filtrate. This procedure was repeated for the remaining mixture and the filtrate washed twice with two different washing buffers, firstly with 500ul of washing solution 1 (Buffer AW1), and then with washing solution 2 (Buffer AW2). The extracted DNA was then eluted into 100-150ul of the elution buffer provided in the kit.

Mycobacterial DNA Amplification

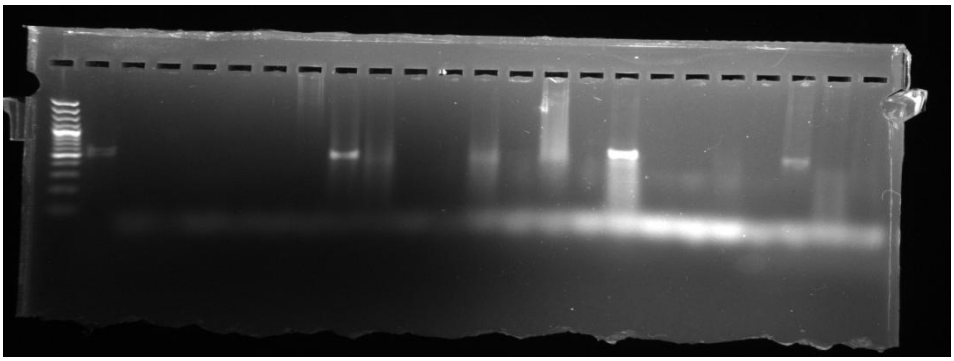
Extracted DNA was amplified using the QIAGEN PCR kit as per instructions provided by the manufacturer. Briefly 4ul of extracted *mycobacterial* DNA was amplified in a final 20-ul volume PCR reaction mix (1_ *Taq*PCR buffer, deoxynucleoside triphosphates (0.2 mM each), 1 U of Ampli*Taq*Gold DNA polymerase (Perkin-Elmer Applied Biosystems), each primer at a concentration of 0.5 _M (represented in the PCR QIAGEN kit as master mix) and Q solution in addition to 1 ul of forward Primer pMU1 and 1ul of reverse pMU2 in addition to10ul of Master Mix solution. The reaction was carried out using an Applied Biosystems thermal cycler starting with denaturation for 10 min at 95°C. PCR was performed for 40 cycles of 30 seconds at 96°C, 30 seconds at 60°C and 68 for 18 seconds .The reactions were terminated by incubation at 72°C for15 minutes.

Visualization of PCR products

PCR products were analyzed by agarose gel electrophoresis using 2% agarose gel incorporated with ethidium bromide and visualized with a UV trans-illuminator. They were estimated by comparing the bands to a 1-kb ladder. The samples were run concurrently with negative and

positive controls. A sample positive for IS2404 was indicated by the alignment of its band with the band produced by the positive control sample as shown in figure 9 below.

Figure 9: Plate of agarose gel showing electrophoresis of amplified PCR products



Lane 1: 1 kb base pair markers

Lane 2: positive control

Lane 3: negative control

Lanes 9 & 17: Clinical samples strongly positive for IS2404PCR

Spatial Mapping

E-trex Garmin Geographical positioning system (GPS) receiver machine was used to mark the location of each suspected buruli ulcer patient. Houses, water bodies, field/crops, gardens, footpaths, rivers, road and any other feature of importance were also marked with the GPS receiver machine. In order to have accurate data from the Garmin eTrex Legend GPS receiver, the research assistants were trained on the use of the GPS receiver. On the main menu page on

the machine, the setup option was selected, opened the time page and selected the time zone. Selected the *Units* icon to open the *Units* page and the following preferences were selected:

a. Position Format – hddd.ddddd (decimal degrees)

b. Map Datum – WGS84 (World Geodetic System, 1984)

c. Distance/Speed – Metric

d. Elevation (Vert. Speed) – Meters

e. Depth – Meters

When the receiver had acquired signals from 4 or more satellites (3D fix), the coordinates were displayed on the screen. To make sure the location (accuracy) shown in the upper left side of the Satellite page was 8 meters (25 feet) or less for greatest positional accuracy. However, the GPS receiver was held until the positional accuracy shown was less than or equal to 4meters (12.5 feet). Coordinates for north latitude and east longitude are denoted by positive numbers and south latitude and west longitude coordinates are represented by negative numbers. GIS software utilizes positive and negative numbers instead of compass directions.

Accuracy Check Point (ACP) on the GPS receiver was set. This was done to ensure that accurate values were recorded during the field work. It was used as a reference point for the GPS observations. In addition, the ACP was used to verify whether the set up was correct and the GPS receiver was working accurately. The main entrance of the School of Public Health, University of Ghana security post was used to establish the ACP. Before leaving for the field sites where GPS observations were made, each research assistant and principal investigator stand

at the security point, switched on the power to the GPS receiver and Check the GPS receiver settings (DD, WGS-84, and WAAS-enabled) and the number of satellites acquired. At least four or more satellites were acquired and the accuracy reading on the Satellite Page shows 4 meters (12.5 feet) or less.

The established geographic coordinates for the ACP and all future GPS coordinate readings at the established ACP matched to the first 3 places behind the decimal (e.g. 33.782, - 118.002) was ensured. If there was any discrepancy, the set up preferences in the GPS receiver was reset and the ACP co-ordinates rechecked for consistency before leaving for the field. The Waypoints collected in the field were recorded to all 5 places behind the decimal to ensure good positional accuracy (e.g. 33.78253o, - 118.00269o).

To obtain geographic coordinates for point locations, a designated point was used to mark the reading. When determining the geographic coordinates for a premise, the front gate geoposition was used example the driveway entrance to the property or main gate of the facility. However, when mapping a facility, a point near the main entrance was chosen and for water bodies, the main entrance to the water body was used. The center of the cover on the borehole was used. For any other feature, standing by the exact location the Satellite page was examined in each case to make sure that the receiver's accuracy level was relatively stable within the acceptable range. The rocker key was pressed and held for a few seconds to create a new waypoint.

GPS receiver machine was used to take the GPS co-ordinates of the controls as well. This activity was carried out twice in a week until each geographic study unit was completed.

The team then moved to the next geographic study unit and the same procedure carried out until the whole study area was covered.

3.7 Data Quality Checks

Questionnaires were designed with consistency checks to prevent errors at the stage of data collection and at the point of data entry. The questionnaire was pre-tested at Obom a known buruli ulcer endemic community in Ga South Municipality to evaluate the following;

1. Acceptability of the questions being asked
2. Willingness of respondents to answer and collaborate with the study
3. Reliability of the questionnaires
4. Sequencing of questions and their clarity
5. Accuracy in interpretation of questions.

In the main study all questionnaires were cross-checked by the field supervisor and principal investigator before the questionnaire being entered into the data base. All questionnaires were checked for incompleteness and any form of error. Questionnaires that were incomplete were taken back to the field within 24 hours for rectification. Completed questionnaires were kept in a big envelope under lock and key. The principal investigator selected some completed questionnaires and re-administered them to ensure accuracy of the data collect. Data was entered into Microsoft Access and analyzed with SPSS version 16.

Geoposition coordinates taken for cases, controls and various attributes of importance in the study had an ACP set and before departure to the field, field workers ensure that the rechecked ACP value was accurate up to three points after the decimal. In addition to recording the coordinates of the attributes in the GPS receiver, the values were also recorded into a design hard copy with special box for each figure, template on appendix.

GIS co-ordinates data taken for the cases, controls, houses, water bodies, field/crops, gardens, footpaths, rivers and roads were entered into an excel spread sheet and analyzed using ArcGIS.

3.8 Data Analysis

To import GPS waypoint data into ArcGIS software for analysis, the GPS receiver was connected to the USB port of the computer using the Garmin data cable that was packaged with the eTrex Legend H receiver. The GPS receiver was powered up, opened the ArcGIS software by double-clicking on the icon on the computer to import the waypoints data for it to plot on the map of the study area (georeferencing). Geographic Information System (GIS) maps were developed showing buruli ulcer case distributions along the Densu river. Maps were generated based on buruli ulcer cases per population of the communities and districts. Buffer zones, which were diameters created around the place where Buruli ulcer cases reside to identify features particularly environmental factors within buffer zone that were associated with Buruli ulcer. Further analysis was done to determine the presence of clustering among the Buruli ulcer cases.

Results of the case control part of the study was presented in frequency tables, 2x2 tables, charts and appropriate graphs. Chi square test and Odds ratio were used to measure the association between the exposure variables and Buruli ulcer. Buruli ulcer development was the dependent variable and demographic, host related, environmental and behavioural risk factors as the independent variables. Significance level was set at a p-value less than 0.05. Univariate analysis was done to explore the association between the exposure variables and Buruli ulcer. All variables that obtained p-values of 0.1 or less from the univariate analysis were retained for backward elimination procedure form of logistic regression model in the multivariate analysis. This procedure using SPSS takes care of interaction and confounders (Norman and Streiner, 2007). The variables which were not statistically significant were removed from the model one at a time and only those that were significant were retained.

3.9 Ethical Consideration

Ethical approval

Ethical clearance was obtained from the Noguchi Memorial Institute for Medical Research Institutional Review Board (IRB) and Ghana Health Service Ethical Committee. The approval was renewed yearly during the period of the study.

Study area approval

All the various Regional, Municipal and District Directors of Health Services were duly informed for their consent namely the Regional Director of Health Services of the Eastern Region, the Municipal Health Director of Akuapem South, Municipal Health Director of Ga West, Municipal Health Director Ga South and District Director of Health of Suhum Kraboa Coaltar. The research was introduced to the District Health Management Teams (DHMTs) of the various districts for information and support for the active case search for buruli ulcer in their respective areas since each of the DHMT's had an active disease control unit. Lastly, the Municipal and District chief executives of the study areas were informed to enable us get access to the communities we were going to work in as well as gather useful information to help in our field activities Permission was sought from the National Buruli Ulcer Control Programme Manager and support solicited for the study.

Subjects involved

Informed consent was sought from parents or guardians for under aged wards. Participation in the study was voluntary and subjects could withdraw at any time they wished. Participants did not benefit directly but results of the study would help in the identifying the risk profile for Buruli Ulcer. Informed consent forms were administered to parents and guardians on behalf of their wards, and those who agreed to participate consented to the study by signing or thumb printing.

Privacy/Confidentiality

The objectives and the procedures for the study were fully explained to them. All potential and enrolled subjects in the study were respected by permitting withdrawal from the research, protecting privacy through confidentiality. The identity of participants was always protected even when there was the need to link the identification of the cases to the community and national buruli ulcer control programme, there was no link of names of participants, the GPS coordinate of the participants' locations to the internet to ensure participants protection and privacy. Participants' names or identity will not be revealed in any report.

Proposal and funding information

The study proposal was developed by me after going through revisions to make it scientifically robust by my supervisors. It was funded jointly by me and the DAAD –ACBRIDGE project.

3.10 Limitations

An active cases search was deployed to identify the Buruli ulcer patients however most of the case patients have been living with the disease for more than two years. In some cases they have been living with the disease for more than ten years. Cases that were at early stages of the infection or preclinical stages may likely be excluded in the study and may lead to bias. In addition, the use of prevalent cases rather than incident cases for a chronic and rare disease like Buruli Ulcer may pose a problem that some of the factors that may be identified will be associated with the persistence of the disease and not its development. Since the questions being

asked depends on the recall ability of the patient, recall bias remains a major limitation to this study. Case patients are more likely to recall events preceding to the disease than their respective control. Individuals who have had the infection for many years are more likely to forget about the antecedent events. The beliefs of the respondents regarding the disease may also affect the response of the participants but due to the education about Buruli ulcer in the communities eroded those beliefs such as witchcraft and evil spirit causing the disease. However, their reasons for not seeking earlier care ranges from geographical accessibility to financial accessibility.

The aspect of interviewer bias may also exist, however all the research assistants were trained and were made to understand that in administering the questionnaires, probing or leading questions should not be asked. The same research assistants were used throughout the study period for consistency purposes. Each case-patient was matched to a community control but avoided using relations in order to prevent overmatching.

CHAPTER FOUR - RESULTS

4.1 Spatial Distribution of Buruli ulcer

Active case search for Buruli ulcer was carried out from the origin of the Densu river (Atiwa forest) along its entire course till the Weija lake. A total of 257 suspected buruli ulcer cases were identified from over 400 communities. One hundred and forty-one (141) of the suspected cases were in Akuapem South and Suhum Kraboa Coaltar Districts and 116 from Ga west and Ga South Municipalities.

Figure 9 shows the distribution of all the Buruli ulcer cases identified during the active case search. The distribution of the cases were mainly in Akuapem south municipality and Ga West Municipalities. A buffer of 500meters and 1000meters were created around each case to look at the special features around the cases as shown in Figure 10. In Figure 11. clustering of buruli ulcer cases was observed in Akuapem South Municipality and Ga West Municipality.

District prevalence of buruli ulcer clearly shows that, Akuapem South Municipality was the most prevalent district followed by Ga West Municipality, Suhum Kraboa Coltar district and the least Ga South Municipality, this is shown in Figure 12.

