WHO ANNUAL MEETING ON BURULI ULCER

31 March – 2 April 2008

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COMMON SESSIONS
Dear Distinguished Participants,

First of all, I would like to thank you all for the opportunity to participate in this meeting and to present a summary of my encounter with Buruli ulcer. I sincerely thank WHO for making it possible for me to be here.

Let me start with a little bit of my background:

My father, a peasant farmer, had two wives (this is not an uncommon practice in northern Ghana). Together with my siblings, we were twelve living in a small house. I did enjoy and still remember those days when I was growing up. I am the oldest child. The entire family – men, women, boys, girls and even the very little ones – were always on the farm assisting our father. We, the children, most often enjoyed catching crabs and sometimes fishing in the ponds and swamps around and on the way to the farm. That was a main means of protein for our evening meals.

My father painstakingly enrolled me in the local primary school when I was six years old. As I passed through school, I however found myself sometimes mending shoes, weaving cane baskets and running errands in order to make some income to supplement the family budget and to get some pocket money. I excelled in school and this made my family very happy. Being the only family member to receive a formal education, I was treated sometimes like a “king” and brought honour to my poor family. But life was not easy at all. From time to time, my parents had to sacrifice basic needs or even sell valuables items in order to meet my educational needs.

In 1998, I was admitted to the Acherensua Secondary School (a high school) in the Brong Ahafo region to study business. It was a new world all together for me. I excelled academically in high school too and I was always among the best 10 students. During vacation I usually returned to my village to continue with my shoe making, cane basket weaving, etc. so I could raise some money for my school fees as well as to support my family. That I did at the expense of my education because I could not enroll in vacation classes. But looking back, I had no option but to do some vacation work as a necessity. That cycle of vacation jobs continued until my final year in high school.

Now let me come to my encounter with Buruli ulcer:

In the year 2000, whilst I was getting ready to write my final high school exams, I was unfortunately struck by a strange illness. We did not know what the disease was. As a result, I missed the opportunity to write my final high school examination. I think I had the disease at Acherensua where I attended secondary school. I did not know whether someone had the disease at that time. But today, I know a number of people who have had the disease in that community.
This flesh eating disease started on September 2000. I remember having seen a small boil-like lesion on my right elbow joint. This ‘boil’ was painless and within a few days and without any warning my whole right arm began to swell. My friends drew my attention but it was so painless that I did not pay any particular attention. I was convinced that it would soon go away. I was more interested in getting ready for my exams.

Unfortunately, things did not go as I expected. Within another few days, I realized that my whole right arm had swollen, to the extent that I could not stand upright for a few minutes. The whole of my arm and part of my trunk were very heavy yet painless. I later learnt that was the oedematous form of the disease. Most of my student friends were scared to see a swollen limb and trunk, yet without any pain. Some of friends related it to witchcraft, juju (black magic), a curse, etc. As to the cause, I still did not know because I could not remember anything that could have caused the disease. With all the remarks of witchcraft, curse, etc and still not feeling the pain, I also became very scared. I often asked myself, “What is happening to me? Lord, help me.” I prayed and prayed.

When my parents heard the news, they were very upset. My dad quickly came to pick me from school to seek medical attention. Initially, I was taken to three main hospitals viz., St. Elizabeth Hospital, Hwidiem; Goaso Government Hospital and Sunyani Regional Hospital. I was given all sorts of drugs but there was no improvement. Indeed, things even got worse despite taking all the prescribed drugs. My fears were heightened further as suspicion of a curse or bewitching was strongly implicated in my condition. My father therefore took me to consult oracles, fetishes and shrines. Several goats, sheep, chicken and cash were lavished on these shrines but my condition did not improve. In fact, my situation was getting worse every day. People who saw me will look at me again and again. Children ran away when they saw me. Even my own brothers and sisters were reluctant to come near me. I was more or less isolated from society!

After about 9 weeks of hopping from one shrine to the other, a family friend advised my father to take me to the St. Martin’s Catholic Hospital at Agroyesum in the Amanse West District of Ashanti Region, about 750 kilometers from my village. The family friend told my parents that the St. Martin’s hospital was (and is still) well known for treating chronic ulcers. With desperation setting in, my father reluctantly agreed to send me to Agroyesum. At the hospital, we (my father and I) were told I would need admission and that I would be hospitalized for at least 3 months. We had to return home in order to prepare for the hospital admission the following week. My father though desperate was much afraid of the cost implications involved and was especially frightened by the mention of surgery. Instead of my father taking me back to the hospital, he rather took me to my hometown in the north where he thought he could fight my disease spiritually. In my hometown it was also revealed that we have wronged the gods of the land so the necessary sacrifices were made with the hope that I was going to be well. Alas! The situation was getting worse as the whole of my chest, down to my scrotum had swollen. The size of the swollen scrotum was almost equivalent to my head. “What a disease! What a tragedy!!” I always asked myself.

For two weeks I could not sleep. Severe pain has set in. The swollen parts had begun to rapture (ulcerate) with the release of some fluid. I smelled terribly bad!

Back at home I was given a separate room. My arm begun smelling due to razor blade marks made by the traditional healers, and the other part of the arm started rotting. The stench was horrible. I was alive but basically rotten.

On 11th October, 2000, I told my father to take me back to the St. Martin’s Hospital, Agroyesum because; I would rather prefer to die in a hospital than in a fetish priest’s house. So that day we traveled all of my hometown in the north through Kumasi to Agroyesum. It was a momentous 750-km journey lasting two days. No vehicle was ready to allow me in. Other passengers got off as I entered the vehicle and none joined after I had entered. My daddy could not also afford a chattered vehicle nor an ambulance. What a nightmare! After much struggle and humiliation, we were
able to get a vehicle to travel with. We arrived at Agroyesum the following day very weak and exhausted.

I wept bitterly upon my arrival at the hospital when all expressions on everyone’s face doubted my survival within the next few days; that only by divine intervention that I could be saved. The doctor could not believe that we had delayed so long in returning to the hospital as promised. I lost all hope. Nonetheless, I was admitted and given all the necessary attention. I thought I received some TB drugs and other antibiotics for a long time. In fact, I thought within myself “my days on earth were numbered” and seeing how I was suffering, I preferred death to life. Life at the hospital was not easy. We arrived at the hospital completely broke. All the money my dad had on him was spent on transport from the north to Agroyesum. How were we going to survive when the hospital operates on “cash and carry” and patients had to feed themselves? Luckily people came to our aid – health staff, Catholic sisters, and even some patients. But I gathered some courage and was consoled by the fact that other patients especially, children who had extensive ulcers were in severe pain and others had had their arms or legs amputated due to this horrible disease.