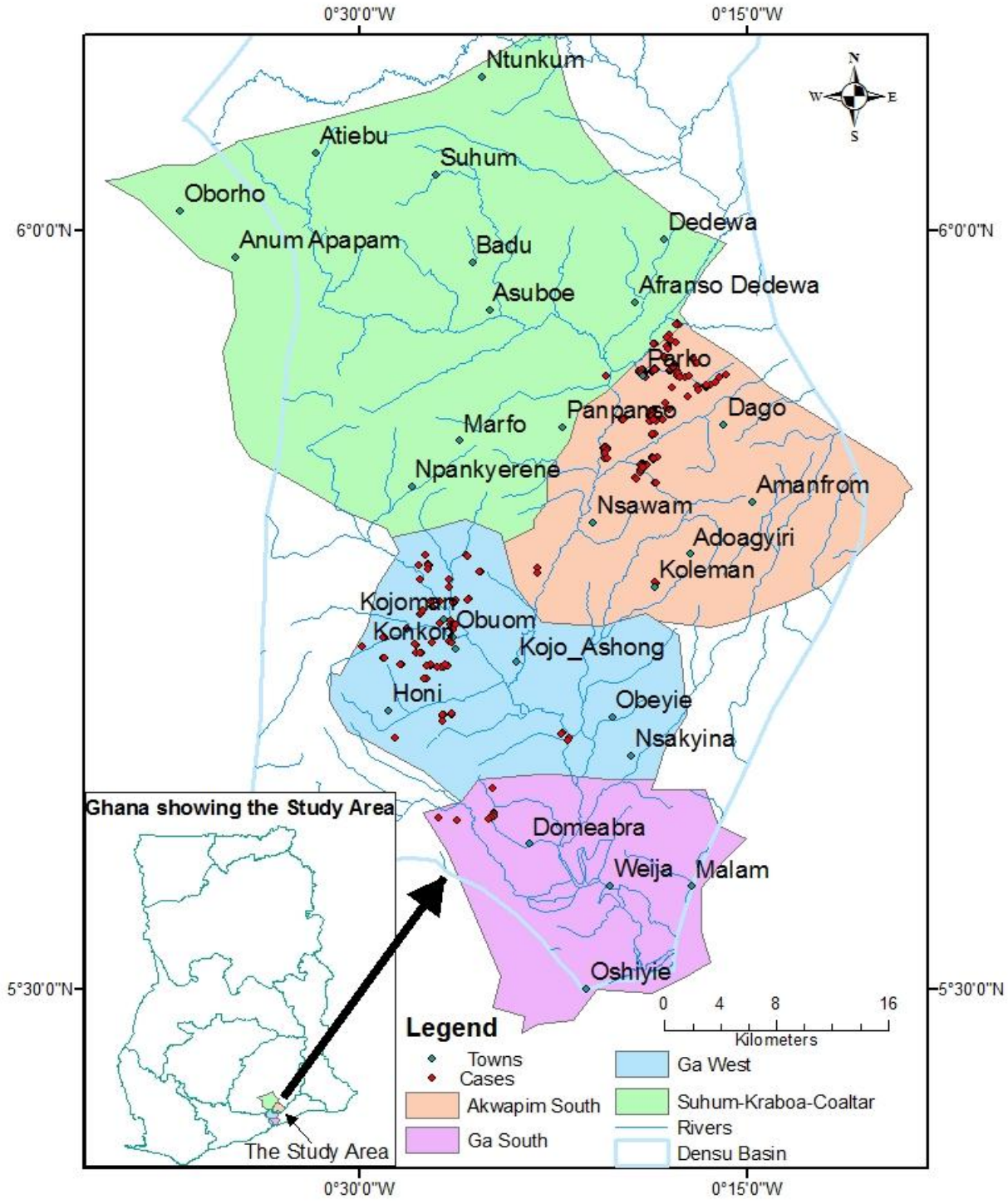


Figure 10: Map of Study Area with distribution of Buruli ulcer cases

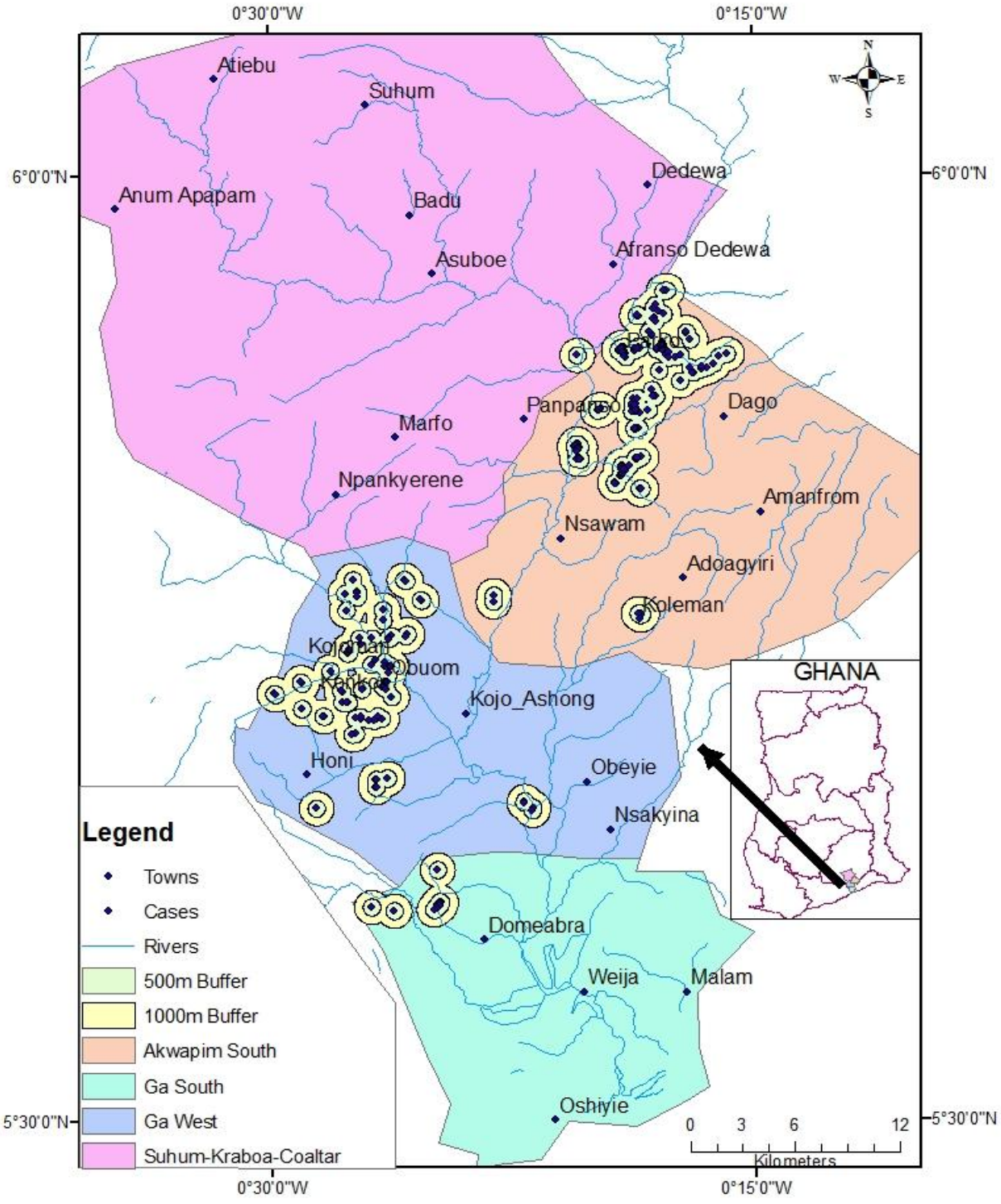


Figure 11: Distribution of Buruli ulcer cases with 500 and 1000 meters buffer

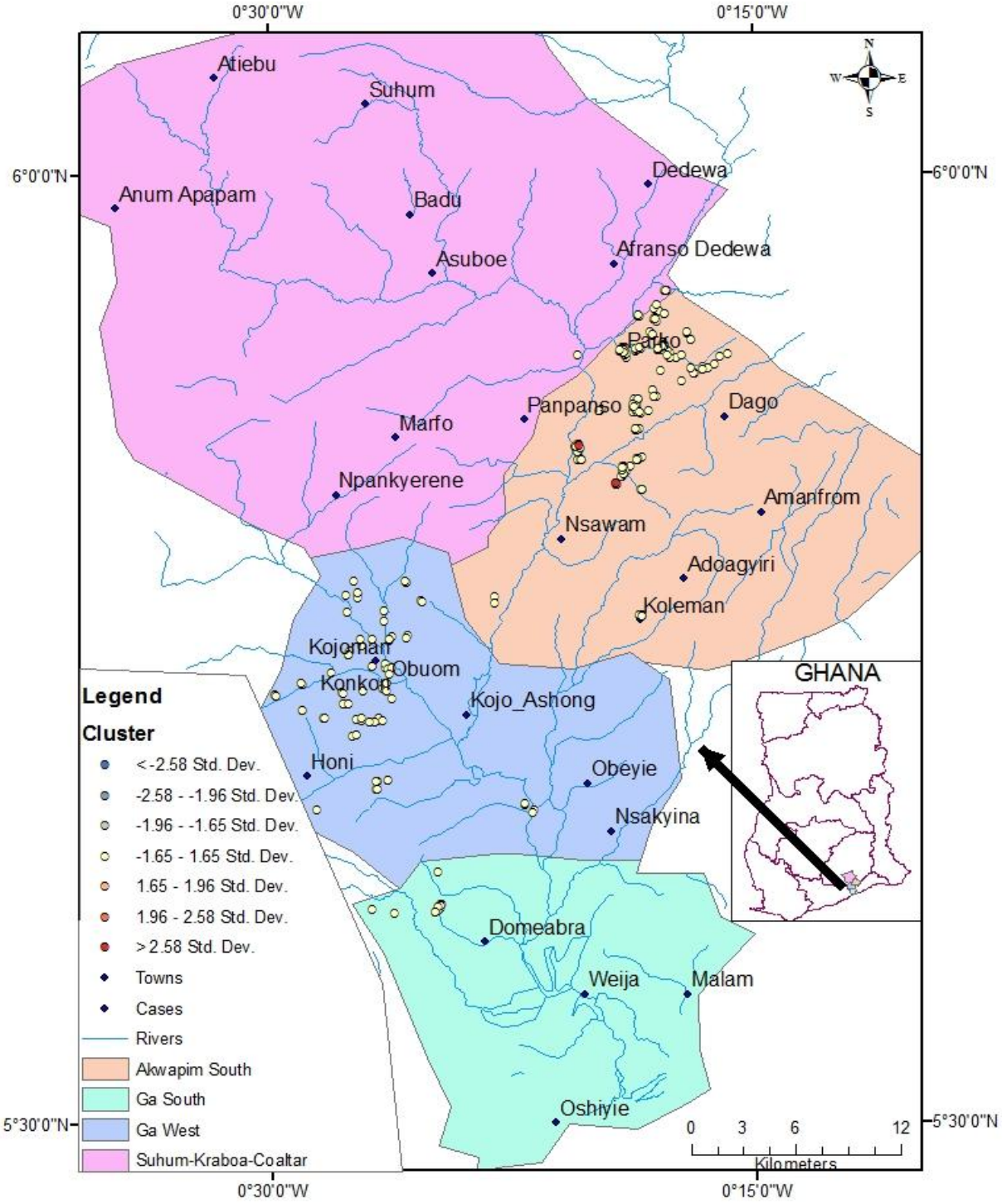


Figure 12: Distribution of Buruli ulcer cases with Clustering

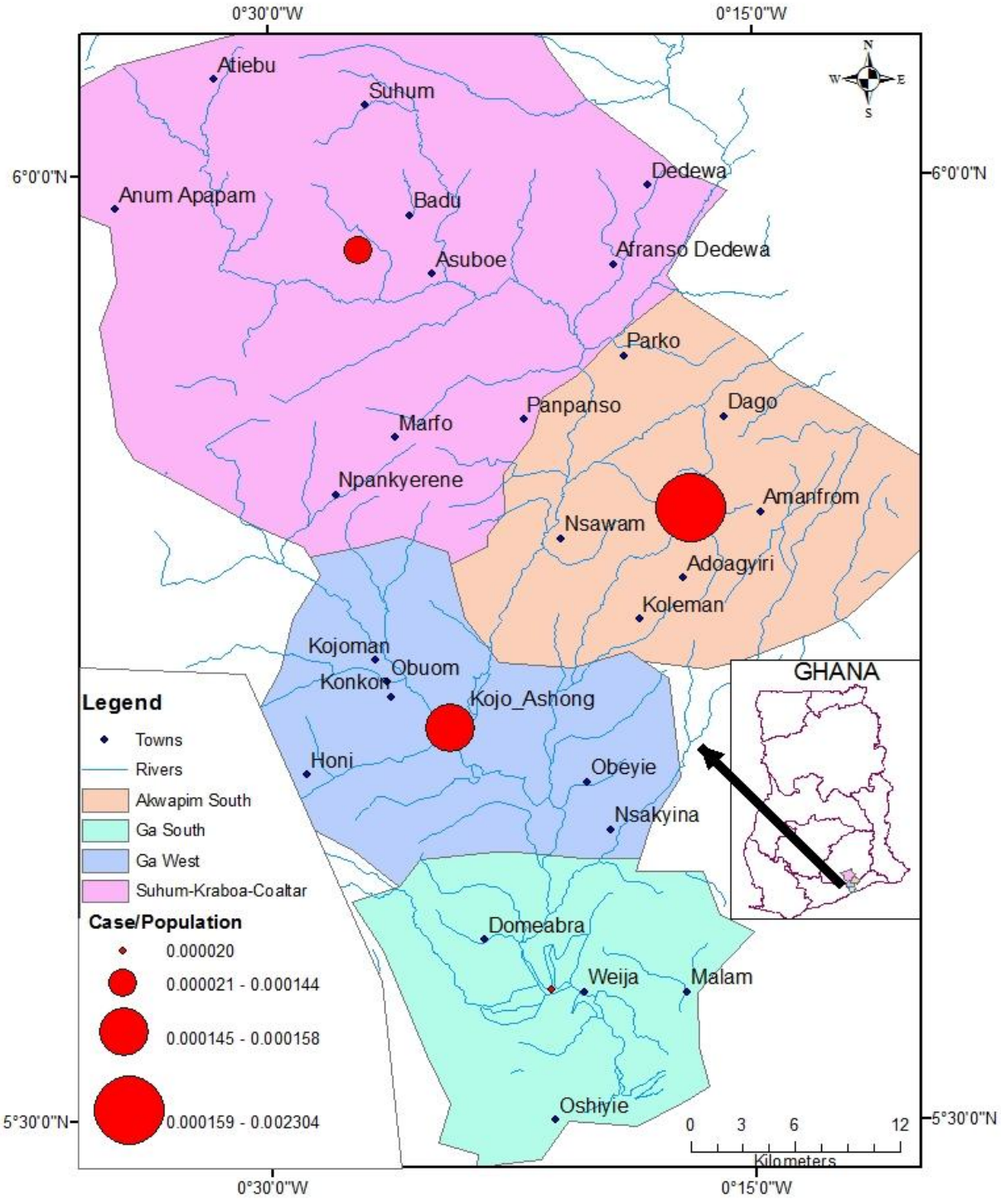


Figure 13: Distribution of Buruli ulcer Cases per population (Prevalence) of Districts

4.2 Demographic Characteristics

A total of 141 probable Buruli ulcer patient were enrolled in the case-control aspect of the study and out of these 113 (80.1%) were confirmed by a positive Polymerase Chain reaction (PCR). Among the 113 confirmed positive PCR, 66 (58.4%) of them were also positive for Ziehl-Nelsen test. The rest of the 28 (19.9%) probable buruli ulcer cases were negative for both PCR and Ziehl-Nelson test. No significant difference was observed between the probable cases and the confirmed cases with respect to demographic data, type of first lesion and localization of lesion (Table 3).

Table 3: Characteristics of total cases, probable and confirmed cases

Characteristics	Total Cases (n, %)	Probable Cases (n, %)	Confirmed cases (n, %)	P-value*
N	141 (100)	28 (19.9)	113 (80.1)	
Sex				
Female	68 (48.2)	11 (39.3)	57 (50.40)	0.3†
Male	73 (51.8)	17 (60.7)	56 (49.6)	
Age(median, range)	27(2 -102)	29 (2 -98)	28 (2-102)	
<10	19 (13.5)	6 (21.4)	13 (11.5)	0.1†
10-14	27 (19.2)	8 (28.6)	19 (16.8)	
15-24	25 (17.7)	6 (21.4)	19 (16.8)	
≥ 24	70 (49.6)	8 (28.6)	62 (54.9)	

***Probable vs. Confirmed cases, using Fisher's exact test; † Nonsignificant association**

Table 3b: Characteristics of total cases, probable and confirmed cases

Characteristics	Total Cases (n, %)	Probable Cases (n, %)	Confirmed cases (n, %)	P-VALUE*
First Lesson				
Papule	3 (2.1)	0 (0)	3 (2.7)	0.2*†
Plaque	3 (2.1)	0 (0)	3 (2.7)	
Nodule	110 (78.0)	27 (96.4)	83 (73.4)	
Oedema	15 (10.7)	1 (3.6)	14 (12.4)	
Active Ulcers	8 (5.7)	0 (0)	8 (7.0)	
Don't Know	2 (1.4)	0 (0)	2 (1.8)	
Localization±				
Leg	94 (67.1)	18 (64.3)	76 (67.9)	0.9*†
Arm	32 (22.9)	8 (28.5)	24 (21.4)	
Trunk (Breast)	1 (0.7)	0 (0)	1 (0.9)	
Head and Neck	8 (5.7)	1(3.6)	7 (6.2)	
Leg and Arm	5 (3.6)	1 (3.6)	4 (3.6)	
Distal	94 (67.1)	18 (64.3)	76 (67.9)	0.9*†
Proximal	41 (29.3)	9 (32.1)	32 (28.6)	
Both	5 (3.6)	1 (3.6)	4 (3.5)	
Right side	68(48.6)	14 (50)	54 (48.2)	0.98*†
Left side	61(43.6)	12 (42.9)	49 (43.8)	
Both	11 (7.8)	2 (7.1)	9 (8.0)	

*Probable vs. Confirmed cases, using Fisher's exact test; † Nonsignificant association; ± one missing piece of data

The median age of all the recruited patients was 27 years (range: 2 to 102 years) and that of the confirmed cases was 28 years (range: 2 to 102 years). Sixty two out of the 113 confirmed BU cases (54.9%) were more than 24 years of age and trend analysis of the age group among the probable and confirmed cases show that there was an association between age and confirmed cases of buruli ulcer. An age above 24 years had 3.6 odds of being confirmed to be BU among all suspected cases of Buruli ulcer.

Contracture deformities were observed in 12 of the cases with active lesions, extensive scar due to Buruli ulcer in 5 of the cases and one patient has had amputation of the right little toe. Only 18 case patients had visited the health center with their lesions, 20 were receiving some form of treatment from the herbalist and the rest were self medication. None of those with deformities were receiving any form of physiotherapy.

Ninety eight percent (111/113) of the case patients could remember the initial form of the BU lesion however, seventy eight percent of them (88/113) could not link the appearance of the initial lesion with any particular event but 22.1% (22/113) associated it with either injury or insect bite. The first form of BU lesion in 73.4% of the confirmed cases presented as nodules (83/113) and 67.9% (76/112) of all the lesions appeared on their legs. Only 1.8% of the patients (2/113) could not remember how the lesion started. The initial lesions occurred less frequently on the trunk 0.9% (1/113), head and neck 6.2% (7/113). More of the patients had their initial lesion on the distal extremity thus from the elbow to the hand and from the knee to the foot,

70.4% (76/108) than on the proximal extremity, trunk or head 29.6% (32/108). Distribution of the lesions was almost even on both sides of the body 48.2% (54/112) on the right side, 43.8% (49/112) on the left side and 8.0% (9/112) on both sides.

During the active case search, most of the lesions diagnosed were active ulcers 77.0% (87/113) which was entirely opposite of the appearance of the first lesion as nodules 73.5% (83/113).

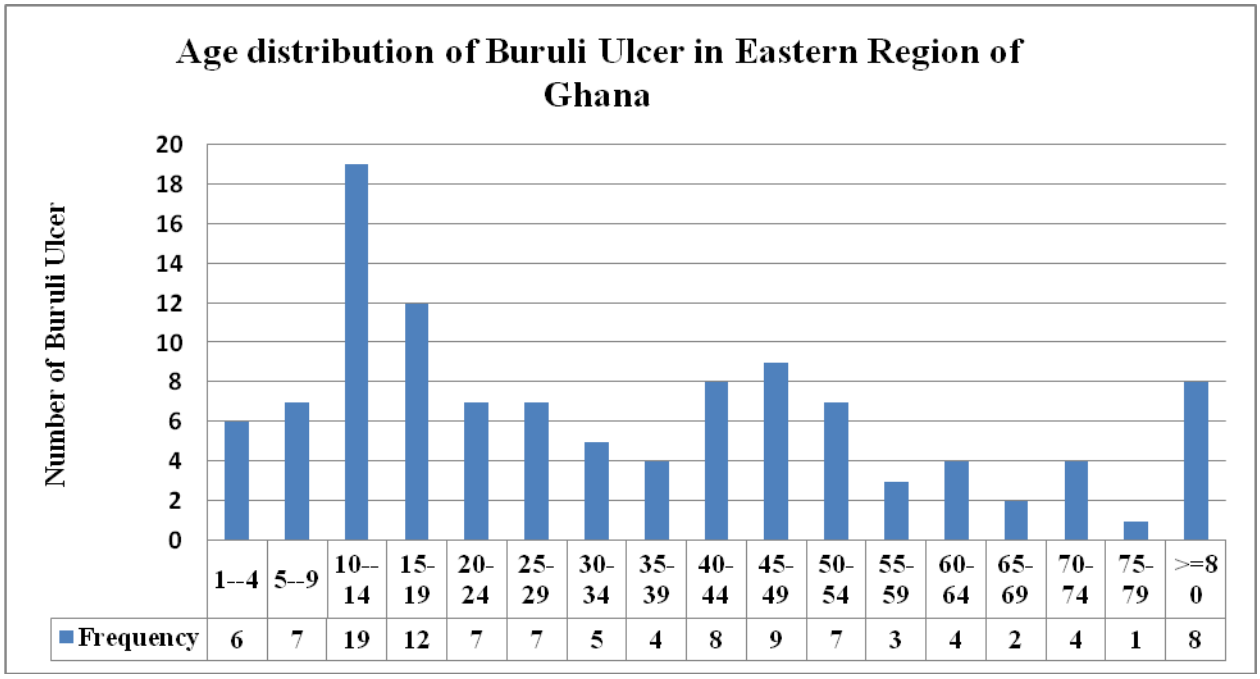
Plaque, papule and nodule were known to be early lesions and oedema and active ulcers noted to be late lesions. Comparing the form of the lesion at diagnosis and its initial appearance to early and late lesions show that there was a significant association between late presentation of lesions and diagnosis of lesion, Mantel-Haenszel Chi^2 - 92.2 and P-value <0.0001 (Table 4)

Table 4: 2x 2 tables showing clinical presentation at diagnosis and the form of the lesion

Clinical Presentation	**Late form of the lesion(oedema, active ulcers)**	**Early form of the lesion (Plaque, papule and Nodule)**	**Total**
Presentation at diagnosis	95	18	113
Initial presentation of the lesion when patient first noticed it	22	89	111
Total	117	109	224

Mantel-Haenszel Chi^2- 92.2 and P-value <0.0001

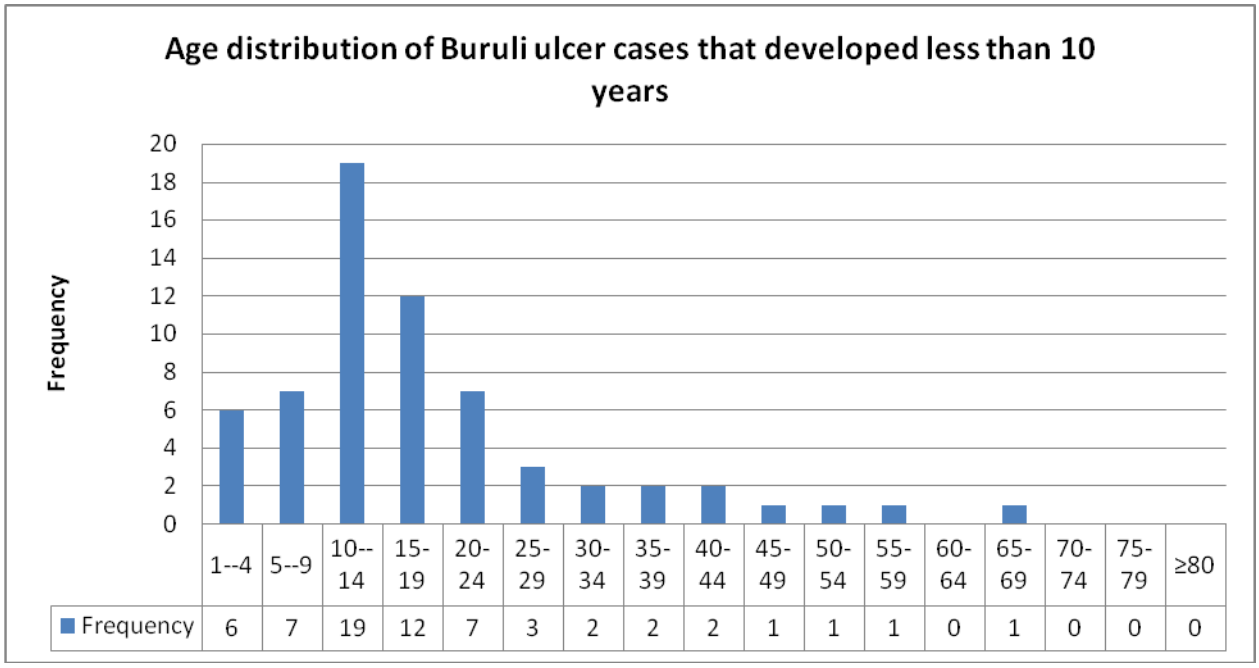
Figure 14: Distribution of Buruli Ulcer by Age group in Suhum Kraboa Coaltar and Akuapem South districts of Eastern Region of Ghana, 2012



Age group 10-14 years was the modal age group 16.8% (19/113) for the BU cases, followed by 15-19 years group 10.6% (12/113) and the least 75-79 years group 0.9% (1/113). Children and teenagers form more than one third of the cases 38.9% (44/113).

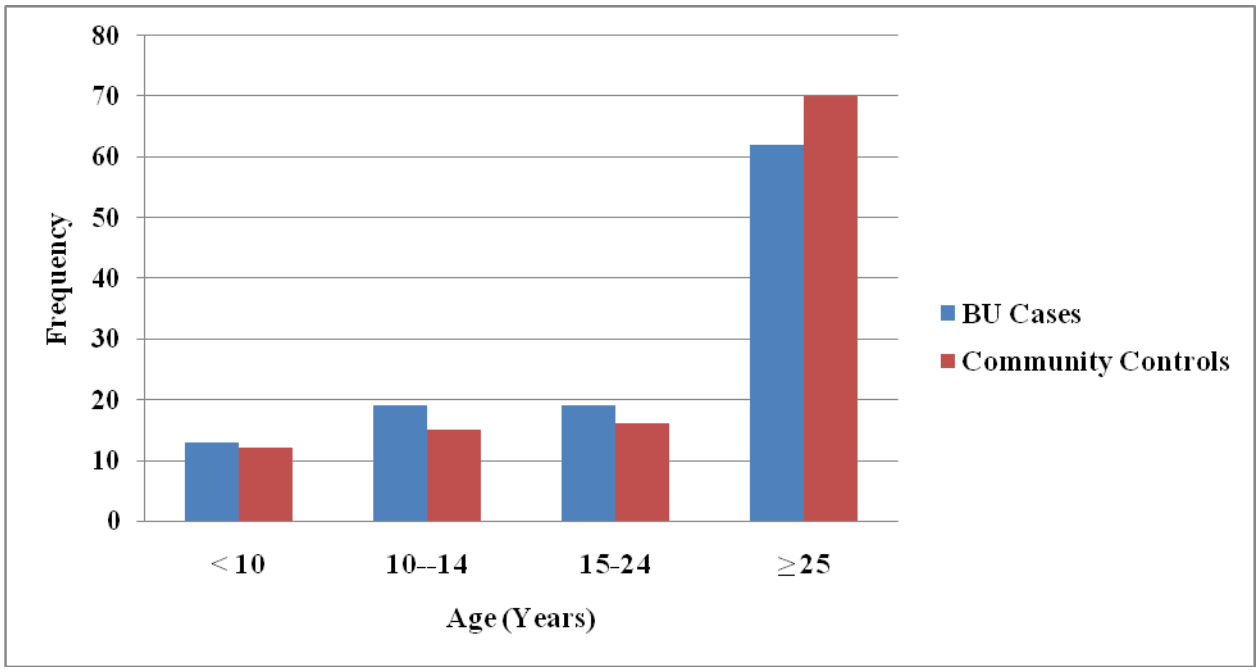
Of the 113 positive PCR cases, 113 community controls matched by sex and 5 year age range were recruited. Among the case patients 50.4% (57/113) were females and among the community controls 50.4% (57/113) were females. The median ages of the case patients and community controls were 28 years (range: 2 to 102) and 30 years (3 to 98) respectively.

Figure 15: Distribution of BU by age group for those who developed the disease within the last 10years



From the graph of recent infection using 10 years as a cut off point 50% (32/64) of all the new infections were in children less than 15 years. Fifty one (51) Buruli ulcer patients contracted the disease more than 10 before the study.

Figure 16: Distribution of Confirmed Buruli ulcer patients and the Community controls



There was no difference in the age range distribution of the BU case patients and the community controls. Half of the study participants were 25 years and above. Fourty Percent (25/62) of the BU case patients who were 25 years and above developed the lesion over 10 years. The ulcers often do not heal completely.

Table 5 shows that the predominant ethnic group among the case patients was Ewe 50.4% (57/113) followed by Akan 35.4% (40/113).

Among the community controls the Akan ethnic group dominated followed by Ewe 49.6% (56/113) and 42.5% (48/113) respectively.

Table 5: Distribution of cases and community controls

Ethnic group of respondents	Case patients (%)	Community Controls (%)	Total (%)
Akan	40 (35.4)	56 (49.5)	96 (42.5)
Ewe	57 (50.4)	48 (42.5)	105 (46.5)
Ga/Adangme	12 (10.6)	7 (6.2)	19 (8.4)
Hausa	1 (0.9)	0 (0)	1 (0.4)
Krobo	1 (0.9)	1 (0.9)	2 (0.8)
Kyerepon	0 (0)	1 (0.9)	1 (0.4)
Larteh	1 (0.9)	0 (0)	1 (0.4)
Others	1 (0.9)	0 (0)	1 (0.4)

Table 6: Trend analysis on educational status of cases and community controls for individuals above 14 years

Educational Status	Cases	Control	OR	P-value
No Education	18	5	1.0	
Primary/JHS	52	52	0.28	0.0006
Secondary/Tertiary	11	24	0.13	

The Chi^2 for linear trend – 12.08

There was a significant association between level of education and risk of Buruli ulcer. Individuals with higher education were protected from developing BU as compared to those without education. Various variables were analysed to assess whether they were associated with buruli ulcer, below are the summary results tables.

Table 7: Univariate analysis of selected variables for BU in Eastern Region, Ghana

Characteristic	No. (%) of Control Subject (n=113)	No. (%) of Case Subject (n=113)	Univariate OR (95% CI)	P-Value
Demographic				
Ethnic group of the father Akan/Others	56 (49.6)	40 (35.4)	0.56 (0.33-0.95)	0.04*
Ethnic group of the mother Akan/Others	58 (51.3)	41(36.3)	0.54(0.32-0.92)	0.03*
Education level: secondary or more/primary	24 (21.2)	11 (9.7)	0.40 (0.19-0.86)	0.03*
Economic level				
Expenditure: ≥ than GH¢10/≤ GH¢10	49 (43.4)	40 (35.4)	0.72 (0.42-1.2)	0.28
Health				
BCG Scar: Yes/No	95 (84.1)	86 (76.1)	0.60 (0.31-1.2)	0.18
Family history of TB: Yes/No	4 (3.5)	9 (8.0)	2.4(0.71-7.9)	0.25
History of TB: Yes/No	2 (1.8)	6 (5.3)	3.1 (0.62-15.8)	0.28
History of blood in urine: Yes/No	18 (15.9)	24 (21.2)	1.4 (0.72-2.8)	0.39

*Significant association between the variable and buruli ulcer.

4.2.1 Demographic factors

An association between the ethnic group of the parents of the case patents and the community controls was assessed and found out that BU was less common in the Akan ethnic group compared to the other ethnic group. The Akan maternal and paternal ethnic group had univariate (OR = 0.54, 95% CI = 0.32- 0.92) and (OR = 0.56, 95% CI = 0.33- 0.95) respectively. Buruli ulcer was less common in individuals who had had higher education thus secondary education

and opposed to primary or no education ($p < 0.001$). Educational trend analysis shows increasing odds for BU with lower educational status.

4.2.2 Economic factors

A disease which was prevalent in poor communities was thought to be associated with the economic status of the inhabitants. Irrespective of the amount of money spent per months, there was no difference in the frequency of BU among the subjects who spend more than Gh¢ 10.00 per month or less (OR = 0.72, 95% CI = 0.42 -1.2).

4.2.3 Health related factors

Bacillus Calmette Guėrin (BCG) vaccination was assessed by observing for the presence of a scar on the left shoulder around the deltoid region, as vaccination cards were difficult to assess and in some cases they were missing. Even though BCG scars were more frequently observed in the control group it was not significantly associated with BU (OR= 0.60, 95% CI = 0.31- 1.2, p = 0.18). Some of the subjected reported of past history of tuberculosis but this was not significantly associated with BU (OR=3.1, 95% CI = 0.62 -15.8; p = 0.28) and family history of tuberculosis was also not significantly associated with BU (OR= 2.4, 95% CI = 0.71- 7.9, p = 0.25). Questions on blood in the urine to assess for schistosomiasis within the study areas showed that, there were reported cases of blood in the urine however it was not significantly associated with BU (OR=1.4, 95% CI = 0.72 -2.8, p = 0.39).

Table 8: Univariate analysis of environmental variables for BU in Eastern Region, Ghana

Characteristic	No. (%) of Control Subject (n=113)	No. (%) of Case Subject (n=113)	Univariate OR (95% CI)	P-Value
Environment				
Mud wall: Yes/No	89 (78.8)	68 (60.2)	0.41 (0.23-0.73)	0.004*
Mud floor: Yes/No	52 (46.0)	42 (37.2)	0.69 (0.41-1.18)	0.22
Presence of Cocoa Plantation in immediate neighborhood: Yes/No	52 (46.0)	36 (31.9)	0.55 (0.32-0.94)	0.04*
Presence of Coffee Plantation in immediate neighborhood: Yes/No	3 (2.7)	1 (0.9)	3.1 (0.31-29.8)	0.62†
Presence of bush in immediate neighborhood: Yes/No	84 (74.3)	100 (88.5)	2.7 (1.3-5.4)	0.01*
Presence of wood in immediate neighborhood: Yes/No	92 (81.4)	97 (85.8)	1.4 (0.68-2.8)	0.47
Presence of wetland : Yes/No	37 (32.7)	87 (77.0)	6.9 (3.8-12.4)	<0.001*
Presence of river in the immediate neighborhood: Yes/No	57 (50.4)	81 (71.7)	2.5 (1.4-4.3)	0.002*
Share living space with goat: Yes/No	59 (52.2)	67 (59.3)	1.3 (0.79-2.26)	0.35
Share living space with poultry: Yes/No	70 (61.9)	85(75.2)	1.9(0.6-5.8)	0.04*
Share living space with pigs: Yes/No	5 (4.4)	9 (8.0)	1.9 (0.6-5.8)	0.41
Share living space with cats :Yes/No	35 (31.0)	52 (46.0)	1.9 (1.1-3.3)	0.03*
Share living space with dogs : Yes/No	49 (43.4)	42 (37.2)	0.77 (0.45-1.3)	0.42
No. of people in HH:<8/≥8	45 (39.8)	61 (54.0)	0.75 (0.41-1.4)	0.44
Drinking water : protected/unprotected water	11(9.7)	25(22.1)	2.6(1.2-5.7)	0.02*

*Significant association between the environmental variable and buruli ulcer

4.2.4 Environmental factors

A number of questions on environmental factors were used to assess their association with BU, the case patients reported less frequently of staying in mud wall house than the community control (OR=0.41, 95% CI = 0.23 -0.73, p = 0.004) and around cocoa plantation in their immediate environment (OR=0.55, 95% CI = 0.32 – 0.94, p = 0.04). The case patients were living near wetland (OR=6.9, 95% CI = 3.8 -12.4, p < 0.001), had river in their immediate neighborhood (OR=2.5, 95% CI = 1.4 - 4.3, p = 0.002), had bush in their immediate environment (OR=2.7, 95% CI= 1.3 - 5.4, p = 0.01) share living space with poultry (OR=1.9, 95% CI = 0.6 - 5.8, p = 0.04), share living space with cats (OR=1.9, 95% CI = 1.1 - 3.3; p=0.03) and drinking water (OR=2.6, 95% CI = 1.2 - 5.7, p = 0.02). There were no significant difference between case patients and the community controls with respect to sharing living space with dogs (OR=0.77, 95% CI= 0.45 -1.3, p = 0.42), sharing living space with pigs (OR=1.9, 95% CI = 0.6 -5.8, p = 0.41) number of people in their households (OR=0.75, 95% CI= 0.41-1.4, p = 0.44), presence of wood in the immediate environment (OR=1.4, 95% CI= 0.68-2.8, p = 0.47) and mud floor (OR=0.69, 95% CI = 0.41-1.18, p=0.22).

Table 9: Univariate analysis of Behavioural variables for BU in Eastern Region, Ghana

Characteristic	No. (%) of Control Subject (n=113)	No. (%) of Case Subject (n=113)	Univariate OR (95% CI)	P-Value
Insect Bite/Behavior				
Insect bite in water/mud Yes/No	7(61.9)	96 (85.0)	3.5 (1.8-6.6)	<0.001
Use of bed net :Yes/No	77(68.1)	87 (77.0)	1.6 (0.87-2.8)	0.18
Use of mosquito coils: Yes/No	70(61.9)	71 (62.8)	1.0 (0.6-1.8)	1.0

*Significant association between the behavioural/environmental variable and buruli ulcer

4.2.5 Insect bite/behavior

The case-patients reported frequently of insect bite in water or wading in mud than the community controls (OR=3.5, 95% CI = 1.8 - 6.6, p<0.001). There was no significant association between the use of bed net and buruli ulcer (OR=1.6, 95% CI = 0.87-2.8, p = 0.18). We also did not find any significant association between the use of mosquito coils and buruli ulcer (OR=1.0, 95% CI = 0.6 -1.8, p = 1.0).

Table 10: Univariate analysis of Water contact activities variables for BU in Eastern Region, Ghana

Characteristic	No. (%) of Control Subject (n=113)	No. (%) of Case Subject (n=113)	Univariate OR (95% CI)	P-Value
Water contact activities				
Wading on the Densu river : Yes/No	34 (30.1)	68 (60.2)	3.5 (2.0-6.1)	<0.001*
Wading in river or stream: Yes/No	36 (31.9)	46 (40.7)	1.5 (0.85-2.5)	0.2
Washing of clothes: Yes/No	107 (94.7)	103 (91.2)	0.5 (0.2-1.6)	0.44
Fetching of water: Yes/No	70 (61.9)	74 (65.5)	1.2 (0.68-2.0)	0.68
Farming : Yes /NO	93 (82.3)	70 (61.9)	0.35 (0.19-0.65)	0.001*
Farming with long sleeves : Yes/No	89 (78.8)	47 (41.6)	0.19 (0.11-0.35)	< 0.001*
Farming with short sleeves : Yes/No	30 (26.5)	66 (58.4)	3.9 (2.2-6.8)	<0.001*
Wearing of long pants to farm: Yes/No	91 (80.5)	60 (53.1)	0.27 (0.15-0.50)	<0.001*
Wearing of short pants to farm: Yes/No	25 (22.1)	54 (47.8)	3.2 (1.8-5.7)	<0.001*
Do you fish: Yes/No	13 (11.5)	16 (14.2)	1.3 (0.58-2.8)	0.7
Fishing in Densu river: Yes/No	8 (7.1)	11 (9.7)	1.4 (0.55-3.7)	0.63
Fish with long sleeves: Yes/No	5 (4.4)	6 (5.3)	1.2 (0.36-4.1)	1.0
Fish with short sleeves: Yes/No	10 (8.8)	14 (12.4)	1.5 (0.62-3.4	0.52
Fish with long pants: Yes/No	8 (7.1)	8 (7.1)	1.0 (0.36-2.8)	1.0

*Significant association between the water contact activities variable and buruli ulcer

4.2.6 Water contact activities

Wading in the Densu river was more frequent among the case patients than the community controls and was significantly associated with BU (OR=3.5, 95% CI = 2.0 - 6.1, p < 0.001). Current farming status was assessed even though all the respondents have had some experience with farming most of the adults were now involved in sand weaning which has to do with similar

activities in farming. There was significant association between current farming activity and BU (OR=0.35, 95% CI = 0.19 - 0.65, p < 0.001) even though current farming was less common among case-patients than community controls.

Univariate odds ratios of farming with long sleeves (OR=0.19, 95% CI = 0.11 - 0.35, p < 0.001), long pants (OR=0.27, 95% CI = 0.15 - 0.5, p < 0.001), short sleeves (OR=3.9, 95% CI = 2.2 - 6.8, p < 0.001) and short pants (OR=3.2, 95% CI = 1.8 - 5.7, p < 0.001) among the case-patients and community controls were significantly associated with BU. Other water related activities were not significantly related to BU. Even though wading in other rivers or streams, fetching of water and fishing in Densu river were more frequent among the case-patients than the community controls, they were not significantly associated with BU. Irrespective of the type of clothes the subjects wear, they were not associated with BU during fishing. There was no difference between case-patents and community controls with respect to washing of clothes.

Table 11: Univariate analysis of Hygiene practices variables for BU in Eastern Region, Ghana

Characteristic	No. (%) of Control Subject (n=113)	No. (%) of Case Subject (n=113)	Univariate OR (95% CI)	P-Value
Immediate treatment for past injuries				
Use of soap and water: Yes/No	50 (44.2)	21 (18.6)	0.29 (0.16-0.53)	<0.001*
Rubbing the area with alcohol after bite: Yes/No	49 (43.4)	9 (8.0)	0.11 (0.05-0.25)	<0.001*
Use of leaves on injury site : Yes/No	45 (39.8)	74 (65.5)	2.9 (1.7-4.9)	<0.001*
Use of adhesive bandage: Yes/No	14 (12.4)	32 (28.3)	2.8 (1.4-5.6)	0.005*
Bath				
Bath for hygiene: Yes/No	113 (100)	112 (99.1)		1.0
Bath for hygiene , but not in Densu: Yes/No	103 (91.2)	97 (85.8)	0.59 (0.26-1.4)	0.3
Bath in Densu for hygiene : Yes/No	19 (16.8)	25 (22.1)	1.4 (0.7-2.7)	0.4
Bath for hygiene, not in open borehole: Yes/No	103 (91.2)	102 (90.3)	0.9 (0.37-2.2)	1.0
Bath for hygiene in open borehole: Yes/No	7 (6.2)	21 (18.6)	3.5(1.4-8.5)	0.008*
Swim/dive/playing in water: Yes/No	20(17.7)	32(28.3)	1.8 (0.98-3.5)	0.08
Swimming: Yes/No	28 (24.8)	34 (30.1)	1.3 (0.73-2.3)	0.46
Swimming but not in Densu: Yes/No	14 (12.4)	20 (17.7)	1.5 (0.73-3.2)	0.35
Swimming in Densu: Yes/No	16 (14.2)	24 (21.2)	1.6 (0.82-3.3)	0.22

*Significant association between the variable and buruli ulcer.

4.2.7 Treatment when hurt

In order to assess any success with the type of treatment used when the subjects get hurt, the study took into consideration the use of soap and water (OR=0.29, 95% CI= 0.16 - 0.53, p < 0.001), rubbing the area with alcohol when hurt or after a bite (OR=0.11, 95% CI = 0.05 - 0.25, p < 0.001), use of leaves on injury site (OR=2.9, 95% CI= 1.7 - 4.9, p < 0.001) and use of adhesive

bandage (OR=2.8, 95% CI= 1.4 -5.6, p = 0.005) were all associated with buruli ulcer. Whereas case-patients more frequently used leaves and adhesive bandage when hurt than the community controls, the community control reported of frequent use of soap and water and rubbing the area with alcohol when hurt than case-patients.

4.2.8 Bath

No difference was observed between the frequency of bathing for hygiene among case-patients and community controls. With the exception of bathing for hygiene in an open borehole thus using borehole water which was more frequent among case patients than community controls was significantly associated with BU (OR=3.5, 95% CI = 1.4 - 8.5, p = 0.008). Bathing for hygiene in Densu river was more common with the case-patients but this was not significantly associated with BU. Swimming/diving/playing in water in either in Densu river or not were more frequently observed in the case-patients than the community controls were not significantly associated with BU.

4.3 Main Findings

All the variables assessed in the univariate analysis that attained p-value <0.1 were retained for the multivariate conditional logistic regression and used the backwards elimination procedure.

Table 12: Multivariate backward elimination model of logistic regression for risk factors for Buruli ulcer disease in Eastern Region, Ghana

CHARACTERISTIC	Multivariate OR (95% Cl)	P- VALUE
Presence of wetland	3.9 (1.9-8.2)	<0.001*
Insect bite in water/mud	5.7 (2.5- 13.1)	<0.001*
Rubbing the area with alcohol after bite	0.21 (0.008-0.57)	0.002*
Use of adhesive bandage	2.7 (1.1-6.8)	0.035*
Washing in Densu river	2.3 (1.1-4.96)	0.028*
Farming clothes with long sleeves	0.29 (0.14-0.62)	0.001*
House wall built with mud	2.6 (1.1-5.9)	0.022*
Bath for hygiene in open borehole	0.29 (0.86-0.90)	0.033*

*Statistically significant

Bathing for hygiene in open borehole (0.29; 95% CI = 0.86 – 0.90), rubbing the area with alcohol after bite (0.21; 95% CI = 0.008 – 0.57) and farming with long sleeve clothes (0.29; 95% CI 0.14 – 0.62) were found to be protective factors for BU in the final multivariate model. The Odds ratio associated with "insect bite in water/mud" was very high (OR= 5.7, 95% CI = 2.5 – 13.1). Presence of wetland (OR=3.9, 95% CI = 1.9 – 8.2), use of adhesive bandage (OR=2.7, 95% CI = 1.1 – 6.8), wading in Densu river (OR= 2.3, 95% CI = 1.1 – 4.96) and house wall built with mud (OR= 2.6, 95% CI = 1.1 – 5.9) were risk factors associated with BU.