The first operation was performed on me within the first four days upon admission. I received one pint of blood. This operation was followed by another major operation in the next two weeks, involving excision of the whole of my right arm and chest leaving a big wound.

In fact, the second operation nearly paralyzed me. It made me very weak. At that point, I became hopeless; even the medical team had little hope for me. All my daily activities of living was performed on my hospital bed with the help of the nurses and my parents. For one and a half years, I was bedridden at the hospital. During that period, the financial situation of my family worsened. My mother who had then joined me at the hospital had to leave me alone to weed people’s farm and sometimes beg from sympathetic people before I could eat.

While in that state, I had no appetite, therefore I could not eat and thus grew very lean. I became anemic and weak. I was then transferred from the main males’ ward to isolated ward meant for TB patients. As a matter of fact, there was no difference between an end stage AIDS patient and myself.

One day, I asked Dr. Etuaful, the doctor in charge of my care and who is seated here today, how the large wound was going to heal. He said, “We have to take a skin from my thighs to cover the whole wound since that was the only method”. Meanwhile, I was too slim for that purpose. After a year and ten months, I was taken to the theatre again for my recovery operation after receiving two pints of blood before the surgery and additional two inside the operation room. During the 3-hour operation, skin was taken from both thighs to cover the whole wound.

One month after that operation, I had recovered a little bit but had to learn how to walk again like a little child and one could imagine how I was tottering. I had lost weight and my legs were so weak after many months in bed.

I became hopeful after my third operation so I decided to learn how to use my left hand to write so that one day, I could return to school.

A series of minor operations were also carried on me in addition to the first three major ones alongside regular “appropriate technology” physiotherapy. There were not physiotherapy services at that time. I was finally discharged from the hospital on 3rd March 2003, in relatively good condition. My right upper limb was swollen although the healing was complete. I was assured that the swelling would reduce over time and that I should regularly apply vaseline over the healed wound so that it does not become dry. I was asked to come for regular reviews.

After I was discharged from the hospital, my arrival at my village should have been joyous with celebrations. Rather, it turned into sorrow after discovering that my dad had died whilst I was in the hospital. In fact I wept a lot. I never knew he even died a year after my admission to the hospital and that the hospital authorities and my mum decided to hide it from me.
I was not only sad because my father had died, but how to continue my education since I could not do routine activities I used to do to raise some money. I was financially handicapped.

I thought life was still not treating me fairly. Churches, individuals and other benevolent persons were approached to help me in my education but yielded nothing.

Finally, the National Buruli Ulcer Control Programme upon my interaction with Dr. Asiedu and Dr. Etuaful came to my rescue and supported me to complete my two year secondary/high school. Thanks to the Matuoka Fund, Japan, I was able to finish my high school. I also got some support from certain individuals.

As a result of my determination to obtain higher education, I am happy to say that I am a second year student at in Kumasi Polytechnic in Ghana where I am enrolled in a Higher National Diploma (first degree) with focus on accounting. I hope to finish in about 2 years’ time. Again, I thank the Matuoka Fund in Japan for taking care of my educational expenses. I also thank individuals who are helping me in various ways. I hope to be a chartered accountant one day and begin to contribute to caring for BU victims.

Dear distinguished participants at this 10th anniversary meeting of the Global BU Initiative, I am honoured to be here today with you. I have never dreamt of flying let alone to come to a beautiful place like this. My joy started when I received my invitation letter, my ticket and more especially the day I got the Swiss visa in my passport. I asked myself whether have I been unfortunate or lucky. I can't answer this question. But I know there are others who have suffered like me but have not been fortunate like me to have an education.

To conclude, ladies and gentlemen, I am happy to be alive today and to be part of this important meeting. Although I cannot bend my right elbow nor fully raise my shoulder, these are minor problems to me compared to the suffering I have gone through. My story is not for myself. My story is for all those who have suffered, are suffering and will suffer as a result of Buruli ulcer. The neglect, the shame, the pain and humiliation that come with the disease can be very big. But was it or is it our fault to contract the disease or could we have done something better to avoid getting the disease? I am sure the distinguished audience gathered here today has no definite answer to these questions but, I know a lot of work has been done within the past 10 years. It is my hope that one day (not too long), you will have the right answers.

Through my story, let us unite in the common front to remove pain, shame, neglect and bring hope and smile to people like me.

Thank you all for your attention and God Bless you.

**Acknowledgments**

The Matuoka Fund, Japan; Dr. Samuel Etuaful; Dr. Kingsley Asiedu; Dr. Edwin Ampadu; Mrs. Ellen A.S. Whitney; Mr. Joseph Adomako; Mrs. Hiroe Soyagime 

Dr. Gerald Mumma; Mr. William Opare; Mag. Banko Orasche; Staff of St. Martin’s Hospital at Agroyesum and WHO
# My life at a glance

<table>
<thead>
<tr>
<th>Date</th>
<th>What happened</th>
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<tr>
<td>June 25, 1981</td>
<td>Date of birth</td>
</tr>
<tr>
<td>1986</td>
<td>Started primary school</td>
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<tr>
<td>1993</td>
<td>Completed primary school</td>
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<tr>
<td>1994</td>
<td>Started junior high school</td>
</tr>
<tr>
<td>1997</td>
<td>Completed junior high school</td>
</tr>
<tr>
<td>1998</td>
<td>Started senior high school</td>
</tr>
<tr>
<td>September 2000</td>
<td>Contracted Buruli ulcer</td>
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<tr>
<td>October 2000</td>
<td>Admitted to St Martin’s Hospital, Agroyesum</td>
</tr>
<tr>
<td>March 3rd 2003</td>
<td>Discharged from St. Martin’s Hospital</td>
</tr>
<tr>
<td>November 1st 2003</td>
<td>Re-entered into Acherensua Secondary School for two years</td>
</tr>
<tr>
<td>July 2005</td>
<td>Completed senior high school Acherensua Secondary school</td>
</tr>
<tr>
<td>November 2006</td>
<td>Entered Kumasi Polytechnic for HND (first degree-Accounting)</td>
</tr>
<tr>
<td>March 2008</td>
<td>Second year, second semester at Kumasi Polytechnic</td>
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<tr>
<td>March 30th –April 4th</td>
<td>Geneva, Switzerland for a WHO meeting on BU</td>
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Mycobacterium ulcerans infection in French Guyana from 1969 to 2007

Pr Pierre Couppié

Dermatology Service, Cayenne hospital centre; Antilles-Guyana Faculty of Medicine. French Guyana.