CHAPTER FIVE - DISCUSSIONS

This is the single study that has mapped out all active cases of Buruli ulcer in communities from the source of the Densu river along its entire course till it ends in the Weija Dam over a period of 18 months. Though not the first active case search for Buruli ulcer in Ghana (Amofah et al., 2002), the unique nature of the planning, involvement of all stakeholders and linking the patients identified into care makes this study unique in the country. The other important feature of the study is the use of two approaches to arrive at a conclusion which will inch us towards understanding the spatial distribution and the prevailing risk factors in the study area. Again, the first part of the work was a descriptive study which helped us in generating the hypothesis we tested with the case-control (analytical) part of the study. The entire study looked at the distribution of the Buruli ulcer cases within the communities along the Densu river and the characteristics of the individuals who develop the disease.

The first hypothesis "There is no difference in the distribution of Buruli ulcer in the communities along the Densu river" was clearly refuted with the mapping of the cases shown by figures 10, 11 and 12. Other studies have also shown varying distribution of buruli ulcer(Duker, 2005). A similar approach has been used by other researchers to show where interventions are needed most (Saxena et al., 2010; Chang et al., 2009; Blanton et al., 2006 Corresponding et al., 2005). In Koraput district in Orissa- India, a GIS based study was carried out for identification of risk factors based on ecological parameters for decision support in formulation of appropriate control strategies for malaria (Daash et al., 2009). Srivastava et al. (2009) used the same approach to identify malaria hot spots for focused intervention in tribal state of India. The study

concluded that GIS mapping would make it easy to update information, identify hot spots at community levels within a district and this information can be graphically presented to policy makers to formulate focused and cost effective malaria control strategy (Srivastava et al., 2009).

During the community active case search it was observed that from the origin of the Densu river, the river was very clear and could observe fishes swimming. Interestingly, in that entire area, no active case of Buruli ulcer was identified. The river served as the source of drinking water for the communities in that area. Though, the water was not tested for its quality, the Chief of the community said scientific work had been done and they were informed that it was safe for drinking. Active cases were found in the regions where the turbidity of the water had changed along the course of the river. These were areas where soil degradation has occurred, sand winning activities were ongoing, most of the trees in the area had been were cut down and the river was not clear. Pictures of the differences in the turbidity of the river are attached in appendix. Previous studies had found an association between aquatic ecosystem and Buruli ulcer (Debacker et al., 2006; Merritt et al., 2005; Marsollier et al., 2003; Portaels et al., 1999) but none of those studies made reference to any specific characteristics of the water associated with Buruli ulcer. Perhaps, the turbidity of the water helps in the growth of *Mycobacterium ulcerans* and that explains why individuals from this point of the river were found to have the active disease. Since the turbidity of water bodies are associated with increased sedimentation and euthrophication which are known to have low dissolved oxygen concentration that may enhance the growth of *M. ulcerans* (Merritt et al., 2010; Palomino and Portaels, 1998).

In Figure 11, a buffer of 500 meters and 1000meters around the location of the cases was created. Most of the cases were in close proximity with water bodies as was demonstrated by Aiga et al. (2004). However, a large scale landscape-based model for predicting *Mycobacterium ulcerans* infection (Buruli ulcer Disease) presence in Benin found no association between percent of water land cover surrounding villages and the distance to the nearest river were not associated with increased probability of Buruli ulcer presence at the village level (Wagner et al., 2008). The challenge with such a large scale study is its inability to have fine resolutions and therefore the study did not take into account water body types e.g. pond, wetland but only major rivers. In this study all water bodies were considered.

This study demonstrated clustering of Buruli ulcer cases in Akuapem South District with Z-score > 2.58 which shows statistically significant hot spot for Buruli ulcer. Of the four districts where the study was performed the hottest spot was Akuapem South, followed by Ga west then Suhum Kraboa Coaltar and the least was Ga South. The prevalence of Buruli ulcer for each district and risk level is on figure 13. Though Ga West has been the district with the largest burden of Buruli ulcer over the years, this study has shown that the various interventions put in place in that district have been effective. This was especially evident in one of the reported highly endemic communities, Kojo Ashong, which had the lowest number of active Buruli ulcer cases. On the other hand areas like Akuapem South and Suhum Kraboa Koaltar which were not known to be endemic with Buruli ulcer are now becoming the hot spots requiring urgent attention. This brings to light how GIS can help policy makers pictorially identify problem areas in order to channel resources more effectively and use it efficiently in disease control and prevention.

The second hypothesis tested was "There is no difference in the characteristics of people who contract Buruli ulcer and those who do not". For this a case-control study with community based controls was performed. The study design could not investigate the effect of age and sex as risk factors for Buruli ulcer since the cases and the controls were matched by age and sex. Only (28.3%) 32 out of the 113 case patients were less than 15 years. Though most studies reported the disease in children less than 15 years (Pouillot et al., 2007; Phanzu et al., 2006; Wansbrough-Jones and Phillips, 2006; Aiga et al., 2004; Debacker et al., 2004; Asiedu and Etuaful, 1998), the finding here was similar to what was reported in Togo and Australia (Johnson, Azuolas, et al., 2007; Quek et al., 2007 James et al., 2003; Veitch et al., 1997). Most of the case-patients identified in this study were more than fifteen years because they have been living with the disease for many years. Often they treat the ulcers with herbal medications and bandage them as they go about their regular activities. However, a sub-analysis of individuals who contracted the disease for less than ten years indicate that 50% (32/62) of the cases were less than 15 years which was similar to finding in most studies (Pouillot et al., 2007a; Phanzu et al., 2006; Wansbrough-Jones and Phillips, 2006; Aiga et al., 2004b; Debacker et al., 2004a; Asiedu and Etuaful, 1998). Eighty four percent (95/113) of the cases presented with ulcers which implies that most of the cases present late or were diagnosed late probably due to other factors identified by Grietens et al. (2008) such as transportation costs, feeding costs and productivity loss (Ackumey et al., 2011; Peeters Grietens et al., 2008).

It was found that 67.9% (76/113) of the case patients had the lesion on their legs (Pouillot et al., 2007; Noeske et al., 2004; Marston et al., 1995; van der Werf et al., 1989; The, 1971) but no

preference to either the right or the left side of the body. However, Van der Werf et al. (1989), reported that the left leg was more frequently affected than the right, a finding which other studies did not confirm(Hospers et al., 2005; Noeske et al., 2004)

The association between the ethnic group of the parents of the cases and the community controls was assessed and this showed that BU was less common in the Akan ethnic group compared to the other ethnic groups. This was done to find out if any ethnic group had any predisposition for Buruli ulcer or genetic link to Buruli ulcer. The univariate (OR= 0.54, 95% CI = 0.32- 0.92) for maternal Akan ethnic group and (OR = 0.56, 95% CI = 0.33- 0.95) for paternal Akan group were found to be protective against Buruli ulcer but were all not significant in the multivariate analysis. This finding could probable be due to the activities they engage in. Most of the Akan's in the communities do petty trading rather than sand winning or farming. No genetic relationship was studied in this research though Sopoh et al. (2010) in their study in Benin concluded that a combination of genetic factors and behavioural factors may increase the susceptibility for developing Buruli ulcer(Sopoh et al., 2010). This was the first study to show the existence of family associations with Buruli ulcer but previous studies did not find any statistically significant association (Nackers et al., 2007 Raghunathan et al., 2005; Marston et al., 1995).

Analysis for educational status and Buruli ulcer was only done for individuals above fourteen years since children were not expected to have attained higher level of education. A low level of education was associated with Buruli ulcer in the univariate analysis however it was not significant finding in the multivariate analysis. Though Pouillot et al. (2007) in their large scale

case control study in Cameroon found low level of education as a significant risk factor for Buruli ulcer, this effect was observed only from the teenagers and adults records (Pouillot et al., 2007). As expected with higher level of education, individuals become health conscious, able to take good care of themselves and take health education issues more seriously. However, individuals with low level or no education may not be able to read most of the educational materials available and may not become health conscious as educated individuals.

The main socio-economic factor assessed in this study was the amount of money spent per month. This form of assessment has a lot of challenges since it assumes individuals economic prowess was linked to income generation and subsequent expenditure. It did not factor in individuals who may not spend money but rather receive income from farm proceeds and feed on their farm products. With a disease which was prevalent in poor communities, it was thought to be associated with the economic status of the inhabitants. Socioeconomic status in a rural African setting may be related to land ownership, household size, livestock ownership and duration of residence but such details were not assessed in this study. However it was shown that the amount of money spent per month as a measure of socio-economic status, had no association with Buruli ulcer, which was similar to other studies by Pouillot et al. (2007) and Raghunathan et al. (2005). Asiedu et al. (1998) in their study were able to link Buruli ulcer with low socio-economic status, poverty and living in remote areas (Asiedu and Etuaful, 1998).

The protective role of Bacillus Calmette Guėrin (BCG) vaccination was assessed in this study by observing for the presence of a scar on the left shoulder around the deltoid region or its

documentation in the vaccination cards. Though protective with (OR=0.60, 95% CI = 0.31-1.2, p = 0.18), it was not significantly associated with Buruli ulcer, similar to previous reports (Pouillot et al., 2007; Quek et al., 2007; Debacker et al., 2006; Nackers et al., 2006; Raghunathan et al., 2005 Amofah et al., 1993). The strains of *M. ulcerans* and host immune response were not studied here but Converse et al. (2011) in their BCG-mediated protection against *M. ulcerans* infection concluded that vaccination with BCG may protect some hosts more effectively than others(Converse et al., 2011). In addition, the protection may depend on the strain of *M. ulcerans* prevalent in a given community but our study did not assess that. Furthermore, a sub-analysis of the of cases that had developed bone infection as a result of the Buruli ulcer was not performed because of there was only one case that the disease had involved the bone hence it was not possible to determined if there is any conferred protection by BCG vaccination in that sub group. However, Portaels and colleagues in their *Mycobacterium bovis* BCG vaccination as prophylaxis against *Mycobacterium ulcerans* osteomyelitis in Buruli ulcer disease study reported some protection against osteomyelitis (Portaels et al., 2004, 2002).

This study showed no significant association of past history of tuberculosis and Buruli ulceran infection as reported by Pouillot et al. (2007). It however confirmed what Pouillot et al. (2007) found about the fact that there was no significant association between presence of blood in the urine and Buruli ulcer.

In assessing environmental factors associated with Buruli ulcer, it was observed that staying in mud house offered protection compared to those who were staying in houses made of wood etc.

However, this finding was not significant in the multivariate analysis. During the study it was observed that most of the individuals staying in mud houses had their kitchens attached to the main building. Probably the heat generated from the cooking may not allow any viable *M. ulcerans* to thrive. This finding about staying in a mud wall house was similar to what was reported by Pouillot et al. (2007).

Having a cocoa plantation in the immediate environment was related to reduced risk of Buruli ulcer infection. This was contrary to what Pouillot et al. (2007) found. In my interaction with the respondents, it was clear that government supports spraying of cocoa farms and perhaps the insecticides that are sprayed create an unfavourable environment for *M. ulcerans* to thrive. Though increased level of risk for Buruli ulcer was identified for presence of wood in the immediate neighborhood, it was not statistically significant. Similarly, Pouillot et al.(2007) found presence of wood in the immediate environment as a risk factor for Buruli ulcer in the univariate analysis but not statistically significant in the multivariate analysis. The presence of bushes around the immediate environment was found to have a relationship with development of Buruli ulcer in the univariate analysis of this study but no significant relationship in the multivariate analysis which confirmed earlier studies (Pouillot et al., 2007; Raghunathan et al., 2005).

The study confirmed that individuals living near wetland have increased risk of Buruli ulcer as reported in earlier studies (Wagner et al., 2008; Walsh et al., 2008; Debacker et al., 2006; Duker et al., 2006 Noeske et al., 2004; Marston et al., 1995)

Pouillot et al. (2007) reported of increased risk of Buruli ulcer when individuals share living space with some livestocks in their univariate analysis but not significant in their multivariate analysis. Findings from this study revealed a similar trend, the results showed an increased risk in the univariate analysis for sharing living space with poultry and cats but not in the multivariate analysis. However, no significant relationship was found between developing Buruli ulcer and sharing living space with dogs, goat and pigs. This study did not compare risk level with respect to sharing indoor living space with livestock with handling or owning livestock. In a reported study on risk level comparison revealed some form of protection when sharing indoor living space with livestock, compared with handling or owning livestock.

Previous studies on the effect of protected water like water sources from wells and pipe borne water on Buruli ulcer found an association (Nackers et al., 2007; Debacker et al., 2006; Barker and Carswell, 1973) but in this multivariate analysis there was no association similar to other studies (Pouillot et al., 2007; Raghunathan et al., 2005 Aiga et al., 2004; Amofah et al., 1993a). However the univariate analysis revealed that individuals using protected water were rather at a higher risk, which was quite surprising. Working visit to the water treatment centre which supplied pipe borne water to less that 50% of the population revealed that the supply of treated

water supply was erratic and not reliable. Probably these individuals though were supposed to use pipe borne water might have used other sources of water. The study assessment solely looked at the primary source of drinking water and did not consider details on their alternate source. Furthermore, the study cannot ascertain the level of protection of these sources of water since the study did not carry out any biochemical studies on the various sources of water classified under protected and unprotected. In a sub-analysis for only individuals who used pipe borne water, there was no association with its use and Burui ulcer contrary to other studies reporting decreased risk (Wagner et al., 2008; Debacker et al., 2006)

The case-patients reported frequently of insect bite in water or wading in mud than the community controls which was statistically significant as reported by other studies (Pouillot et al., 2007; Quek et al., 2007). Like in all case control studies cases were more likely to recall any incidence of insect bite either by mosquito or insect while wading in water. In assessing this part in our study, neither prompting nor follow –up questions were asked.

There was no significant association between the use of bed net and Buruli ulcer which confirmed Raghunathan's finding but was contrary to other studies (Nackers et al., 2007; Pouillot et al., 2007; Quek et al., 2007). As part of the national malaria control strategies, free insecticide impregnated nets are distributed throughout the country however some individuals do not sleep under the net and thus do not enjoy or offer protection against mosquito bites. This explains the high proportion of the bed net used. The study failed to ascertain the type of net that the individuals were using since they are other bed nets which are not insecticide treated, these nets cannot offer protection the same as insecticide treated nets have been proven to offer.

There was no evidence of association between the use of mosquito coils and Buruli ulcer which confirmed what had been reported earlier by other researchers (Pouillot et al., 2007; Raghunathan et al., 2005).

From the analysis on the type of treatment undertaken when hurt, it was clear that management of injury plays a significant role in developing Buruli ulcer. One of the current hypothesis is that the organism enters into the body through a broken skin, therefore an appropriate initial treatment when injured can offer protection against development of Buruli ulcer. In this study individuals who wash the injured area with soap and water when hurt immediately were protected from Buruli ulcer in the univariate analysis but not in the multivariate analysis. This protection could be linked to the fact that washing the area with soap and water may immediately wash away the *M. ulcerans* organism that has possibly been introduced into the injured site. This finding was contrary to what was reported by Pouillot et al. (2007) in their study.

Similarly rubbing the area with alcohol when hurt or after a bite significantly protected them from Buruli ulcer. In order to be sure if indeed it was alcohol that they used to clean the wound when hurt, we asked for exhibits and all those who were using alcohol produced the locally prepared alcohol called "Akpeteshie". The plausible explanation could be the percentage of alcohol used will kill most bacteria that may have been introduced into the injured site and hence prevent them from causing disease.

Locally, the use of certain leaves ("Acheampong") known in the local area as one of the local herbs for treatment of acute injuries is common. The univariate analysis shows that, this form of treatment rather increases the risk of Buruli ulcer. Typically, the leaves were ground and the paste used to dress the injured site or the fluid from the paste was squeezed and used to clean the injured site. Since we cannot ascertain whether the leaves are washed before grinding and not sure about the active ingredients in the leaves, it becomes difficult to determine if it has any effect on *M.ulcerans* when introduced into the site of injury. In assessing the use of adhesive bandage when injured, it was observed that adhesive bandage use when an individual is hurt increases the odds of developing Buruli ulcer. Most of the adhesive bandages being used by the respondents were very old and dirty. Such old and dirty adhesive bandages do not have any antiseptic agent in them and when used cannot protect against Buruli ulcer. Also because some of the case patients were living with the disease for many years, exiting cases were more likely to use adhesive bandage to dress their wounds.

Wading in the Densu river was more frequent among the case patients than the community controls and was significantly associated with Buruli ulcer in the univariate analysis similar to other studies (Pouillot et al., 2007; Raghunathan et al., 2005).

Swimming was not found to be associated with Buruli ulcer in this study which confirms earlier study by Marston et al. (1995). In 2004, Aiga and colleagues found swimming to be a risk in a case control study carried out in Ghana.

I assessed fishing as a possible risk factor for Buruli ulcer and found out that it was not a risk factor in this study contrary to other studies (Pouillot et al., 2007b; Raghunathan et al., 2005; Marston et al., 1995). The type of fishing undertaken in these areas has little or no contact with water. They either use hook or small nets that were placed at the bank of the river. However, the study assessed whether the type of clothes that the subjects wear (long pants, short pants, long sleeves and short sleeves) had any relationship with Buruli ulcer during fishing. The negative finding in the study was similarly reported by other studies (Pouillot et al., 2007; Raghunathan et al., 2005; Marston et al., 1995).

The study looked at the current farming status of adult respondents and noticed that almost all of them have had some experience with farming but some of them were involved in sand winning which had to do with similar activities as farming. There was a significant association between current farming activity and BU. Similar to what has been reported in other studies, individuals who farm with long sleeves and long pants were protected against Buruli ulcer (Pouillot et al., 2007; Raghunathan et al., 2005; Marston et al., 1995). In addition, wearing of short sleeves and short pants were significantly associated with increased risk of Buruli ulcer.

Fetching of water in the Densu river was more frequent among case-patients than the community controls but there was no significant association with Buruli ulcer. The frequency of Densu river contact and occurrence of Buruli ulcer was not assessed in this study since it was difficult to

assess objectively but Sopoh et al. (2010) reported that daily contact with a natural water source was a risk factor for Buruli ulcer.

In terms of personal hygiene there was no difference between the frequency of bathing for hygiene among case-patients and community controls. It is known that daily washing with soap reduces the risk of Buruli ulcer as bacterial contamination of skin surfaces may facilitate *M. ulcerans* infection. The use of soap and water for washing was found to be associated with a decreased risk of *M. ulcerans* infection (Nackers et al., 2007; Raghunathan et al., 2005). Though this study did not assess the various types of soaps used, Raghunathan et al. (2005) reported that the regular use of toilet soap (i.e., wrapped bar soap) while bathing reduces the risk of Buruli ulcer (Nackers et al., 2007; Raghunathan et al., 2005). However, no protective association was observed among those who used less costly mass-produced Key soap or homemade amonkye soap (Raghunathan et al., 2005). Further analysis of the source of the water used for bathing for hygiene revealed that use of water from an open borehole increases the risk of Buruli ulcer than that from the Densu river but the increased risk with the use of the Densu river was not statistically significant.

There was no difference between case-patents and community controls with respect to washing of clothes in this study however washing of clothes was found to protect against *M. ulcerans* infection (Marston et al., 1995; Pouillot et al., 2007a).

CHAPTER SIX - CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The goal of the study was to determine the risk profile of Buruli ulcer and show the spatial distribution of the disease in the communities along the Densu river. The GPS maps generated clearly show where the cases are coming from, the clustering nature of the disease and the risk level per population of the districts. In addition, this study has clearly shown that Buruli ulcers cases were not present upstream of the Densu river but rather were only seen from the point where the river was turbidity and contaminated.

Findings in this study are consistent with the existing hypotheses of *M. ulcerans* transmission i.e. insect bites and /or contamination following or accompanying trauma. The study identified that the various forms of treatment carried out after trauma strongly support the hypothesis of contamination of trauma site. This study confirms that the presence of wetland, insect bite in water/mud, use of adhesive bandage when hurt, washing in Densu river and wall of house built with mud are risk factors for Buruli ulcer whereas rubbing the injured area with alcohol, farming with long sleeves and bath for hygiene with open borehole water were identified as protective factors.