The tenth anniversary of the creation of the working group on Buruli ulcer provides an opportunity for an overview of the epidemiological situation and of the research under way in French Guyana.

Guyana is the area of the Americas in which incidence is highest. Between 1969 and 2007, the annual number of cases varied from 0 to 26. As the population increased three fold over this period, global incidence has declined during these 38 years, if we compare the periods pre- and post 1988. During the last three years, there have been only 2 cases per year.

In clinical terms, the predictive value of the « lower limb ulceration» is quite low in Guyana on account of the high frequency of cutaneous leishmaniasis, incidence of which is 10 to 50 times higher. A few cases of leishmaniasis- BU association have been reported at a previous meeting. For three years now, the treatment administered has been a rifampicin-amikacin combination. The four patients treated cured without the need for surgery.

A research project* associating the Cayenne Hospital Centre, IRD and the Pasteur Institute into variations in spatial and temporal case distribution is under way. The studies also concern detection of the bacterium in the environment. It has so far not been possible to isolate it.

*The EREMIBA project (funded by ANR)

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1 French Institute for Research and Development

2 French National Research Agency
Buruli ulcer (BU) is an endemic disease in French Guiana, a French overseas territory located north of Brazil, where BU cases have been recorded since 1965. In order to better understand the role of contacts with the environment in *Mycobacterium Ulcerans* transmission, a case-control study was set up within the dermatology department of Cayenne Central Hospital. The study included every BU patient admitted to Cayenne Hospital from January 2002 to July 2004. For each BU patient, two controls were selected among hospital patients treated for other disease. Cases and controls were matched on age and date of admission. Covariates included occupation, leisure activities, ethnic group, type of residence, geographical distance to several types of water bodies from living place and workplace, making a total set of about 100 covariates. The study was analyzed via conditional logistic regression, a method able to take into account several explanatory variables and their covariation. In this oral communication, we discuss the influence of the main factors that may explain BU disease transmission among French Guiana citizens.
From golf courses to possum traps - a 10 year review of the epidemiology of Buruli ulcer in Victoria, Australia


Austin Health; University of Melbourne; Victorian Infectious Diseases Laboratory; Monash University; Department of Primary Industries, Attwood.

In Australia there are currently active endemic foci of Mycobacterium ulcerans transmission in coastal Victoria (Bairnsdale ulcer), Far North Queensland (Daintree ulcer), and near Rockhampton in southern Queensland. Single cases also occur elsewhere in the wet tropics. However, it is mainly in temperate Victoria where Buruli ulcer has emerged as a significant public health problem.

Cases linked to the Bairnsdale region have been recognized since the 1930s, and continue to occur infrequently. Emergent disease is most associated with central coastal Victoria, 250 km further to the west, near the major population centers of the state. During an outbreak at Phillip Island (1992-5) we noted intense geographical clustering of cases only in the eastern half of the town of Cowes. Cases lived near or visited a newly formed wetland and a golf course that used a mixture of ground and recycled water to spray-irrigate the fairways. Drainage of the wetland and separation of recycled water from ground water at the golf course appeared to substantially reduce the number of new cases, and there are now only very occasional human cases linked to east Cowes [Veitch et al 1997].

The discovery of the M. ulcerans-specific insertion sequence IS2404 in 1995 allowed us to develop methods to identify M. ulcerans in environmental samples collected at the peak of the east Cowes outbreak. PCR-positive results from the golf course irrigation system and wetland seemed to confirm the hypothesis that M. ulcerans is a water-borne organism that may be transmitted by aerosol arising from contaminated aquatic environments [Ross et al 1997]. At least 6 possums with proven or suspected M. ulcerans infection were identified over the 5 years following the end of the human outbreak, and it was assumed that they had also been infected by exposure to contaminated water.

A decade later there have been two further significant outbreaks, involving the towns of St Leonard’s (1998-2002) and Point Lonsdale (2002-present). Both towns superficially resemble east Cowes—they are low-lying holiday resorts with sandy soils, wetlands and dense stands of Tea-Tree. Both have golf courses but neither used recycled water and the cases did not seem to cluster around the golf courses or local wetlands as they had at east Cowes. Furthermore, environmental PCR results obtained from a small lake in the centre of St Leonards and the golf course at Point Lonsdale were negative. Hence, we were initially unable to explain these outbreaks.

During the last 10 years the discovery of mycolactone, increasing knowledge of the genetics of M. ulcerans and the discovery of the plasmid encoding mycolactone production have combined to alter our understanding of the likely ecology M. ulcerans. Following reports of PCR-positive aquatic insects captured in endemic areas in west Africa, we set out to take a fresh look at transmission in Victoria by trapping and testing mosquitoes captured at Point Lonsdale. Over a 16-month period we showed that M. ulcerans DNA is detectable in 4.3/1000 mosquitoes on average, belonging to 5 different species, but mainly Aedes camptorhynchus (Southern salt marsh mosquito). A case-control study performed at the same time demonstrated increased odds of disease in those reporting a history of frequent mosquito bites, and reduced odds if insect repellent was used regularly [Quek et al, EID, 2007]. We then focused on possible sources of mosquito contamination. The further adaptation of the original IS2404 environmental PCR to a real time format enabled us to not only detect M. ulcerans but also to estimate quantity. With this tool, we estimated that PCR +ve mosquitoes may harbor 10-100
cells per individual. While searching for a reservoir of *M. ulcerans* in the environment that could lead to contamination of mosquitoes, we collected and tested several samples of possum excreta and showed that they often high concentrations of *M. ulcerans* DNA, in one sample we estimated > 10⁷ *M. ulcerans* cells per gram. We have since identified *M. ulcerans* in the gut of one brush tail possum that died naturally, and directly cultured the organism from its liver. At the time of death, the possum did not display any evidence of cutaneous ulcerative disease. By performing surveys of possum excreta obtained in endemic and non-endemic areas we have now shown that the presence of high concentrations of *M. ulcerans* DNA in possum excreta is epidemiologically linked to outbreaks of human disease.