Most of the cases were found to have active ulcer supporting the fact that there is still late case detection which may be due to weakness in the existing surveillance system or inadequate health education with emphasis on early case detection, effectiveness of treatment with antibiotics to

reduce sequalae and the identified risk factors of Buruli ulcer. Public health messages about these risk factors can be added to the existing knowledge in the population and will help guide public health strategy to reduce the burden of Buruli ulcer.

6.2 Recommendations

In view of the findings from the study I recommend the following:

1. National Buruli Ulcer Control Programme in collaboration with the Buruli Ulcer endemic District Assemblies should support health workers and the Community Based Volunteers to undertake regular active case search

2. National Buruli Ulcer Control Programme and the various District Health Management Teams should embark on regular training of Community Based Volunteers on the Buruli ulcer and risk factors

3. National Buruli Ulcer Control Programme should incorporate these findings into educational materials on prevention of BU in endemic communities

4. District assemblies in the endemic communities should ensure that the Densu river is not polluted by individuals living close to it by providing residents with water and sanitation facilities

5. Research institutions undertaking research in Buruli ulcer should also focus on water and the genetic aspects of Buruli ulcer

REFERENCES

Ackumey, M.M., Gyapong, M., Pappoe, M., Kwakye-Maclean, C., Weiss, M.G., 2012. Illness meanings and experiences for pre-ulcer and ulcer conditions of Buruli ulcer in the Ga-West and Ga-South Municipalities of Ghana. BMC Public Health 12, 264.

Ackumey, M.M., Kwakye-Maclean, C., Ampadu, E.O., de Savigny, D., Weiss, M.G., 2011. Health services for Buruli ulcer control: lessons from a field study in Ghana. PLoS Neglected Tropical Diseases 5, e1187.

Aiga, H., Amano, T., Cairncross, S., Adomako, J., Domako, J.A., Nanas, O.-K., Coleman, S., 2004. Assessing water-related risk factors for Buruli ulcer: a case-control study in Ghana. Am. J. Trop. Med. Hyg 71, 387–392.

Amofah, G., Bonsu, F., Tetteh, C., Okrah, J., Asamoa, K., Asiedu, K., Addy, J., 2002. Buruli ulcer in Ghana: results of a national case search. Emerging Infectious Diseases 8, 167.

Amofah, G.K., Sagoe-Moses, C., Adjei-Acquah, C., Frimpong, E.H., 1993. Epidemiology of Buruli ulcer in Amansie West district, Ghana. Trans. R. Soc. Trop. Med. Hyg. 87, 644–645.

Annick Chauty, Marie-Françoise Ardant, Ambroise Adeye, Hélène Euverte, Augustin Guédénon, Christian Johnson, Jacques Aubry, Eric Nuermberger, and Jacques Grosset, 2007. Promising Clinical Efficacy of Streptomycin-Rifampin Combination for Treatment of Buruli Ulcer (*Mycobacterium ulcerans* Disease) Antimicrobial Agents and Chemotherapy, November 2007, p. 4029-4035, Vol. 51,

Asiedu, K., Etuaful, S., 1998. Socioeconomic implications of Buruli ulcer in Ghana: a three-year review. The American journal of tropical medicine and hygiene 59, 1015–1022.

Aujoulat I, Johnson C, Afram-Gyening C. Psychosocial aspects of health seeking behaviors of patients with Buruli ulcer in Southern Benin. *Tropical Medicine and International Health* 2003;8:750-759.

Barker, D.J., Ninkibigaya, V., 1972. Buruli disease and patients' activities. East African medical journal 49, 260.

Barker, D.J.P., Carswell, J.W., 1973. Mycobacterium ulcerans infection among tsetse control workers in Uganda. International journal of epidemiology 2, 161–165.

Barnett, H.C., 1960. The incrimination of arthropods as vectors of disease, in: Proceedings of the 11th Congress on Entomology, Vienna, Austria. pp. 341–5.

Barogui, Y., Johnson, R. C., van der Werf, T. S., Sopoh, G., Dossou, A., Dijkstra, P. U., Stienstra, Y. (2009). Functional Limitations after Surgical or Antibiotic Treatment for Buruli Ulcer in Benin. *Am J Trop Med Hyg* 81: 82-87

Bayley, A.C., 1971. Buruli ulcer in Ghana. British Medical Journal 2, 401–402.

Benbow, M.E., Williamson, H., Kimbirauskas, R., McIntosh, M.D., Kolar, R., Quaye, C., Akpabey, F., Boakye, D., Small, P., Merritt, R.W., 2008. Aquatic Invertebrates as Unlikely Vectors of Buruli Ulcer Disease. Emerg Infect Dis 14, 1247–1254.

Blanton, J.D., Manangan, A., Manangan, J., Hanlon, C.A., Slate, D., Rupprecht, C.E., 2006. Development of a GIS-based, real-time Internet mapping tool for rabies surveillance. Int J Health Geogr 5, 47.

Ceballos, G., García, A., Ehrlich, P.R., 2010. The Sixth Extinction Crisis Loss of Animal Populations and Species. Journal of Cosmology 8, 1821–1831.

Chang, A.Y., Parrales, M.E., Jimenez, J., Sobieszczyk, M.E., Hammer, S.M., Copenhaver, D.J., Kulkarni, R.P., 2009. Combining Google Earth and GIS mapping technologies in a dengue surveillance system for developing countries. Int J Health Geogr 8, 49.

Chauty A, Ardant MF, Adeve A, Euverte H, Guédénon A, Johnson C, Aubry J, Nuermberger E, Grosset J: Promising clinical efficacy of the combination streptomycin – rifampin for the treatment of Buruli ulcer (Mycobacterium ulcerans disease). Antimicrobial Agents Chemother 2007; 51: 4029–4035.

Clements, A.C.A., Bosqué-Oliva, E., Sacko, M., Landouré, A., Dembélé, R., Traoré, M., Coulibaly, G., Gabrielli, A.F., Fenwick, A., Brooker, S., 2009. A comparative study of the spatial distribution of schistosomiasis in mali in 1984-1989 and 2004-2006. PLoS Negl Trop Dis 3, e431.

Converse, P.J., Almeida, D.V., Nuermberger, E.L., Grosset, J.H., 2011. BCG-mediated protection against Mycobacterium ulcerans infection in the mouse. PLoS Neglected Tropical Diseases 5, e985.

Converse, P.J., Nuermberger, E.L., Almeida, D.V., Grosset, J.H., 2011. Treating Mycobacterium ulcerans disease (Buruli ulcer): from surgery to antibiotics, is the pill mightier than the knife? Future Microbiology 6, 1185–1198.

Corresponding, A.S., Nagpal, B.N., Saxena, R., Dev, V., Subbarao, S.K., 2005. Prediction of Anopheles minimus habitat in India—a tool for malaria management. International Journal of Geographical Information Science 19, 91–98.

Daash, A., Srivastava, A., Nagpal, B.N., Saxena, R., Gupta, S.K., 2009. Geographical information system (GIS) in decision support to control malaria--a case study of Koraput district in Orissa, India. J Vector Borne Dis 46, 72–74.

Debacker, M., Aguiar, J., Steunou, C., Zinsou, C., Meyers, W.M., Scott, J.T., Dramaix, M., Portaels, F., 2004. Mycobacterium ulcerans disease: role of age and gender in incidence and morbidity. Trop. Med. Int. Health 9, 1297–1304.

Debacker, M., Portaels, F., Aguiar, J., Steunou, C., Zinsou, C., Meyers, W., Dramaix, M., 2006. Risk factors for Buruli ulcer, Benin. Emerging Infect. Dis 12, 1325–1331.

Dega H, Bentoucha A, Robert J, Jarlier V & Grosset J (2002) Bactericidal activity of rifampin-amikacin against Mycobacterium ulcerans in mice. Antimicrobial Agents and Chemotherapy 46, 3193–3196.

Demangel, C., Stinear, T.P., Cole, S.T., 2009. Buruli ulcer: reductive evolution enhances pathogenicity of Mycobacterium ulcerans. Nat. Rev. Microbiol. 7, 50–60.

Duker, A.A., 2005. Spatial analysis of factors implicated in Mycobacterium ulcerans infection in Ghana. Citeseer.

Duker, A.A., Portaels, F., Hale, M., 2006. Pathways of Mycobacterium ulcerans infection: a review. Environ Int 32, 567–573.

Durnez, L., Suykerbuyk, P., Nicolas, V., Barrière, P., Verheyen, E., Johnson, C.R., Leirs, H., Portaels, F., 2010. Terrestrial small mammals as reservoirs of Mycobacterium ulcerans in benin. Applied and environmental microbiology 76, 4574–4577.

Eisen, L., Lozano-Fuentes, S., 2009. Use of Mapping and Spatial and Space-Time Modeling Approaches in Operational Control of Aedes aegypti and Dengue. PLoS Negl Trop Dis 3, e411.

Espey, D. K., G. Djomand, I. Diomande, M. Dosso, M. Z. Saki, J. M. Kanga,R. A. Spiegel, B. J. Marston, L. Gorelkin, W. M. Meyers, F. Portaels, M. S.Deming, and C. R. Horsburgh, Jr. 2002. A pilot study of treatment of Buruli ulcer with rifampin and dapsone. Int. J. Infect. Dis. 6:60–65

Etuaful S, Carbonnelle B, Grosset J, Lucas S, Horsfield C, Phillips R, Evans M, Ofori-Adjei D, Klustse E, Owusu-Boateng J, Amedofu GK, Awuah P, Ampadu E, Amofah G, Asiedu K, Wansbrough-Jones M, 2005. Efficacy of the combination rifampin-streptomycin in preventing growth of *Mycobacterium ulcerans* in early lesions of Buruli ulcer in humans. *Antimicrob Agents Chemother 49:* 3182–3186.

Ezzedine, K., Pistone, T., Cottin, J., Marsollier, L., Guir, V., Malvy, D., 2009. Buruli ulcer in long-term traveler to Senegal. Emerging infectious diseases 15, 118.

Farnsworth, M.L., Wolfe, L.L., Hobbs, N.T., Burnham, K.P., Williams, E.S., Theobald, D.M., Conner, M.M., Miller, M.W., 2005. Human land use influences chronic wasting disease prevalence in mule deer. Ecological Applications 15, 119–126.

Flood, P., Street, A., O'Brien, P., Hayman, J., 1994. Mycobacterium ulcerans infection on Phillip Island, Victoria. The Medical journal of Australia 160, 160.

Forman, R.T.T., 1995. Land mosaics: the ecology of landscapes and regions. Cambridge Univ Pr.

Fyfe, J.A.M., Lavender, C.J., Handasyde, K.A., Legione, A.R., O'Brien, C.R., Stinear, T.P., Pidot, S.J., Seemann, T., Benbow, M.E., Wallace, J.R., McCowan, C., Johnson, P.D.R., 2010. A major role for mammals in the ecology of Mycobacterium ulcerans. PLoS Negl Trop Dis 4, e791.

Fyfe, J.A.M., Lavender, C.J., Johnson, P.D.R., Globan, M., Sievers, A., Azuolas, J., Stinear, T.P., 2007. Development and application of two multiplex real-time PCR assays for the detection of Mycobacterium ulcerans in clinical and environmental samples. Applied and environmental microbiology 73, 4733–4740.

George, K.M., Chatterjee, D., Gunawardana, G., Welty, D., Hayman, J., Lee, R., Small, P.L.C., 1999. Mycolactone: a polyketide toxin from Mycobacterium ulcerans required for virulence. Science 283, 854–857.

Grietens, K.P., Boock, A.U., Peeters, H., Hausmann-Muela, S., Toomer, E., Ribera, J.M., 2008. "It is me who endures but my family that suffers": social isolation as a consequence of the household cost burden of Buruli ulcer free of charge hospital treatment. PLoS Negl Trop Dis 2, e321.

HAYMAN, J., 1991. Postulated epidemiology of Mycobacterium ulcerans infection. International journal of epidemiology 20, 1093–1098.

Herbinger, K.-H., Adjei, O., Awua-Boateng, N.-Y., Nienhuis, W.A., Kunaa, L., Siegmund, V., Nitschke, J., Thompson, W., Klutse, E., Agbenorku, P., Schipf, A., Reu, S., Racz, P., Fleischer, B., Beissner, M., Fleischmann, E., Helfrich, K., van der Werf, T.S., Lüscher, T., Bretzel, G., 2009. Comparative Study of the Sensitivity of Different Diagnostic Methods for the Laboratory Diagnosis of Buruli Ulcer Disease. Clinical Infectious Diseases 48, 1055 –1064.

Hill, A.B., 1965. The environment and disease: association or causation? Proceedings of the Royal Society of Medicine 58, 295.

Horsburgh Jr, C.R., Meyers, W.M., 1997. Buruli ulcer. Pathology of emerging infections. ASM Press, Washington, DC 119–126.

Hospers, I.C., Wiersma, I.C., Dijkstra, P.U., Stienstra, Y., Etuaful, S., Ampadu, E.O., Graaf, W.T.A. van der, van der Werf, T.S., 2005. Distribution of Buruli ulcer lesions over body surface area in a large case series in Ghana: uncovering clues for mode of transmission. Transactions of the Royal Society of Tropical Medicine and Hygiene 99, 196–201.

Howick, J., Glasziou, P., Aronson, J.K., 2009. The evolution of evidence hierarchies: what can Bradford Hill's "guidelines for causation"contribute? JRSM 102, 186–194.

Jackson, L.E., Hilborn, E.D., Thomas, J.C., 2006. Towards landscape design guidelines for reducing Lyme disease risk. International journal of epidemiology 35, 315–322.

James, K., Attipou, K.K., James, Y.E., Blakime, M., Tignokpa, N., Abete, B., 2003. [Buruli ulcer in Togo: a hospital study]. Sante 13, 43–47.

John Travis, 1999. Africa's Latest Scourge: *A flesh-devouring bacterium begins to reveal its secrets The Weekly Newsmagazine of Science* Volume 156, Number 3 (July 17, 1999)

Johnson, P., Lavender, C., Azuolas, J., Brown, L., Fyfe, J., 2009. Vector borne diseases, mosquitoes and Buruli ulcer in Victoria, Australia, in: WHO Annual Meeting on Buruli Ulcer. p. 97.

Johnson, P.D., Azuolas, J., Lavender, C.J., Wishart, E., Stinear, T.P., Hayman, J.A., Brown, L., Jenkin, G.A., Fyfe, J.A., others, 2007. Mycobacterium ulcerans in mosquitoes captured during outbreak of Buruli ulcer, southeastern Australia. Emerging infectious diseases 13, 1653.

Johnson, P.D., Veitch, M.G., Leslie, D.E., Flood, P.E., Hayman, J.A., others, 1996. The emergence of Mycobacterium ulcerans infection near Melbourne. The Medical Journal of Australia 164, 76.

Johnson, P.D.R., Hayman, J.A., Quek, T.Y., Fyfe, J.A.M., Jenkin, G.A., Buntine, J.A., Athan, E., Birrell, M., Graham, J., Lavender, C.J., others, 2007. Consensus recommendations for the diagnosis, treatment and control of Mycobacterium ulcerans infection (Bairnsdale or Buruli ulcer) in Victoria, Australia. Medical journal of Australia 186, 64.

Johnson, P.D.R., Stinear, T., Pamela, L.C., Pluschke, G., Merritt, R.W., Portaels, F., Huygen, K., Hayman, J.A., Asiedu, K., 2005. Buruli ulcer (M. ulcerans infection): new insights, new hope for disease control. PLoS medicine 2, e108.

Johnson, R.C., Nackers, F., Glynn, J.R., de Biurrun Bakedano, E., Zinsou, C., Aguiar, J., Tonglet, R., Portaels, F., 2008. Association of HIV infection and Mycobacterium ulcerans disease in Benin. Aids 22, 901.

Johnson, R.C., Sopoh, G.E., Boko, M., Zinsou, C., Gbovi, J., Makoutode, M., Portaels, F., 2005. Distribution de l'infection à Mycobacterium ulcerans (Ulcère de Buruli) dans la commune de Lalo au Bénin. Tropical Medicine & International Health 10, 863–871.

Johansson E, Long NH, Diwan VK, Winkvist A. Gender and tuberculosis control: perspectives on health seeking behaviour among men and women in Vietnam. *Health Policy* 2000;52:33-51

Kibadi, K., Panda, M., Tamfum, J.-J.M., Fraga, A.G., Longatto Filho, A., Anyo, G., Pedrosa, J., Nakazawa, Y., Suykerbuyk, P., Meyers, W.M., Portaels, F., 2008. New foci of Buruli ulcer, Angola and Democratic Republic of Congo. Emerging Infect. Dis 14, 1790–1792.

King, R.S., Baker, M.E., Whigham, D.F., Weller, D.E., Jordan, T.E., Kazyak, P.F., Hurd, M.K., 2005. Spatial considerations for linking watershed land cover to ecological indicators in streams. Ecological Applications 15, 137–153.

Kotlowski, R., Martin, A., Ablordey, A., Chemlal, K., Fonteyne, P.A., Portaels, F., 2004. One-tube cell lysis and DNA extraction procedure for PCR-based detection of Mycobacterium ulcerans in aquatic insects, molluscs and fish. Journal of medical microbiology 53, 927–933.

Lavender, C.J., Stinear, T.P., Johnson, P.D.R., Azuolas, J., Benbow, M.E., Wallace, J.R., Fyfe, J.A.M., 2008. Evaluation of VNTR typing for the identification of Mycobacterium ulcerans in environmental samples from Victoria, Australia. FEMS microbiology letters 287, 250–255.

Liefooghe R, Baliddawa JB, Kipruto EM, Vermeire C, de Munynck AO. From their own perspective. A Kenyan community's perception of tuberculosis. *Tropical Medicine and International Health* 1997;2(8):809–821.

Linderman, M.A., An, L., Bearer, S., He, G., Ouyang, Z., Liu, J., 2006. Interactive effects of natural and human disturbances on vegetation dynamics across landscapes. Ecological Applications 16, 452–463.

Lozano-Fuentes, S., Elizondo-Quiroga, D., Farfan-Ale, J.A., Loroño-Pino, M.A., Garcia-Rejon, J., Gomez-Carro, S., Lira-Zumbardo, V., Najera-Vazquez, R., Fernandez-Salas, I., Calderon-Martinez, J., Dominguez-Galera, M., Mis-Avila, P., Morris, N., Coleman, M., Moore, C.G.,

Beaty, B.J., Eisen, L., 2008. Use of Google Earth to strengthen public health capacity and facilitate management of vector-borne diseases in resource-poor environments. Bull. World Health Organ 86, 718–725.

Marion, E., Eyangoh, S., Yeramian, E., Doannio, J., Landier, J., Aubry, J., Fontanet, A., Rogier, C., Cassisa, V., Cottin, J., others, 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, e731.

Marsollier, L., Aubry, J., Coutanceau, E., André, J.P.S., Small, P.L., Milon, G., Legras, P., Guadagnini, S., Carbonnelle, B., 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, 935–943.

Marsollier, L., Aubry, J., Saint-André, J.P., Robert, R., Legras, P., Manceau, A.L., Bourdon, S., Audrain, C., Carbonnelle, B., 2003. Écologie et mode de transmission de< i> Mycobacterium ulcerans</i>. Pathologie Biologie 51, 490–495.

Marsollier, L., Brodin, P., Jackson, M., Korduláková, J., Tafelmeyer, P., Carbonnelle, E., Aubry, J., Milon, G., Legras, P., Saint André, J.P., others, 2007. Impact of Mycobacterium ulcerans biofilm on transmissibility to ecological niches and Buruli ulcer pathogenesis. PLoS pathogens 3, e62.

Marsollier, L., Robert, R., Aubry, J., Saint André, J.P., Kouakou, H., Legras, P., Manceau, A.L., Mahaza, C., Carbonnelle, B., 2002. Aquatic insects as a vector for Mycobacterium ulcerans. Applied and environmental Microbiology 68, 4623–4628.

Marsollier, L., Stinear, T., Aubry, J., Saint André, J.P., Robert, R., Legras, P., Manceau, A.L., Audrain, C., Bourdon, S., Kouakou, H., others, 2004. Aquatic plants stimulate the growth of and biofilm formation by Mycobacterium ulcerans in axenic culture and harbor these bacteria in the environment. Applied and environmental microbiology 70, 1097–1103.