The ability to study new naturally occurring outbreaks, improved technology and a new understanding of the likely ecology of *M. ulcerans*, has led us to develop a completely new hypothesis to explain the extraordinarily focal nature of *M. ulcerans* outbreaks in coastal Victoria. We now propose that *M. ulcerans* initially colonizes and spreads among possums, and that humans become infected through transmission via mosquitoes. Whether mosquitoes directly transmit the organism from possums or pick it up passively through contact with soil or water contaminated by possum excreta has yet to be determined. In 2008 with funding support from DHS Victoria, we plan to begin investigating this new hypothesis by trapping, testing, tagging and releasing possums in the Point Lonsdale endemic area.
Study of risk factors for Buruli ulcer and awareness-raising campaigns: the experience of Cameroon

S. Eyangoh,1 C. Nsom Mba,2 A. Um Boock,3 J. Aubry4

1 Mycobacteria laboratory, Centre Pasteur (Cameroon); 2 National Buruli Ulcer Control Programme/Ministry of Public Health, Cameroon; 3 Regional Office for Africa, Leprosy Relief Emmaus-Switzerland, Cameroon; 4 University of Nantes, U601 Inserm, F-44000 Nantes.

Epidemiological data link Buruli ulcer (BU) with wetland aquatic environments but are unable to pinpoint the associated human activity. The reservoir and mode of transmission of the mycobacterium and the mechanism by which the disease develops are still imperfectly understood. It is thus difficult to propose and introduce effective prevention strategies.

Over the past 10 years or more, a number of studies have been carried out in endemic countries to identify risk factors associated with human behaviours and the environment, principally in Ghana (1993, 2004 and 2005), Côte d'Ivoire (1995) and Benin (2006a, 2006b). These studies have identified certain risk factors and methods of primary prevention for populations at risk, such as wearing long clothing in Côte d'Ivoire and using protected water sources in Benin, but no education or awareness-raising campaigns have been undertaken or reported.

In Cameroon, research into BU risk factors carried out in 2007 revealed that wearing long clothing while working in the fields, using a mosquito net and proper wound care could afford protection against BU. These findings were then applied in practice by means of new public information campaigns, awareness-raising days in endemic areas, and radio and television programmes and articles in the press, with a view to informing and educating local people about simple primary prevention methods.

Ten years after the launch of the Global Buruli Ulcer Initiative, and pending clarification of the mode of transmission of the disease, hygiene and education ought to be considered as new weapons in the fight against BU. New messages could thus be immediately developed and disseminated among the populations concerned, as is being done in Cameroon.
Challenges of determining the epidemiological profile of *Mycobacterium ulcerans* disease (Buruli ulcer) in DRC: the Kasongo experience


In 1950, the first laboratory confirmed Buruli ulcer (BU) case on the African continent was reported by Van Oye and Ballion in the Democratic Republic of Congo (DRC). Thereupon, more than 500 BU cases had been reported in 6 dispersed foci in DRC before 1980. From 2000 until present, after a 20 year period with no notified cases, an increasing number of laboratory confirmed cases have been reported in previously described foci in Bas-Congo and Bandundu Province. However, no recent data are available from other historical foci of this huge country. Furthermore, poor financial and logistic resources hampered the PNLUB-DRC to conduct any significant epidemiologic survey since its creation in 2002.

In this presentation we will (i) discuss the general and specific difficulties to determine the exact prevalence and burden of BU in DRC and (ii) formulate specific recommendations for DRC. Examples will illustrate current efforts, especially the last Kasongo-experience:

From 2 to 9 October 2007, fifty years after the first report of BU, we conducted a preliminary cross-sectional survey in the Kasongo focus, Maniema Province, East DRC.

This small-scaled assessment of BU was part of a larger study on the ecological niche of *M. ulcerans*. Two out of 28 clinically diagnosed BU active cases were laboratory confirmed. Therefore, we assume that the Kasongo area is still an active, but low prevalent, BU focus.
Osteomyelitis in *Mycobacterium ulcerans* disease: A review of of 106 patients treated in Zagnanado (Benin)

*Portaels F*, *Aguiar J*, *Debacker M*, *Johnson C*, *Meyers WM*

1 Institute of Tropical Medicine, Antwerp, Belgium  
2 Centre Sanitaire et Nutritionnel, Gbemoten, Zagnanado, Bénin  
3 Buruli Ulcer National Control Program, Cotonou, Benin  
4 Armed Forces Institute of Pathology, Washington DC, USA

Buruli ulcer (BU) is best known for its characteristic cutaneous lesions. *M. ulcerans*, however, often also causes serious lesions in bone, particularly in Africa.

Thus far, bone lesions have received little attention so far, even though in Benin, as many as 13% of all BU cases have osteomyelitis (Debacker et al., 2003).

From 1996 until 2007, 106 cases of *M. ulcerans* osteomyelitis, confirmed bacteriologically and/or histologically, were analyzed. All patients were treated by surgery (excision, curettage and skin grafting) at the CDTUB (Centre de Traitement de l’Ulcère de Buruli) of Zagnanado (Benin). Moreover, 22 of them received antibiotic treatment (rifampin and streptomycin) for 8 to 12 weeks depending on the severity of the disease (one week before surgery followed by 7 or 11 weeks after surgery).

A total of 27 patients (25.5%) initially presented at the CDTUB without bone lesions. Of these 27 patients, 11 developed bone lesions during hospitalization and 12 after the skin lesions had healed.

A total of 31 patients (29.2%) presented on admission to the hospital with only a single bone lesion and 48 patients (45.3%) presented with metastatic osteomyelitis.

The median hospital stay was 56 days for patients with bone lesions, compared to 46 days for those with skin lesions only, seen during the period of this study (Mann-Whitney test, p < 0.001).

Delay in presentation to the hospital following onset of disease was 167 days for those with osteomyelitis and 61 days for those with skin lesions only (Mann-Whitney test, p < 0.001).

Of the 106 patients, 101 were cured by surgery, and 5 patients died: 3 due to complications unrelated to BU and 2 because of other opportunistic infections related to HIV positivity. Amputation of a limb (13 patients) or portions thereof (8 patients) was required.

Antibiotic treatment with rifampicin and streptomycin for at least 8 weeks did not prevent dissemination of *M. ulcerans* to other bones. Indeed, 12 out of 22 (54.5%) patients who were treated by surgery plus antibiotherapy developed additional bone lesions during or after the medical treatment, while 50 out of 84 (59.5%) patients treated only by surgery developed additional bone lesions after the first surgical intervention.

Patients were followed up by visits to the village or by check-up at the hospital. The median period for follow-up was 3 years (1-12 years).

The recurrence rate of patients with bone lesions after a follow-up of up to 12 years was 16.0% (17 patients out of 106) but only 6.6% occurred within 1 year of completing treatment (7 patients out of 106).
Risk factors for bone lesions were identified:

- presence of a scar typical of BU that had not been treated surgically
- a markedly prolonged delay in presentation to the hospital
- absence of BCG vaccination (Portaels et al., 2004)
- coexistence of other tropical diseases such as schistosomiasis (Scott et al., 2004), sickle cell anemia (Nackers et al., 2007)

A case-control study suggested that HIV infection does increase the risk for BU. However, bone involvement was not found to be more frequent among HIV-positive patients (Johnson et al., 2008).

Comparison between *M. ulcerans* strains isolated from patients with and without bone lesions did not reveal differences in virulence for mice nor in their ability to grow at 37°C (Eddyani et al., 2007).

Other risk factors such as genetic factors and the immune status of BU patients deserve more investigation to explore their possible influence on the development of bone lesions.

References


Contribution to BU control efforts of the NGO Water for All Children

Solange Yayi Allechi

Owing to their extent and seriousness, diseases caused by the environment represent a significant public-health problem, particularly in Africa. In many African countries, health systems must operate in a constantly deteriorating environment where endemic and epidemic diseases proliferate. Women and children are those principally affected. Civil society in Africa is gradually organizing itself to tackle these health problems. Against this backdrop, the NGO Water for All Children, which was founded in the United States in 2007, is pursuing the dual objective of controlling waterborne diseases by installing water and sanitation infrastructure, and helping to control Buruli ulcer.

As part of its interest in BU, Water for All Children intends to pursue initiatives in accordance with the strategies defined and recommended by the World Health Organization. Accordingly it has focused its efforts on BU detection and treatment centres in Benin, specifically ensuring the availability of information, education and communication materials. Strategically, the NGO is planning to focus on two priority areas:

- Advocacy of BU control efforts vis-à-vis decision-makers in endemic countries and social mobilization against the disease;
- Social and occupational rehabilitation and reintegration of former BU patients.

As part of its advocacy efforts, Water for All Children has lobbied the highest political authorities in Benin in the cause of BU awareness. This lobbying has paid off. Thanks to the advocacy of Water for All Children, the President of the Republic of Benin, His Excellency Dr Yayi Boni, has agreed to convene and sponsor an international conference on BU. The conference, to be held 10 years after the Yamoussoukro Conference, will bring together the most senior political authorities in the endemic countries and all stakeholders involved in BU control. The President of the Republic of Benin, through Water for All Children, is counting on the support and mobilization of all stakeholders to ensure the success of this major advocacy event in the field of BU control.
ALM’s involvement with Buruli ulcer: a ten-year review

Paul Saunderson, American Leprosy Missions

In 2000/2001, ALM developed links with MAP in Côte d’Ivoire and Dr Pius Agbenorku in Ghana. In Côte d’Ivoire, ALM support went initially to institutions which were already treating patients. Over the last three years we have assisted MAP in developing a community program for early diagnosis and decentralized treatment, at Taabo, in Tiassalé District, an area previously without any local facilities for managing BU. This has progressed well and through TLM-Ireland, funding to scale up this work to 5 new areas has been granted by Irish Aid.

In Ghana, the focus initially was on training of surgeons and their support staff in district hospitals. This was quite successful in making treatment more widely available in district hospitals. In order to develop other work in Ghana (for example, community-based early case detection and treatment), we are working with MAP to set up an office in Ghana.

During the period 2001-4, ALM supported research into serological diagnosis at Emory University. ALM also acted as a conduit for funds from a donor in the US for two building projects – BU wards at Treichville University Hospital in Abidjan and at Apromase Hospital in Ghana.

ALM started to support I.M.E. Kimpese, in Bas Congo, in 2004. Support has been in two parts: firstly support for the treatment of cases in the hospital and secondly, support for a community outreach program to train peripheral health staff in early diagnosis and treatment.

ALM now focuses particularly on two main issues: disability prevention and community-based early case detection. Support for training and for WHO publications in these fields has also been given. It has been very positive to find that our knowledge and experience from working in leprosy have been helpful in the development of appropriate strategies in BU.
Involvement of the Luxembourg Raoul Follereau Foundation in Buruli ulcer control: progress since 1998 and outlook

Emile China

Since the launch of the Global Buruli Ulcer Initiative in 1998 by the World Health Organization, the Luxembourg Raoul Follereau Foundation, in support of its partner organization the Raoul Follereau Association of Benin, has stepped up its involvement in controlling and managing BU and reducing the adverse social and economic impact of the disease.

Thus in 2002, with funding from the Luxembourg Raoul Follereau Foundation and following a feasibility study, a Buruli ulcer detection and treatment centre with capacity for 30 patients was opened at Allada. Five years on, the centre handles 10 times as many patients every year (from 30 patients in 2002 to over 350 in 2007). In addition, 10 peripheral centres dispense outpatient care to BU patients, with financial support from the Luxembourg Raoul Follereau Foundation and supervised by the Allada BU detection and treatment centre. As a result of these initiatives, the Raoul Follereau Association of Benin and the Luxembourg Raoul Follereau Foundation have been able to address the issue of availability of patient care in the Atlantique and Littoral departments of Benin, and partially to address the issue of accessibility of care, in the spirit of the recommendations of the Yamoussoukro Conference.

In order to make treatment affordable to patients, the Luxembourg Raoul Follereau Foundation continues to support the BU detection and treatment centre and its peripheral centres in respect of medicines, consumables, technical medical equipment and transport.

It also promotes:

- Prevention through local and mass detection and communication activities targeted at different populations;
- Quality of care by strengthening the technical capacity of health workers through training at the Allada BU treatment centre and its peripheral facilities.