Marston, B.J., Diallo, M.O., Horsburgh Jr, C.R., Diomande, I., Saki, M.Z., Kanga, J.M., Patrice, G., Lipman, H.B., Ostroff, S.M., Good, R.C., others, 1995. Emergence of Buruli ulcer disease in the Daloa region of Cote d'Ivoire. The American journal of tropical medicine and hygiene 52, 219
.

McGuire, J.M., Bunch, R.L., Anderson, R.C., Boaz, H.E., Flynn, E.H., Powell, H.M., Smith, J.W., 1952. Ilotycin, a new antibiotic]. Schweizerische medizinische Wochenschrift 82, 1064.

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. Frontiers in Ecology and the Environment 3, 323–331.

Merritt, R.W., Craig, D.A., Walker, E.D., Vanderploeg, H.A., Wotton, R.S., 1992. Interfacial feeding behavior and particle flow patterns ofAnopheles quadrimaculatus larvae (Diptera: Culicidae). Journal of insect behavior 5, 741–761.

Merritt, R.W., Dadd, R.H., Walker, E.D., 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annual Review of Entomology 37, 349–374.

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 Negl Trop Dis 4.

Moloney, K.A., Levin, S.A., 1996. The effects of disturbance architecture on landscape-level population dynamics. Ecology 375–394.

Moss, M.P., Schell, M.C., Goins, R.T., 2006. Using GIS in a first national mapping of functional disability among older American Indians and Alaska Natives from the 2000 census. Int J Health Geogr 5, 37.

Murray, E.J., Marais, B.J., Mans, G., Beyers, N., Ayles, H., Godfrey-Faussett, P., Wallman, S., Bond, V., 2009. A multidisciplinary method to map potential tuberculosis transmission “hot spots” in high-burden communities. Int. J. Tuberc. Lung Dis 13, 767–774.

Nackers, F., Dramaix, M., Johnson, R.C., Zinsou, C., Robert, A., BAKEDANO, E.D.E.B., Glynn, J.R., Portaels, F., Tonglet, R., 2006. BCG vaccine effectiveness against Buruli ulcer: a case-control study in Benin. The American journal of tropical medicine and hygiene 75, 768–774.

Nackers, F., Johnson, R.C., Glynn, J.R., Zinsou, C., Tonglet, R., Portaels, F., 2007. Environmental and health-related risk factors for Mycobacterium ulcerans disease (Buruli ulcer) in Benin. Am. J. Trop. Med. Hyg 77, 834–836.

Noeske, J., Kuaban, C., Rondini, S., Sorlin, P., Ciaffi, L., Mbuagbaw, J., Portaels, F., Pluschke, G., 2004. Buruli ulcer disease in Cameroon rediscovered. Am. J. Trop. Med. Hyg. 70, 520–526.

Norman, G.R., Streiner, D.L., 2007. Biostatistics: the bare essentials. Pmph USA Ltd.

Palomino, J.C., Portaels, F., 1998. Effects of decontamination methods and culture conditions on viability of Mycobacterium ulcerans in the BACTEC system. Journal of clinical microbiology 36, 402–408.

Peeters Grietens, K., Um Boock, A., Peeters, H., Hausmann-Muela, S., Toomer, E., Muela Ribera, J., 2008. "It Is Me Who Endures but My Family That Suffers": Social Isolation as a Consequence of the Household Cost Burden of Buruli Ulcer Free of Charge Hospital Treatment. PLoS Negl Trop Dis 2.

Phanzu, D.M., Bafende, E.A., Dunda, B.K., Imposo, D.B., Kibadi, A.K., Nsiangana, S.Z., Singa, J.N., Meyers, W.M., Suykerbuyk, P., Portaels, F., 2006. Mycobacterium ulcerans disease (Buruli ulcer) in a rural hospital in Bas-Congo, Democratic Republic of Congo, 2002-2004. Am. J. Trop. Med. Hyg 75, 311–314.

Plowright, R.K., Sokolow, S.H., Gorman, M.E., Daszak, P., Foley, J.E., 2008. Causal inference in disease ecology: investigating ecological drivers of disease emergence. Frontiers in Ecology and the Environment 6, 420–429.

Portaels, F., Aguiar, J., Debacker, M., Guedenon, A., Steunou, C., Zinsou, C., Meyers, W.M., 2004. Mycobacterium bovis BCG vaccination as prophylaxis against Mycobacterium ulcerans osteomyelitis in Buruli ulcer disease. Infection and immunity 72, 62–65.

Portaels, F., Aguiar, J., Debacker, M., Steunou, C., Zinsou, C., Guedenon, A., Meyers, W.M., 2002. Prophylactic effect of Mycobacterium bovis BCG vaccination against osteomyelitis in children with Mycobacterium ulcerans disease (Buruli ulcer). Clinical and diagnostic laboratory immunology 9, 1389–1391.

Portaels, F., Elsen, P., Guimaraes-Peres, A., Fonteyne, P.A., Meyers, W.M., 1999. Insects in the transmission of Mycobacterium ulcerans infection. Lancet 353, 986.

Portaels, F., Meyers, W.M., Ablordey, A., Castro, A.G., Chemlal, K., De Rijk, P., Elsen, P., Fissette, K., Fraga, A.G., Lee, R., others, 2008. First cultivation and characterization of Mycobacterium ulcerans from the environment. PLoS neglected tropical diseases 2, e178.

Pouillot, R., Matias, G., Wondje, C.M., Portaels, F., Valin, N., Ngos, F., Njikap, A., Marsollier, L., Fontanet, A., Eyangoh, S., 2007. Risk factors for buruli ulcer: a case control study in Cameroon. PLoS Negl Trop Dis 1, e101.

Quek, T.Y.J., Athan, E., Henry, M.J., Pasco, J.A., Redden-Hoare, J., Hughes, A., Johnson, P.D.R., 2007. Risk factors for Mycobacterium ulcerans infection, southeastern Australia. Emerging Infect. Dis 13, 1661–1666.

Raghunathan, P.L., Whitney, E.A.S., Asamoa, K., Stienstra, Y., Taylor, T.H., Amofah, G.K., Ofori-Adjei, D., Dobos, K., Guarner, J., Martin, S., Pathak, S., Klutse, E., Etuaful, S., van der Graaf, W.T.A., van der Werf, T.S., King, C.H., Tappero, J.W., Ashford, D.A., 2005. Risk factors for Buruli ulcer

disease (Mycobacterium ulcerans Infection): results from a case-control study in Ghana. Clin. Infect. Dis 40, 1445–1453.

Renzaho AMN, Woods PV, Ackumey MM, Harvey SK, Kotin J. Community-based study on knowledge, attitude and practice on the mode of transmission, prevention and treatment of the Buruli ulcer in Ga West District, Ghana. *Tropical Medicine and International Health* 2007;12(3):445-458

Revill, W. D., R. H. Morrow, M. C. Pike, and J. Ateng. 1973. A controlledtrial of the treatment of *Mycobacterium ulcerans* infection with clofazimine

Ross, B.C., Johnson, P.D., Oppedisano, F., Marino, L., Sievers, A., Stinear, T., Hayman, J.A., Veitch, M.G., Robins-Browne, R.M., 1997. Detection of Mycobacterium ulcerans in environmental samples during an outbreak of ulcerative disease. Applied and environmental microbiology 63, 4135–4138.

Saxena, R., Nagpal, B.N., Srivastava, A., Gupta, S.K., Dash, A.P., others, 2010. Application of spatial technology in malaria research & control: some new insights. Indian Journal of Medical Research 130, 125.

Semret, M., Koromihis, G., MacLean, J.D., Libman, M., Ward, B.J., 1999. Mycobacterium ulcerans infection (Buruli ulcer): first reported case in a traveler. The American journal of tropical medicine and hygiene 61, 689–693.

Sizaire V, Nackers F, Comte E, Portaels F (2006) *Mycobacterium ulcerans* infection: control, diagnosis, and treatment. Lancet Infect Dis 6: 288–296.

Smith, K.F., Dobson, A.P., McKenzie, F.E., Real, L.A., Smith, D.L., Wilson, M.L., 2005. Ecological theory to enhance infectious disease control and public health policy. Frontiers in Ecology and the Environment 3, 29–37.

Sopoh, G.E., Barogui, Y.T., Johnson, R.C., Dossou, A.D., Makoutodé, M., Anagonou, S.Y., Kestens, L., Portaels, F., 2010b. Family relationship, water contact and occurrence of Buruli ulcer in Benin. PLoS Negl Trop Dis 4, e746.

Sopoh, G.E., Johnson, R.C., Anagonou, S.Y., Barogui, Y.T., Dossou, A.D., Houézo, J.G., Phanzu, D.M., Tente, B.H., Meyers, W.M., Portaels, F., 2011. Buruli ulcer prevalence and altitude, Benin. Emerging Infect. Dis. 17, 153–154.

Sopoh, G.E., Johnson, R.C., Chauty, A., Dossou, A.D., Aguiar, J., Salmon, O., Portaels, F., Asiedu, K., 2007. Buruli ulcer surveillance, Benin, 2003–2005. Emerging infectious diseases 13, 1374.

Srivastava, A., Nagpal, B.N., Joshi, P.L., Paliwal, J.C., Dash, A.P., 2009. Identification of malaria hot spots for focused intervention in tribal state of India: a GIS based approach. Int J Health Geogr 8, 30.

Stienstra, Y., van der Graaf, W.T., te Meerman, G.J., The, T.H., de Leij, L.F., van der Werf, T.S., 2001. Susceptibility to development of Mycobacterium ulcerans disease: review of possible risk factors. Trop. Med. Int. Health 6, 554–562.

Stienstra, Y., van der Graaf, W.T.A., Asamoa, K., van der Werf, T.S., 2002. Beliefs and attitudes toward Buruli ulcer in Ghana. Am. J. Trop. Med. Hyg 67, 207–213.

Stienstra, Y., van der Werf, T.S., van der Graaf, W.T.A., Secor, W.E., Kihlstrom, S.L., Dobos, K.M., Asamoa, K., Quarshi, E., Etuaful, S.N., Klutse, E.Y., King, C.H., 2004. Buruli ulcer and schistosomiasis: no association found. Am. J. Trop. Med. Hyg. 71, 318–321.

Stinear, T., Davies, J.K., Jenkin, G.A., Hayman, J.A., Oppedisano, F., Johnson, P.D.R., 2000. Identification of Mycobacterium ulcerans in the environment from regions in Southeast Australia in which it is endemic with sequence capture-PCR. Applied and environmental microbiology 66, 3206–3213.

Stinear, T.P., Hong, H., Frigui, W., Pryor, M.J., Brosch, R., Garnier, T., Leadlay, P.F., Cole, S.T., 2005. Common evolutionary origin for the unstable virulence plasmid pMUM found in geographically diverse strains of Mycobacterium ulcerans. Journal of bacteriology 187, 1668–1676.

Stinear, T.P., Mve-Obiang, A., Small, P.L.C., Frigui, W., Pryor, M.J., Brosch, R., Jenkin, G.A., Johnson, P.D.R., Davies, J.K., Lee, R.E., others, 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, 1345.

Stinear, T.P., Seemann, T., Pidot, S., Frigui, W., Reysset, G., Garnier, T., Meurice, G., Simon, D., Bouchier, C., Ma, L., others, 2007. Reductive evolution and niche adaptation inferred from the genome of Mycobacterium ulcerans, the causative agent of Buruli ulcer. Genome research 17, 192–200.

Stoffel, V., Barthelme, B., Chague, F., 2005. Tropical ecopathology: up hill and down dale Buruli ulcer]. Santé publique (Vandoeuvre-lès-Nancy, France) 17, 191.

Teelken, M.A., Stienstra, Y., Ellen, D.E., Quarshie, E., Klutse, E., van der Graaf, W.T.A., van der Werf, T.S., 2003. Buruli ulcer: differences in treatment outcome between two centres in Ghana. Acta tropica 88, 51–56.

Thangaraj, H.S., Evans, M.R.W., Wansbrough-Jones, M.H., others, 1999. Mycobacterium ulcerans disease; Buruli ulcer. TRANSACTIONS-ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE 93, 337–339.

The, U.B.G., 1971. Epidemiology of Mycobacterium ulcerans infection (Buruli ulcer) at Kinyara, Uganda. Transactions of the Royal Society of Tropical Medicine and Hygiene 65, 763–775.

Tobias, N.J., Seemann, T., Pidot, S.J., Porter, J.L., Marsollier, L., Marion, E., Letournel, F., Zakir, T., Azuolas, J., Wallace, J.R., Hong, H., Davies, J.K., Howden, B.P., Johnson, P.D.R., Jenkin, G.A., Stinear, T.P., 2009. Mycolactone gene expression is controlled by strong SigA-like promoters with utility in studies of Mycobacterium ulcerans and buruli ulcer. PLoS Negl Trop Dis 3, e553.

van der Werf, T.S., van der Graaf, W.T., Groothuis, D.G., Knell, A.J., 1989. Mycobacterium ulcerans infection in Ashanti region, Ghana. Trans. R. Soc. Trop. Med. Hyg. 83, 410–413.

Vandelannoote, K., Durnez, L., Amissah, D., Gryseels, S., Dodoo, A., Yeboah, S., Addo, P., Eddyani, M., Leirs, H., Ablordey, A., Portaels, F., 2010. Application of real-time PCR in Ghana, a Buruli ulcer-endemic country, confirms the presence of Mycobacterium ulcerans in the environment. FEMS Microbiol. Lett. 304, 191–194.

Veitch, M.G.K., Johnson, P.D.R., Flood, P.E., Leslie, D.E., Street, A.C., Hayman, J.A., others, 1997. A large localized outbreak of Mycobacterium ulcerans infection on a temperate southern Australian island. Epidemiology and infection 119, 313–318.

Wagner, T., Benbow, M.E., Burns, M., Johnson, R.C., Merritt, R.W., Qi, J., Small, P.L.C., 2008. A Landscape-based model for predicting Mycobacterium ulcerans infection (Buruli Ulcer disease) presence in Benin, West Africa. EcoHealth 5, 69–79.

Wallace, J.R., Gordon, M.C., Hartsell, L., Mosi, L., Benbow, M.E., Merritt, R.W., Small, P.L.C., 2010. Interaction of Mycobacterium ulcerans with mosquito species: implications for transmission and trophic relationships. Applied and environmental microbiology 76, 6215–6222.

Walsh, D.S., Portaels, F., Meyers, W.M., 2008. Buruli ulcer (Mycobacterium ulcerans infection). Trans. R. Soc. Trop. Med. Hyg 102, 969–978.

Walsh, D.S., Portaels, F., Meyers, W.M., 2010. Recent advances in leprosy and Buruli ulcer (Mycobacterium ulcerans infection). Curr. Opin. Infect. Dis. 23, 445–455.

Wansbrough-Jones, M., Phillips, R., 2006. Buruli ulcer: emerging from obscurity. The Lancet 367, 1849–1858.

WHO/AFRO. 2006. Guidelines for controlling buruli ulcer in the Africa Region. AFRO%20BU%20Guidelines.pdf. Harare

Williamson, H.R., Benbow, M.E., Nguyen, K.D., Beachboard, D.C., Kimbirauskas, R.K., McIntosh, M.D., Quaye, C., Ampadu, E.O., Boakye, D., Merritt, R.W., Small, P.L.C., 2008a. Distribution of *Mycobacterium ulcerans* in buruli ulcer endemic and non-endemic aquatic sites in Ghana. PLoS Negl Trop Dis 2, e205.

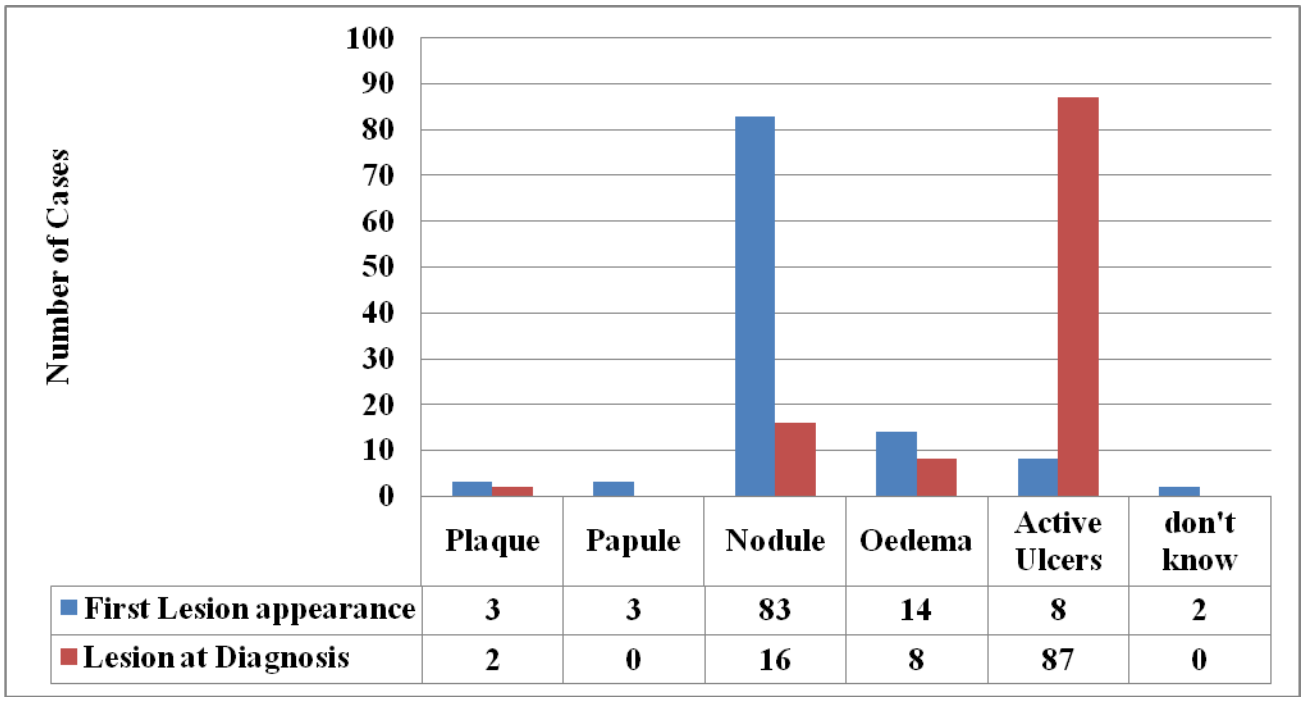
Yeboah-Manu, D., Bodmer, T., Mensah-Quainoo, E., Owusu, S., Ofori-Adjei, D., Pluschke, G., 2004. Evaluation of decontamination methods and growth media for primary isolation of *Mycobacterium ulcerans* from surgical specimens. Journal of clinical microbiology 42, 5875–5876.

Zhou, X.-N., Lv, S., Yang, G.-J., Kristensen, T.K., Bergquist, N.R., Utzinger, J., Malone, J.B., 2009. Spatial epidemiology in zoonotic parasitic diseases: insights gained at the 1st International Symposium on Geospatial Health in Lijiang, China, 2007. Parasit Vectors 2, 10.

APPENDICES

Appendix A – Distribution of Buruli ulcer lesions

Figure 17: Graph showing the distribution of Buruli ulcer lesion forms as first seen by patient and at diagnosis during the active case search



	Plaque	Papule	Nodule	Oedema	Active Ulcers	don't know
First Lesion appearance	3	3	83	14	8	2
Lesion at Diagnosis	2	0	16	8	87	0

Appendix –B Study Materials

CONSENT FORM

DEVELOPMENT OF RISK PROFILE FOR TRANSMISSION OF MYCOBACTERIUM ULCERANS IN SUHUM-KRABOA-COALTAR AND AKUAPEM SOUTH DISTRICTS OF EASTERN REGION, GHANA

Principal Investigator: Ernest Kenu

Address: DEPARTMENT OF EPIDEMIOLOGY AND DISEASE CONTROL

SCHOOL OF PUBLIC HEALTH,

UNIVERSITY OF GHANA

P.O.BOX LG13

LEGON-ACCRA

Introduction

This Consent Form contains information about the research named above. In order to be sure that you are informed about being in this research, we are asking you to read (or have read to you) this Consent Form. You will also be asked to sign it (or make your mark in front of a witness). We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

Reason for the Research

The reason for the research is to find out the distribution of the Buruli ulcer disease within communities in Suhum-Kraboa-Coaltar and Akwapem South districts and find out possible things that put people at risk of developing the disease.

General Information about Research

Buruli ulcer disease (BU) is a skin disease caused by *Mycobacterium ulcerans*. The disease can present as a swelling on the skin or an ulcer. Ghana is one of the countries in the West-Africa that buruli ulcer affects. Amongst the districts, Suhum-Kraboa-Coltar and Akuapem South are also affected. Unfortunately the exact way of getting the disease is not known.

Your Part in the Research

You/ward involvement will require that you/ward answer certain questions on demography (age, sex, place of residence etc), environment /Household factors (type of house you stay in, type of floor, source of drinking water etc), Heath (BCG scar, Past history of tuberculosis) and we will also mark where you/ward stay with a machine called Geographic positioning system (GPS), this will help us find possible things that make people get the disease. This will take approximately 25 minutes.

If you agree to be in the research, you will be required to answer those questions. About 100 people will participate in the research at this site.

Possible Risks

There is no risk in participating in this research.

Possible Benefits

This research will bring benefit to the community by helping the National buruli ulcer control programme to target communities that are severely affected with needed intervention and also prevent possible risk factors where possible.

If You Decide Not to Be in the Research

You are free to decide if you want to be in this research.

Confidentiality

We will protect information about you and your taking part in this research to the best of our ability. You will not be named in any reports. However, the members of our research team may sometimes look at your research records. A court of law could order medical records shown to other people, but that is unlikely.

Compensation

You will not be paid, since you do not have to take part in this research.

Alternatives to Participation

You do not have to participate in the research in order to receive care.

Leaving the Research

You may leave the research at any time. If you choose to take part, you can change your mind at any time and withdraw.

Please call Ernest Kenu (phone 0244592122) or Dr Adanu on 0244238556 or Prof Binka 0208131031

VOLUNTEER AGREEMENT

The above document describing the benefits, risks and procedures for the research on "DEVELOPMENT OF RISK PROFILE FOR TRANSMISSION OF MYCOBACTERIUM ULCERANS IN SUHUM-KRABOA-COALTAR AND AKUAPEM SOUTH DISTRICTS OF EASTERN REGION, GHANA" has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

_____ _____

Date Signature or mark of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

_____ _____

Date Signature of Witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____ _____

Date Signature of Person Who Obtained Consent

Appendix –C QUESTIONNAIRES

QUESTIONNAIARS FOR DEVELOPMENT OF RISK PROFILE FOR TRANSMISSION OF MYCOBACTERIUM ULCERANS IN SUHUM-KRABOA-COALTAR AND AKUAPEM SOUTH DISTRICTS OF EASTERN REGION, GHANA.

Form number
MFORMNUM ☐☐☐

Q.N^0	Questions and filters	Answers	Codes	Skips	FIELDS
1.	ID	☐☐☐☐☐			ID
2.	Date of interview	☐☐☐☐☐☐☐☐			DINT
3.	Do you have BU?	Yes No	1 2	 >>7	BUD
4.	Has the BU been confirmed	Yes No	1 2		CON
5.	What form was the first lesion?	Papule Nodule Plaque Edema Active Ulcers	1 2 3 4 5		LES
5b.	What form is the lesion now?	Papule Nodule Plaque Edema Active Ulcers	1 2 3 4 5		
6.	Which part of the body is the lesion located?	Leg Arm Trunk Head Distal Proximal/Trunk/Head) Right side Left side	1 2 3 4 5 6 7 8		LOC
7.	How old are you? (Age in years)	☐☐			AGEC
8.	Gender	Male Female	1 2		SEX
9.	What is the ethnic group of your father?	Ga/Adangme Ewe Akan Others	1 2 3 4		ETHF
10.	What is the ethnic	Ga/Adangme	1		ETHM

	group of your mother?	Ewe Akan Others	2 3 4		
11.	What is your educational level?	No Education Primary Secondary Tertiary	1 2 3 4		EDUC
12.	What is your marital status?	Married Not married	1 2		MAR
13.	How much money do you spend in this household per month?	Gh¢……..per month			HHS
	HEALTH				
14.	Do you have a BCG scar? Look on the left shoulder.	Yes No	1 2		BCG
15.	Have you had tuberculosis before?	Yes No	1 2		HTB
16.	Do you have a family history of tuberculosis?	Yes No	1 2		FHTB
17.	Have you ever had blood in urine before?	Yes No	1 2		BLD
	HOUSEHOLD / ENVIRONMENT				
18.	Is the wall of your house made of Mud?	Yes No	1 2		WAL
19.	Is the floor of your house made of Mud?	Yes No	1 2		FLO
20.	How many people are in your household?				HHN
21.	Do you have Cocoa plantation in your immediate neighborhood?	Yes No	1 2		COA
22.	Do you have Coffee plantation in your immediate neighborhood?	Yes No	1 2		COF
23.	Do you have bush in your immediate	Yes No	1 2		BUS

	neighborhood?				
24.	Do you have woods in your immediate neighborhood?	Yes No	1 2		WOD
25.	Do you have a wetland (Swamp) that is an area of land, usually fairly large, that is always wet and is overgrown with various shrubs and trees in your immediate neighborhood?	Yes No	1 2		SWP
26.	Do you have river in your immediate neighborhood?	Yes No	1 2		RIV
27.	Do you Share living space (that is staying under the same roof) with goats?	Yes No	1 2		GOT
28.	Do you Share living space, (that is staying under the same roof) with poultry?	Yes No	1 2		POU
29.	Do you Share living space, (that is staying under the same roof) with pigs?	Yes No	1 2		PIG
30.	Do you Share living space, (that is staying under the same roof) with cats?	Yes No	1 2		CAT
31.	Do you Share living space, (that is staying under the same roof) with dogs?	Yes No	1 2		DOG
32.	What is your primary source of drinking water?	River or stream Borehole Pipe borne	1 2 3		DRK

	INSECT BITES/BEHAVIOR				
33.	Have you received insect bite in water/mud?	Yes No	1 2		BIT
34.	Do you use bed nets?	Yes No	1 2		NET
35.	Do you Use mosquito coils?	Yes No	1 2		COIL
	TREATMENT WHEN HURT				
36.	Do you use Soap and water?	Yes No	1 2		SOAP
37.	Do you Rub the area with alcohol after a bite?	Yes No	1 2		ACH
38.	Do you use Leaves on the site of injury?	Yes No	1 2		LEAV
39.	Do you use adhesive bandage when you get hurt?	Yes No	1 2		BAN
	ACTIVITIES				
40.	Do you Wade in the Densu river?	Yes No	1 2		DEN
41.	Do you Wade in a river or stream?	Yes No	1 2		WRIV
42.	Do you Wash your clothes?	Yes No	1 2		WAS
43.	Do you Fetch water?	Yes No	1 2		FET
44.	Do you Farm?	Yes No	1 2		FARM
45.	Do you Farm and wear long upper body clothing/shirt?	Yes No	1 2		WEAR
46.	Do you Farm and wear short upper body clothing/shirt?	Yes No	1 2		WSHT
47.	Do you Farm and wear long	Yes No	1 2		WLOG

	pants/dress?				
48.	Do you Farm and wear short pants/dress?	Yes No	1 2		FWSHT
49.	Do you Fish?	Yes No	1 2		FISH
50.	Do you Fish, but not in the Densu river?	Yes No	1 2		NDEN
51.	Do you Fish in the Densu river?	Yes No	1 2		INDEN
52.	Do you Fish with long upper body clothing?	Yes No	1 2		FLCTH
53.	Do you Fish with short/no upper body clothing?	Yes No	1 2		FSHTC
54.	Do you Fish with long lower body clothing?	Yes No	1 2		FLLC
55.	Do you fish with short/no lower body clothing?	Yes No	1 2		FSLC
BATH (HYGIENE)					
56.	Do you have your bath for hygiene?	Yes No	1 2		BATH
57.	Do you have your bath for hygiene, but not in the Densu river?	Yes No	1 2		BAH
58.	Do you have your bath for hygiene in the Densu river?	Yes No	1 2		BATD
59.	Do you have your bath for hygiene, but not in open borehole?	Yes No	1 2		BNBH
60.	Do you have your bath for hygiene in open borehole?	Yes No	1 2		BOBH
61.	Do you Swim/dive/play in water?	Yes No	1 2		SIW
62.	Do you Swim?	Yes No	1 2		SWIM
63.	Do you Swim, but	Yes	1		SWND

	not in the Densu river?	No	2		
64.	Do you Swim in the Densu river?	Yes No	1 2		SWID

SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
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Fax: +233-21-517501

Cable: UNIVGhana

E-mail: afariea@yahoo.co.uk

P O Box LG13

Legon-Accra

February 22, 2010

The Chairman

Institutional Review Board

Noguchi Memorial Institute for Medical Research

P.O.Box LG 581

Legon

Dear Sir,

REQUEST FOR ETHICAL APPROVAL OF PHD PROPOSAL – ERNEST KENU

I wish to submit the proposal of the above-mentioned, who is a PhD student of the School of Public Health, University of Ghana, for ethical review.

The title of his proposal is "Development of Risk Profile for Transmission of Mycobacterium Ulcerans in Suhum-Kraboa-Coaltar and Akuapem South Districts of Eastern Region, Ghana"

It will be appreciated if the Ethical Review Board will expedite review of the proposal and forward the comments to the School of Public Health.

Thank you for your usual cooperation.

Yours faithfully,

Prof. E. A Afari

Head of Department, Disease Control and Epidemiology

SCHOOL OF PUBLIC HEALTH
COLLEGE OF HEALTH SCIENCES
UNIVERSITY OF GHANA

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Fax: +233-21-517501

Cable: UNIVGhana

E-mail: afariea@yahoo.co.uk

P O Box LG13

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February 22, 2010

The Chairman

Ethical Review Board

Ghana Health Service

Accra

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Thank you for your usual cooperation.

Yours faithfully,

Prof. E. A Afari

Head of Department, Disease Control and Epidemiology

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL

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NMIMR-IRB

P. O. Box LG 581

Legon, Accra

Ghana

My Reference: DF 22

3/3/2010

Ernest Kenu MBChB, MPH,

Department of Epidemiology and Disease Control, SPH

P. O. Box LG 13

Legon, Accra

RE: Our Study #042/09-10 At: NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Ernest Kenu:

Meeting Date: 3/3/2010 At: NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Protocol Title:

Development of Risk Profile for Transmission of Mycobacterium Ulcerans in Akuapim South and Suhum-Kraboa-Coaltar Districts of Eastern Region, Ghana

This is to advise you that the above referenced Study has been presented to the Institutional Review Board, and the following action taken subject to the conditions and explanation

provided below.

Internal #: New Appl

Expiration Date: 3/2/2011

On Agenda For: Initial Submission

Reason 1: Reason 2:

Description: Date Received- 2/16/2010

IRB ACTION: Approved

Condition 1:

Action

Explanation: The study was approved but the consent form should be simplified

Yours Sincerely,

Helena Baidoo

Deputy IRB Administrator

NMIMR-IRB

School of Public Health

College of Health Sciences

University of Ghana

Legon

P.o.Box LG13

2nd April 2010

The Chairman,

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Sir/Madam,

CORRECTIONS ON PHD PROPOSAL SUBMITTED TO NMIMR-IRB

Reference to your letter on the PhD protocol entitled Development of Risk Profile for Transmission of Mycobacterium Ulcerans in Akuapim South and Suhum-Kraboa-Coaltar Districts of Eastern Region, Ghana.

The action taken by the IRB was that the protocol was approved but the consent form needs to be simplified. The simplified consent form has been done and can be found on pages 23 to 25 of the study protocol.

Thank you

Yours Faithfully,

Ernest Kenu

PhD Student

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH
INSTITUTIONAL REVIEW BOARD

(UNIVERSITY OF GHANA)

Phone: +(233) 21 500374 /501178
Fax: +(233) 21 502182
Email: Director@noguchi.mimcom.org
Telex No: 2556 UGL GH

P.O. Box LG581
Legon
Ghana

My Ref. No: DF.22

3rd March, 2010

Your Ref. No:

ETHICAL CLEARANCE

FEDERALWIDE ASSURANCE FWA 00001824 **IRB 0001276**

NMIMR-IRB CPN 042/09-10 **IORG 0000908**

On 3rd March, 2010, the Noguchi Memorial Institute for Medical Research (NMIMR) Institutional Review Board (IRB), at a full board meeting reviewed and approved your protocol titled:

TITLE OF PROTOCOL : **Development of Risk Profile for Transmission of *Mycobacterium ulcerans* in Suhum-Kraboa-Coaltar and Akuapim South Districts of Eastern Region, Ghana**

PRINCIPAL INVESTIGATOR : **Ernest Kenu (PhD Student)**

Please note that a final review report must be submitted to the Board at the completion of the study. Your research records may be audited at any time during or after the implementation.

Any modification of this research project must be submitted to the IRB for review and approval prior to implementation.

Please report all serious adverse events related to this study to NMIMR-IRB within seven days verbally and fourteen days in writing.

This certificate is valid till 2nd March, 2011. You are to submit annual reports for continuing review.

Signature of Chairman: .....
Rev. Dr. Samuel Ayete-Nyampong
(NMIMR – IRB, Chairman)

cc: Professor Alexander K. Nyarko
Director, Noguchi Memorial Institute
for Medical Research, University of Ghana, Legon

GHANA HEALTH SERVICE ETHICAL REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. :GHS-ERC: 3
Your Ref. No.

Research and Development Divison
Ghana Health Service
P. O. Box MB 190
Accra

27th May 2010

Tel: +233- 0302-681109
Fax + 233-0302 685424
Email: Hannah.Frimpong@ghsmail.org

ERNEST KENU- Principal Investigator

ETHICAL CLEARANCE - ID NO: GHS-ERC: 02/4/10

The Ghana Health Service Ethical Review Committee has reviewed and given approval for the implementation of your Study Protocol titled:

"Development of Risk Profile for Transmission of Mycobacterium Ulcerans in Suhum-Kraboa-Coaltar and Akuapem South Districts of Eastern Region, Ghana"

This approval requires that you submit periodic review of the protocol to the Committee and a final full review to the Ethical Review Committee (ERC) on completion of the study. The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Please note that any modification of the project must be submitted to the ERC for review and approval before its implementation.

You are also required to report all serious adverse events related to this study to the ERC within seven days verbally and fourteen days in writing.

You are requested to submit a final report on the study to assure the ERC that the project was implemented as per approved protocol. You are also to inform the ERC and your mother organization before any publication of the research findings.

Please always quote the protocol identification number in all future correspondence in relation to this protocol

SIGNED.....

MR. ANNOR NIMAKO
(GHS-ERC VICE CHAIRMAN)

Cc: The Director, Research and Development Division, GHS, Accra

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH

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NMIMR-IRB

P. O. Box LG 581

Legon, Accra

My Reference: DF 22

Ernest Kenu MBChB, MPH,

Department of Epidemiology and Disease Control, SPH

P. O. Box LG 13

Legon, Accra

RE: Our Study # 042/09-10 At: NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Ernest Kenu:

Meeting Date: 3/2/2011 At: NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Protocol Title:

Development of Risk Profile for Transmission of Mycobacterium Ulcerans in Akuapim South and Suhum-Kraboa-Coaltar Districts of Eastern Region, Ghana

This is to advise you that the above referenced Study has been presented to the Institutional Review Board, and the following action taken subject to the conditions and explanation

provided below.

Internal #: 171

Expiration Date: 3/1/2012

On Agenda For: Renewal

Reason 1: Progress Report Reason 2:

Description:

IRB ACTION: Renewed

Condition 1:

Action

Explanation:

Yours Sincerely,

Helena Baidoo

IRB Administrator

NMIMR-IRB

FORM B

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH INSTITUTIONAL REVIEW BOARD PROGRESS REPORT FORM FOR CONTINUING REVIEW

Title of study: Development of risk profile for transmission of *mycobacterium ulcerans* in Suhum-Kraboa –Coaltar and Akuapem South Districts of Eastern Region, Ghana

Principal Investigator: Ernest Kenu (PhD Student)

Co-Investigators:

Certified Protocol Number (CPN): NMIMR- IRB CPN 042/09-10

Initial Date of Approval: 3rd March 2010

Recent Date of Approval: N/A

Duration of Project: Three years

a) How long has project run? One Year

b) Time remaining: Two years

If requesting for an extension state duration required

Enrollment:

a.Total number of participants enrolled *to date*: __58__
b.Number of participants enrolled *since last renewal*: __58__
c.Estimated number to be enrolled in upcoming year: __22____
d.Number of participants discontinued:

- by investigator: _0_____
- voluntarily: _0____
- due to SAE: _0___
- Other Reasons (Specify): _0_____

e.In case of animal/vector studies

- list number sampled to date __N/A____
- list number yet to be sampled in the upcoming year __N/A

Answer Questions 1 through 7 below and attach a memo explaining any "yes" answers.

Check Boxes with X

	NO	YES	N/A
1. Have there been any complaints received from anyone about the study? [Participants, Parents/Guardians, Community Members, Staff, etc)	☒	☐	☐
2.Have there been any unanticipated problems or serious adverse events involving risk to participations since the last renewal? If yes, include all copies of Serious Adverse Event reports with this submission.	☒	☐	☐
3.Have the risks or benefits changed as a result of any new information?	☒	☐	☐
4.Does this study have a Data Safety and Monitoring Board? If yes, provide the most recent report from that board.	☒	☐	☐
5.Have there been any amendments approved since the last review?	☒	☐	☐

6.Have there been changes in participant population, recruitment, study procedures or consent procedures that were not submitted for approval by the IRB? ☒ ☐ ☐

7.Are you requesting any changes (i.e. protocol amendment) in participant population, recruitment, study procedures or consent procedures as part of this renewal? ☒ ☐ ☐

(If changes are requested, attach revised proposal summary forms, protocol and consent forms.)

NB: A two page detailed report should accompany the progress form. The report should have an introduction, materials and methods, Preliminary results, discussion, further studies to be done, etc)

Signature of Principal Investigator Date

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH

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NMIMR-IRB

P. O. Box LG 581

Legon, Accra

My Reference: DF 22

Ernest Kenu MBChB, MPH,

Department of Epidemiology and Disease Control, SPH

P. O. Box LG 13

Legon, Accra

RE: Our Study # 042/09-10 At: NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Ernest Kenu:

Protocol Title:

Development of Risk Profile for Transmission of Mycobacterium Ulcerans in Akuapim South and Suhum-Kraboa-Coaltar Districts of Eastern Region, Ghana

IRB Meeting Will Be Held On: 3/2/2011

Study Expires On: 3/2/2011 A Response is Requested by 2/16/2011.

The records of our Institutional Review Board indicate that the above referenced Study is due for renewal or closure.

Please complete the NMIMR IRB Progress Report Form B at the website if the study is still in progress. In the report please include number of participants currently enrolled in study, any DSMB involved and any changes since last review (ie: revisions or amendments, SAE's, changes in P.I.'s, etc). Please note that this report must be signed by the P.I. and 14 hard copies must be submitted to the IRB office. As a reminder, a Protocol must remain open for IRB review as long as any enrolled participants remain in follow-up or samples are being collected or analyzed. Please be aware that should the above study not be renewed in the right manner, you will be in conflict with Noguchi IRB regulations; therefore, any data collected after this date will no longer be valid.

If the study has ended and will not require renewal, please fill out the Study closure Form E and submit 14 hard copies to the IRB office.

If you have further questions or concerns, please do not hesitate to contact the IRB office.

Thank you for your continued support and cooperation.

Yours Sincerely,

Helena Baidoo

IRB Administrator

School of Public Health

College of Health Sciences

University of Ghana

Legon

P.o.Box LG13

2nd February, 2012

The Chairman,

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH-IRB

Dear Sir/Madam,

REQUEST FOR CLOSURE OF STUDY

I write to request for a closure of my PhD study titled " Development of risk profile for transmission of *mycobacterium ulcerans* in Suhum- Kraboa –Coaltar and Akuapem South Districts of Eastern Region, Ghana" with a Certified Protocol Number (CPN): NMIMR- IRB CPN 042/09-10

I have successfully completed data collection and have done analysis of the data.