These initiatives by the Luxembourg Raoul Follereau Foundation, acting through the Raoul Follereau Association of Benin, have been successful in fully implementing the strategies proposed by WHO.

The Luxembourg Raoul Follereau Foundation is going beyond these strategies by providing support for psychosocial treatment, as specified in the National BU Control Programme, through promotion of patient nutrition, care of destitute patients and abandoned children, school enrolment and supervision of patients embarking on income-generating activities.

These initiatives will continue with a contribution to the research component, through improved working conditions, better internal and external communications (already operative in the centre in 2007), and proper documentation of activities.

The Allada BU detection and treatment centre now has a broadband satellite internet (VSAT) connection, offering every advantage in the fields of data management and telemedicine.
The extension programme currently under way will bring new benefits to the Centre in 2008. This programme will enable the Allada BU detection and treatment centre to:

- Set up a training unit comprising a training room, workshops, an Internet room and a refectory;
- Improve working conditions of health workers in hospitals, physiotherapy units, laboratories and dressing stations, and the management of clean and dirty areas in operating theatres;
- Inaugurate a guest house to accommodate the various actors and foreign trainees at the Centre;
- Provide patients and home carers with proper facilities for social activities.
Implementation of treatment with rifampicin and streptomycin in Bas-Congo: The experience of the Kimpese Evangelical Medical Institute hospital

Phanzu M.D., Imposo B.B.D., Saunderson P., Portaels F.

Kimpese Evangelical Medical Institute hospital, Bas-Congo, Democratic Republic of the Congo

Until very recently, the only possible treatment for Buruli ulcer was extensive surgical excision, with or without skin grafts.

The Kimpese Evangelical Medical Institute hospital began implementing WHO provisional guidance on rifampicin-streptomycin combination treatment in March 2005.

To date, 128 patients have been given this specific antibiotic treatment, 23 in their villages and 105 at the Kimpese Evangelical Medical Institute itself (compared with 15 out of a total of 41 BU cases in 2005, 36 out of 74 cases in 2006, and 54 out of 75 cases in 2007).

The purpose of this preliminary study is to assess the impact of this treatment in the Kimpese health zone. In the light of experience to date, the assessment will focus on the following essential points (noted by the Technical Advisory Group in the presentation given by Dr Mark Wansbrough-Jones, WHO, April 2007):

- Most BU lesions are cured after 8 weeks of R+S treatment;
- 8 weeks' treatment are sufficient;
- Additional surgery is sometimes needed to accelerate healing of ulcers during or after antibiotic treatment;
- Some extensive ulcerations eventually heal without surgery after sufficient medical treatment;
- The relapse rate is very low (< 2%) following administration of R+S alone or in combination with surgery;
- Side-effects are rare and benign where observed;
- Best results are obtained by forming a team of health workers in an endemic district under the supervision of a dedicated team leader;
- Antibiotics are administered to outpatients not requiring surgery, under the close supervision of local health workers;
- It is advisable to maintain close links with a hospital able to provide specialist services and surgical backup.

Additionally, note will be made of problems arising from implementation of the current WHO guidelines, as well as any specific clinical problems encountered.
RCT for early *Mycobacterium ulcerans* disease comparing 8 weeks treatment with streptomycin and rifampicin, and 4 weeks treatment with streptomycin and rifampicin followed by 4 weeks treatment with clarithromycin and rifampicin – Interim Analysis

**WA Nienhuis¹, Y Stienstra¹, WA Thompson², PC Awuah³, EO Ampadu⁴, NY Awua_Boateng⁶, V Siegmund⁵, G Bretzel⁵, O Adjei⁶, B Fleischer⁷, TS van der Werf¹**

1 Universitair Medisch Centrum Groningen (UMCG), the Netherlands
2 Agogo Presbyterian Hospital, Ghana
3 Nkawie-Toase Governmental Hospital, Ghana
4 NBUCP, Accra, Ghana
5 Department of Infectious Diseases & Tropical Medicine (DITM), Munich, Germany
6 Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), KNUST, Ghana
7 Bernhard Nocht Institute for Tropical Medicine (BNITM), Hamburg, Germany

**Objective**

To evaluate the efficacy of antibiotic therapy with rifampicin and streptomycin given for 8 weeks, and with rifampicin and streptomycin given for 4 weeks followed by an oral combination of clarithromycin and rifampicin, in confirmed, early stage *M. ulcerans* infection in patients over five years of age from Ghana. The ultimate goal is to search for an effective alternative treatment to radical debridement surgery, and to explore possibilities to minimize the use of injectable antimicrobial therapy.

**Study design**

**Inclusion criteria:** patients =/> 5 yrs with early, limited BU disease (onset < 6 months and diameter < 10 cm)

**Confirmation:** 3 punch biopsies for 1) PCR, 2) culture and ZN staining, 3) histopathology. In ulcerative lesions, also 2 swabs for 1) PCR and 2) culture and ZN staining are taken

**Primary clinical end point:** cure (no need for debridement surgery and no recurrence) at 12 months after starting antimicrobial treatment

**Failure:** increase in size of lesion (> 150% of initial surface area); non-closure at 12 months after starting treatment; debridement surgery for any other reason

**Sample size calculation:** 80% power to detect >20% difference (α = 0.05) in cure / failure rate between SR8wk and SR4wk/CR4wk after follow up = 12 months - 148 fully evaluable patients with confirmed early, limited *M. ulcerans* infection to be randomized.
Study sites

Patient recruitment started April 2006 at Nkawie-Toase Governmental Hospital and June 2006 at Agogo Presbyterian Hospital; both Ashanti Region, Ghana.

Treatment and follow up

Treatment (8 weeks in total) is given to outpatients; DOT strategy in the nearest health post. Weekly clinical assessment by BU team in hospital with digital photography. Fortnightly assessment of diameter and surface area of lesion, together with blood tests and hearing tests for toxicity screening.

After treatment, follow up is fortnightly for the first month, thereafter monthly, in total 12 months from start of treatment.

Interim results (dd. 25/01/2008)

No. of screened patients: 198.

No. of confirmed and randomized patients: 144. As follow-up is still in progress for most patients, and only 61 completed 12-mo follow-up, calculations still have wide CI.

No. of patients lost to follow at 12-mo follow-up: 4

No. of patients failed on treatment: 8 (3 treated with debridement surgery because of progression to >150% of initial lesion size, one of these lesions still positive by culture; 1 treated with 4 more weeks of SR because of suspected relapse, not confirmed by culture; 1 treated with small debridement surgery because of opening of scar, not confirmed by culture; 3 not healed after 52 weeks of inclusion, one of these lesions still positive by culture).

No difference in failure rate can be seen yet in the treatment arms.

No nephro- or hepatotoxicity has been observed. No hearing impairment has been detected.

Observations

Pre-ulcerative lesions tend to ulcerate during or even after the 8 weeks treatment period. After ulceration, lesions close spontaneously.

‘New’ lesions (n=7) developed during or after treatment. These new lesions (except for one which was excised) were not examined by punch biopsy, but ulcerated, closed and healed spontaneously without further intervention except for wound dressings.

We hypothesize that either a paradoxical reaction, or an immune response to dead bacilli, can be the cause of late or paradoxical ulceration. As mycolactone production seizes with antimicrobial therapy we believe that, considering the quick wash-out of mycolactone from tissues, ongoing damage by residual mycolactone molecules is less likely to be the cause.
Treatment of Buruli ulcer patients in Ghana with the combination rifampicin-streptomycin for 7 days per week for 8 weeks.

Phillips R1,2, Sarfo FS1, Nsiah R1, Dinko B1, Opare W3, Boateng A4, Adentwe E4, Asiedu K6, Wansbrough-Jones M5

1Komfo Anokye Teaching Hospital, Kumasi, Ghana.
2School of Medical Sciences, KNUST, Kumasi, Ghana
3National Buruli ulcer Control Programme, MOH, Ghana
4Tepa Government Hospital, Ahafo Ano North District, Tepa, Ghana
5St George’s University of London, London
6WHO Geneva Switzerland

Introduction

The combination of rifampicin and streptomycin administered on 7 days per week for 8 weeks (RS7) is the antibiotic regimen currently recommended by the WHO for treatment of humans with Buruli ulcer caused by Mycobacterium ulcerans (Mu) infection. The aim of this study was to determine the success of RS7 in healing Buruli ulcer, reducing viability of M. ulcerans and preventing recurrence of M. ulcerans disease.

Methods

Between September 2005 and December 2006 in a prospective study patients were recruited from the Ahafo Ano North District of Ghana and active Mu disease was diagnosed by microscopy, culture and PCR on 4 mm punch biopsies and/or fine needle aspirates. RS7 was administered to 108 patients. A further biopsy was cultured after 6 and 12 weeks if the lesion was still visible. Clinical response was assessed 2 weekly by measuring the surface area of lesions until healing was complete. Subjects were followed up monthly for 12 months after treatment.

Results

Among 108 patients with confirmed Mu disease, 51 had lesions <5cm diameter (category 1), 34 had lesions 5-15cm diameter (category 2) and 19 had lesions >15cm diameter (category 3) were treated with RS7. Four patients were lost to follow-up so the analyses were done on 104 patients. After 8 weeks, at completion of treatment, 22 (43%) category 1, 12 (35%) category 2 and 1 (5%) category 3 lesion had completely healed. The percentage cumulative healing was 41% for all lesions at 8 weeks increasing to 71% at 12 weeks, 85% at 16 weeks and 97% at 20 weeks. At the 8th week when treatment was complete 35 out of 43 unhealed Category I & II lesion size had significant reduction in size (>50%). At 12 weeks all 17 Category I & II lesions had >50% reduction in surface area.

All category I lesions were healed by the 16th week, category 2 lesions by the 20th week and category 3 lesions by the end of the 28th week without surgery and there were no recurrences 12 months after treatment. One subject with a category 2 ulcer developed a nodular lesion at another site on completion of treatment but it regressed without further RS treatment. Cultures from a punch biopsy were negative and no AFB were seen but PCR and histopathology were not done.

Six positive cultures were obtained out of 66 from whom tissue were obtained during (3 at week 6) or after RS7 administration (3 at week 12). The 3 positive cultures obtained at week 12 were all from
category 3 lesions. These lesions went on to healing with no recurrence to date. Drug susceptibility testing have been planned on these isolates.

**Conclusion**

Standard WHO treatment with RS7 was effective in healing category 1-3 forms of Buruli ulcer without surgery in 104 patients with no recurrences after 12 months despite positive cultures at 6 and 12 weeks in 6 patients.

**Acknowledgement**

Support from the World Health Organisation is gratefully acknowledged.
Oral drug regimens achieve bacteriological cure and prevention of relapse in a mouse model of Mycobacterium ulcerans disease

Deepak V. Almeida, Eric L. Nuermberger, and Jacques H. Grosset. Johns Hopkins University, Baltimore, MD 21231.

Rationale

The current recommended therapy for M. ulcerans (Mu) disease or Buruli ulcer (BU) includes daily treatment with an aminoglycoside, usually streptomycin (SM), and rifampicin (RIF) for two months followed, if necessary, by surgical excision and/or skin grafting. Since many patients with BU reside in remote areas with limited medical facilities, development of a completely or partially oral drug regimen would make treatment cheaper and easier to implement. Use of rifapentine (RPT), a rifamycin derivative with a longer half life than RIF, may enable greater rifamycin exposure and more active regimens.

Aim

To develop an effective oral or partially oral regimen for treatment of BU with currently available drugs

Methods

We conducted two separate experiments to evaluate new treatment regimens for BU.

Experiment 1: 137 Balb/C mice were infected in the right hind footpad with 3.82 log₁₀ cfu of M. ulcerans. Treatment was started seven weeks later upon footpad swelling, when there were 6.33 log₁₀ cfu/footpad. Untreated mice were used as negative controls while the remaining mice were treated with either RIF+SM or RPT+SM for 4 or 8 weeks. Mice were sacrificed at start of treatment and at wks 4, 6 and 8 for cfu counts, and in each treatment group 20 mice were held for observation for 9 months after treatment completion. End points were the change in footpad swelling and cfu counts during treatment, and relapse rate in the follow up period after treatment completion.

Experiment 2: 197 Balb/C mice were infected with 3.94 log₁₀ of M. ulcerans. Untreated mice were used as negative controls and the rest were initiated upon footpad swelling 9 weeks after infection on treatment with various combinations of SM, RIF, RPT and clarithromycin (CLR) for 8-12 weeks. Cfu counts were done at the start of treatment, 4, 8 and 12 weeks, and 20 mice in each group were held for observation for 9 months after treatment completion. End points were the change in footpad swelling and cfu counts during treatment, and relapse rate after treatment completion.