Thank you

Yours Faithfully,

Ernest Kenu

PhD Student

FORM E

NOGUCHI MEMORIAL INSTITUTE FOR MEDICAL RESEARCH INSTITUTIONAL REVIEW BOARD STUDY CLOSURE FORM

Title of study: Development of risk profile for transmission of *mycobacterium ulcerans* in Suhum- Kraboa –Coaltar and Akuapem South Districts of Eastern Region, Ghana

Certified Protocol Number (CPN): NMIMR- IRB CPN 042/09-10

Principal Investigator: Ernest Kenu (PhD Student)

Co- Investigators:

Initial Date of Approval: 3rd March 2010

Duration of Project: Three years

Enrollment:
a. Total number of participants enrolled: 141
b. Number of participants discontinued: 0

- By investigator: 0
- Voluntarily: 0
- Due to SAE: 0
- Other reasons (Please specify): 0

c. Total number of participants who completed the study: 141

Answer the following questions by checking the boxes and attach a memo explaining any yes answers.

	NO	YES
1. Have there been any complaints received from anyone about the study? (Participants, Parents/Guardians, Community Members, Staff, etc)	☒	☐
2. Did the anticipated risks or benefits change during the study?	☒	☐
3. Did this study have a Data Safety and Monitoring Board? If yes, attach the most recent report from the board.	☒	☐
4. Was your study audited or monitored by the NMIMRIRB or any other agency	☒	☐

If yes, please attach a copy of the findings and any corrective actions that have been implemented as a result of this audit or monitoring.

6. Are there any publication regarding this study? ☐ ☒
If yes, please attach an abstract, quoting the reference publication.

Why is this study being closed? (Check one)

Data analysis complete ☒ ☐

Interim analysis determined study is not safe or efficacious ☐

☐

No funding, time constraints or personnel to do the study ☐ ☐

Others (Please explain) ☐ ☐

_____

NB: A cover letter requesting for closure of study and a 2-3 page detailed final report should be attached to the study closure form. The report should have an introduction, methodology, final results, discussion, a section stating whether or not the study objectives have been made, recommendations, conclusions and conclusion

_____

Signature of Principal Investigator Date

DEVELOPMENT OF RISK PROFILE FOR TRANSMISSION OF MYCOBACTERIUM ULCERANS IN SUHUM-KRABOA-COALTAR AND AKUAPEM SOUTH DISTRICTS, GHANA

Ernest Kenu

Background

Buruli ulcer disease (BU) is a skin disease caused by *Mycobacterium ulcerans*. The disease can present as a swelling on the skin or an ulcer. Ghana is one of the countries in the West-Africa that buruli ulcer affects. Amongst the districts, Suhum-Kraboa-Coltar and Akuapem South are also affected. Unfortunately the exact way of getting the disease is not known. Even though Some risk factors associated with contracting the disease have been identified in some previous studies however we do not know what pertains in Suhum-Kraboa-Coaltar and Akuapem South districts of the Eastern. In addition, there is Lack of detailed understanding of how environmental and social conditions interact to cause the disease. Finally, we do not know the spatial distribution of BU in the area as well as the genetic types that are in study area.

The absence of these are hampering the prevention and control of BU within the municipalities and consequently leading to ineffective use of scarce resources hence the need for the research.

Objectives

The general objective of the study is to develop a risk profile for the transmission of BU

Specific Objectives:

1. To determine demographic and behavioral factors associated with BU at the individual and community level

2. To determine environmental factors associated with BU

3. To determine spatial factors associated with BU

Study Design/Method

The study was design as a Case-control with Spatial mapping. Active case search was carried out with community based volunteers in the study area to identify Buruli ulcer patients. A case of buruli ulcer was defined as any person aged 2 years or more who resides in the Suhum-Kraboa-Coaltar and Akuapem South districts diagnosed of Buruli ulcer meeting the WHO clinical case definition for *M. ulcerans* disease. A Control was defined as any person who resides in the community/neighbourhood where the Buruli ulcer patient comes from.

Standardized questionnaires were administered to the cases as well as their controls. Geographic Positioning System (GPS) mapping conducted to locate the cases as well as map environmental characteristics that of particular interest in contracting BU

Results

- A total of 141 cases were recruited and 113 were confirmed to have buruli ulcer using PCR, we selected 113 community matched controls by sex and 5 year age group. Fifty-seven of the cases were females and 56 were males. The median age (ranges) of the cases and controls were 28 (2 – 102) and 30 (3 – 98) years respectively.

- Univariate analysis done showed that BU was associated with the following

Variable	OR	95% CI	P-value
1. Higher education	0.4	0.19 – 0.86	0.03
2. Presence of Cocoa			
Plantation in neighborhood	0.55	0.32 - 0.94	0.04
3. Presence of bush	2.7	1.3 - 5.4	0.01
4. Presence of wetland	6.9	3.8 - 12.4	<0.001
5. Presence of river	2.5	1.4 - 4.3	0.002
6. Share living space with			
Poultry	1.9	1.1-3.3	0.04
7. Insect bite in water/mud	3.5	1.8 - 6.6	<0.001
8. Use of soap and water	0.29	0.16 – 0.53	<0.001
9. Rubbing area with alcohol after bite	0.11	0.05 – 0.25	<0.001
10. Use of leaves on injury site	2.9	1.7 – 4.9	<0.001
11. Use of adhesive bandage	2.8	1.4 - 5.6	<0.001
12. Wading in Densu river	3.5	2.0 - 6.1	<0.001
13. Farming with long sleeves	0.19	0.11- 0.35	<0.001
14. Farming with short sleeves	3.9	2.2 - 6.8	<0.001

15. Wearing long pants to farm	0.27	0.15 - 0.5	<0.001
16. Wearing short pants to farm	3.2	1.8 - 5.7	0.001

A backward elimination logistic regression analysis indicated that presence of wetland in the neighborhood, insect bite in water/mud, use of adhesive when injured, wading in the densu river were significantly associated with the development of BU. Whereas rubbing an injured area with alcohol and wearing long sleeves to farm protects against BU.

From the mapping it was noted that upstream along the Densu River where the river is not polluted, we did not find any BU cases there but we identified all the cases beyond the polluted area of the Densu River. BU showed clustering along the river as well.

Conclusions

Wearing clothes that covers the limbs during farming, rubbing an injured area with alcohol immediately protect against Buruli ulcer. There is clustering of cases of buruli ulcer and they occur in areas where the river was most contaminated

The objectives of the study have been achieved since it confirms some findings in other areas and shows the areas of endemic BU and non-endemic areas. With this critical information appropriate recommendation will be given to the National Buruli control Programme to incorporate the finding into their educational materials and know when areas to focus in buruli control along the Densu River.

Risk Profile for transmission of Mycobacterium Ulcerans in Akuapem South and Suhum Kraboa Coaltar districts, Ghana

Ernest Kenu[1], R. K Adanu[1], O. Razum[2], M. Kaeser[3]

1. School Of Public Health, University of Ghana, 2. Bielefeld University, Germany, 3 Swiss Tropical Institute, Basel

BACKGROUND

Buruli ulcer (BU) disease is a chronic debilitating skin disease caused by *Mycobacterium Ulcerans.*

It is associated with areas where the water is slow-flowing or stagnant.

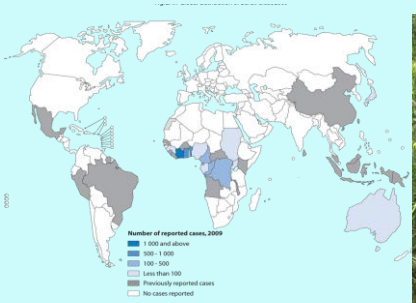
Unfortunately the exact mode of transmission and the development of the disease through human activities is unknown.

We carried out risk profile assessment and mapped the distribution of the disease along the Densu river

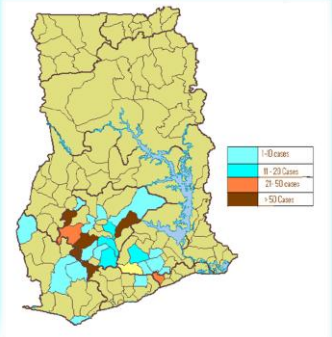
STUDY OBJECTIVE

To develop a risk profile for the transmission of BU in Suhum-Kraboa-Coaltar and Akuapem south Districts of Eastern Region

Global distribution of BU, 2009



Buruli Ulcer Distribution in Ghana



METHODS

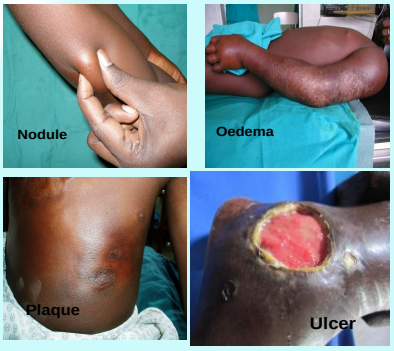
We conducted a case-control study and spatial mapping in Akuapem South and Suhum- Kraboa-Coaltar districts of the Eastern region of Ghana to identify risk factors for BU and its spatial distribution along the densu river. We carried out an active case search for Buruli ulcer patients in the study area to identify the case patients and matched them with a community control of the same sex and 5 year age ranged.

Structured questionnaire on host related, demographic, environmental, behavioural factors was administered to both cases and controls

Using the E-trex Garmin Geographical positioning system (GPS) machine receiver, we marked the location of the case patients, and any important attributes of the community.

Contaminated section of Densu river

Clinical Presentations of Buruli Ulcer



RESULTS

A total of 112 confirmed buruli ulcer cases and 112 community controls were interviewed.

Risk factors identified to be associated with BU were: Low educational background, being married, living in a mud house, presence of wetland, insect bite in water, wearing short pants while farming or fishing and living near cocoa plantation.

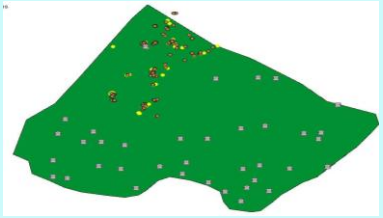
Using bed nets, washing of clothes and wearing clothes covering the limbs protect against BU. Spatially, there was clustering of BU patients downstream of densu river.

Factors associated with BU: Multivariate (Logistic regression) analysis

VARIABLE	OR (95% CI)
Educational background	
•No education	1
•Primary	0.58 (0.28 – 1.19)
•Secondary	0.29 (0.10 – 0.81)
•Tertiary	*0.0 (0.00 – 10.8)
Marital status	
•Married	0.59 (0.33 – 1.04)
•Not married	
Living in Mudhouse	
•Yes	0.41 (0.22 – 0.77)
•No	
Living near/around/in wetland	
•Yes	3.34 (1.85 – 6.05)
•No	
Insect bite	
•Yes	1.91 (1.04 – 3.52)
•No	
Use of bednet	
•Yes	0.93 (0.49 – 1.75)
•No	
Use of mosquito coil	
•Yes	0.87 (0.45 – 1.67)
•No	
Wearing long sleeves for farming	
•Yes	0.18 (0.10 – 0.34)
•No	
Wearing Short sleeves for farming	
•Yes	5.0 (2.73 – 9.22)
•No	
Wearing short pants when farming	
•Yes	3.08 (1.66 – 5.72)
•No	

Aerial Map Showing part of Densu river and communities

Maps showing the relation of Buruli ulcer cases to Densu river andcommunities



CONCLUSION

Wearing clothes that covers the limbs during farming, fishing and use of bednets protect against Buruli ulcer disease.

There is clustering of cases of buruli ulcer and they occur in areas where the river was most contaminated

Funded by DAAD via ACBRIDGE project

Figure 18: Poster presentation made at 7th European Congress on tropical Medicine and International Health, Barcelona

Table 13: GPS ID List

This list contains sensitive data important for the ACBRIDGE Study about Buruli Ulcer.

If you find this list, please call Ernest Kenu (0244 592122) and hand it over to him for a handsome reward.

ID number of patient	GPS coordinates	
	Latitude	Longitude
	.	.
	.	.
	.	.
	.	.
	.	.
	.	.
	.	.
	.	.
	.	.
	.	.

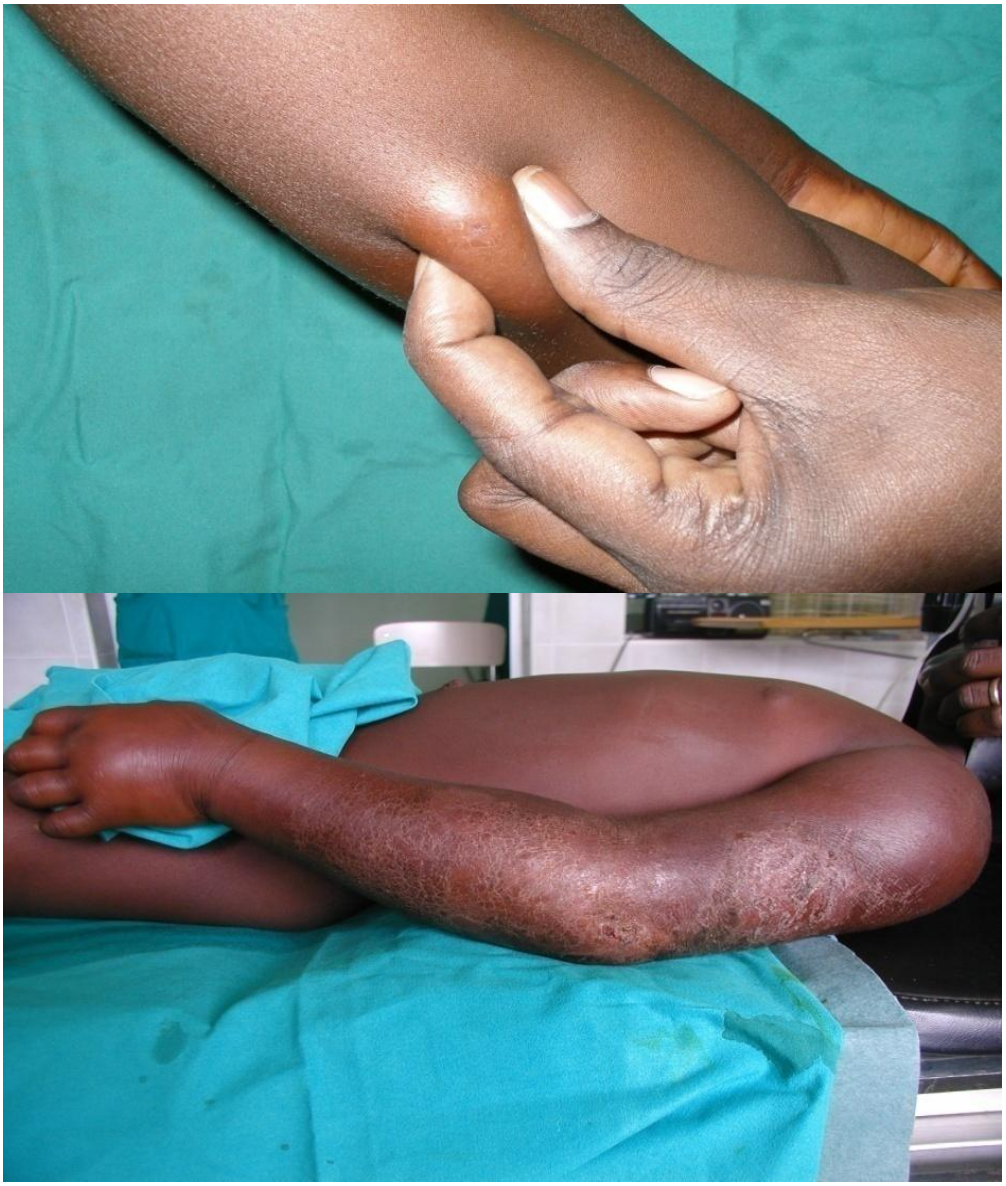


Figure 19: Pre ulcerative forms of Buruli ulcer (Nodule at the top and Oedema at the bottom

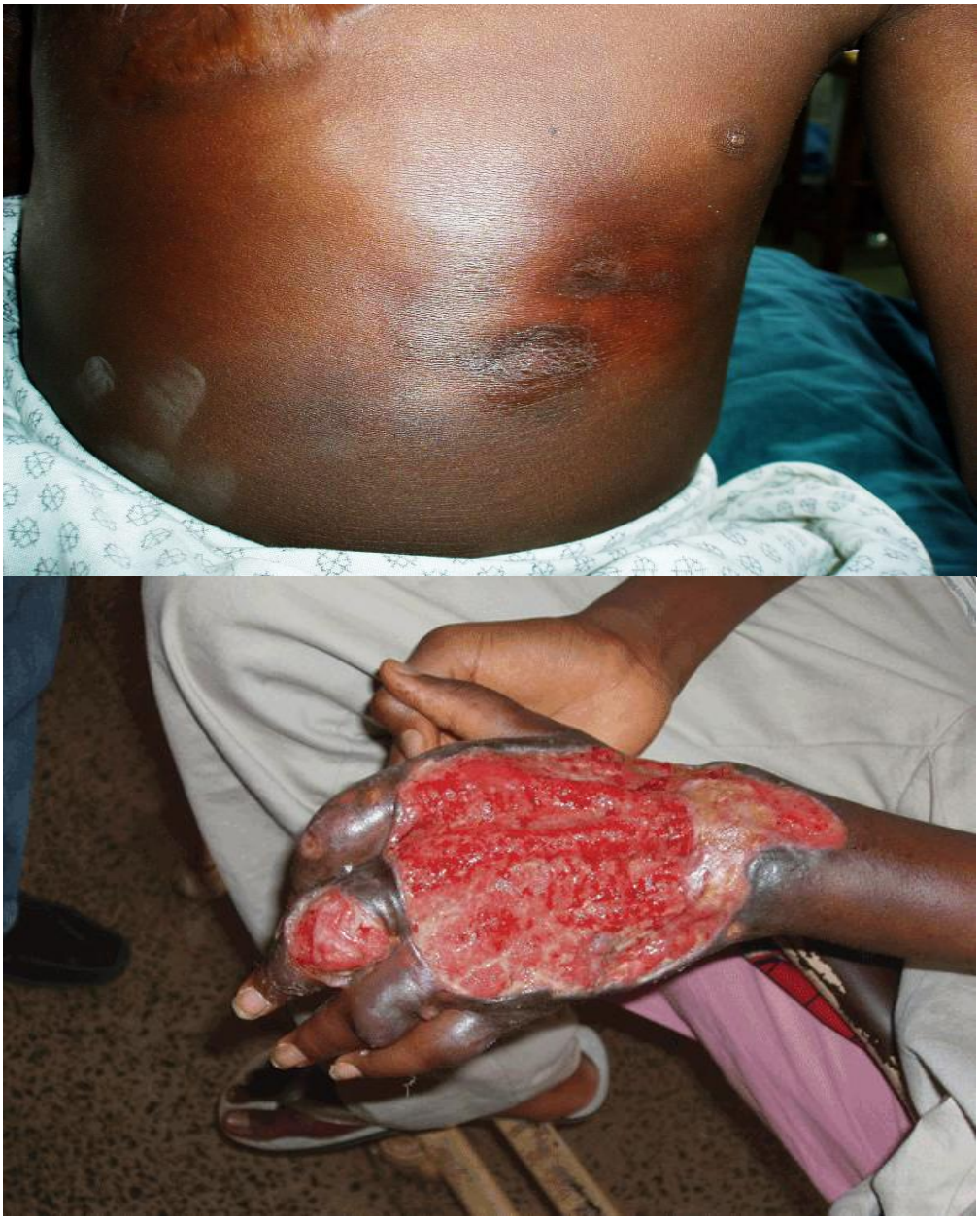


Figure 20: Plaque (at the top) and active ulcer lesions of Buruli ulcer

Figure 21: Upstream portion of the Densu River (no turbidity)

Figure 22: Deforestation in the Atewa forest along the course of the Densu River

Figure 23: Deforestation in the Atewa forest

Figure 24: Chain saw operators cutting down trees

Figure 25: Portion of the Densu River with contamination (Turbidity of the water)

Figure 26: Point of Densu River where the pollution/contamination started

Figure 27: Portions of the contaminated lower stream of Densu River

Figure 28: Contaminated Portions of the Densu River

Figure 29: Down Stream portion of the Densu River