Results:

In experiment 1, untreated control mice exhibited deterioration in footpad swelling and plateau in cfu counts. All treated mice exhibited reduction in footpad swelling. At week 4, four out of five mice treated with RIF+SM were culture negative and the fifth mouse had 8 cfu; all five mice treated with RPT+SM were culture negative. At the weeks 6 and 8 all mice in both treatment groups were culture negative. Among mice kept for nine month observation, none relapsed after 4 and 8 weeks of treatment in both the groups.

In experiment 2, the cfu count at treatment initiation was 6.6 log₁₀ of M. ulcerans. Untreated control
mice exhibited deterioration in footpad swelling and plateau in cfu counts. All treated mice exhibited reduction in footpad swelling. At week 4, all mice treated with RIF+SM were culture negative; the 6 mice treated with 2wk of RIF+SM followed with 2wk of RIF+CLR, and the 6 mice treated with 4wk of RIF+CLR were also culture negative; among the six mice treated with 4 wk of RPT+CLR, one mouse had a single CFU while the other 5 were culture negative. At week 8 mice in all treatment groups were culture negative. The mice treated with 12 weeks RIF+CLR were also culture negative. Among mice kept for 9 months observation (after treatment completion), none relapsed in any of the treatment groups.

**Conclusion**

Based on our results the combination of rifapentine and clarithromycin well substitutes for the combination rifampicin and streptomycin in the curative model of *Mu* disease in Balb/c mice. Further studies are needed with respect to the mouse model used, because our current results do not fit well with previous findings using the kinetic model of C. Sheppard. It is possible that host response to the disease obscures the relative activities of different drug regimens.
Bactericidal and sterilizing activities of several orally-administered combined regimens against *Mycobacterium ulcerans* infection of mice


Treatment with rifampin-clarithromycin or moxifloxacin-clarithromycin for 8 weeks displayed promising bactericidal activity against *M.ulcerans* in mice; none of the mice treated with rifampin-clarithromycin relapsed, whereas 59% of those treated with moxifloxacin-clarithromycin relapsed after stopping treatment. The bactericidal and sterilizing activities of the five-times-weekly (5/7) administration of rifapentine 5 mg/kg, either alone or in combination, were virtually identical to those of the corresponding regimens containing rifampin 10 mg/kg; however, because of the long half-life of rifapentine, accumulation of the drug after 5/7-administration is a concern. The bactericidal activity of the twice-weekly (2/7) administration of rifapentine 20 mg/kg, either alone or in combination, was at least as effective as 5/7-administration of the corresponding regimens containing rifampin 10 mg/kg, suggesting that Buruli ulcer might be treated with intermittently-administered rifapentine-containing combinations.
Hyperbaric oxygen therapy as a complementary treatment in Buruli ulcer

Franco Poggio, Christian Johnson, Ghislain Sopoh, Ange Dossou, Claudio Clemente, Giorgio Leigheb, Elisa Zavattaro

The Allada Hyperbaric Therapy Centre in Benin, the fruit of a partnership between the Luxembourg Raoul Follereau Foundation and the Milan Aquileia Rotary Club, supported by other Italian Rotary Clubs (RC of North Bergamo) and the Rotary Club of New York, has been in operation since 2005.

Many of the amenities were improved in 2007: the air conditioning system was overhauled, the premises were soundproofed to minimize compressor noise, and the outside gate was thermally insulated.

The object of the therapy provided by our Beninese colleagues at Allada Luxembourg Hospital is the treatment of ulcerative skin lesions, specifically Buruli ulcer (hereafter BU). Patients are selected for treatment not only based on an assessment of the seriousness of their clinical picture, but also on the protocol of a pilot study focusing on a theme that has been raised on many occasions during meetings in Geneva, namely a randomized controlled trial designed to explore the efficacy of hyperbaric therapy in conjunction with conventional antibiotic treatment (a combination of rifampicin and streptomycin, with surgery where appropriate) in patients with various stages of BU.

Treatment involves 5 days of hyperbaric therapy a week for 6 weeks and follow-up treatment lasting up to 3 months. The control group is treated with antibiotics only, and surgery where appropriate.

Unfortunately, frequent power cuts in Allada in the course of 2007 have made it difficult to carry out this study. The possibility of equipping the hyperbaric centre with a dedicated electric generator is being looked into.

On the basis of experience to date, as described in the report presented in Geneva in 2007, and notwithstanding the above-mentioned problems, we conclude that hyperbaric therapy, when combined with antibiotic treatment and surgery, should be considered a useful supplementary therapy in cases of BU, particularly Category III cases (i.e. extensive ulcerative lesions, multiple lesions or propagation of the disease). It is of course desirable that the study should be properly completed in order to form a definite opinion regarding the efficacy of complementary hyperbaric therapy in reducing recovery time and achieving better anatomical and functional outcomes.
Thermotherapy of Buruli ulcer revisited: Results of a pilot trial using phase change material as a heat application system.

Junghanss T\textsuperscript{1}, Um Boock A\textsuperscript{2} Weinlaeder H\textsuperscript{3}, Pluschke G\textsuperscript{4}

\textsuperscript{1}Section Clinical Tropical Medicine, University Hospital, Heidelberg, Germany
\textsuperscript{2}ALES, Bureau Régional pour l’Afrique, Yaoundé, Cameroon
\textsuperscript{3}Bavarian Center for Applied Energy Research, Thermal Insulation and Heat Transfer, Weurzburg, Germany
\textsuperscript{4}Dept. Medical Parasitology / Infection Biology, Swiss Tropical Institute, Basle, Switzerland

In a proof of principle study we adapted and explored an innovative heat application system (phase change material [PCM]) to treat \textit{Mycobacterium ulcerans} disease. The device is easy to apply and to recharge in hot water, non-toxic, non-hazardous to the environment and cheap. The clinical study was carried out in Ayos (Cameroon). Ethical clearance has been granted by the ethical committees of Yaounde and Heidelberg University Hospital. We enrolled seven patients clinically diagnosed as having ulcerative BU lesions (WHO 2001). The diameters of the ulcers ranged between 1.0 and 8.7 cm with various degrees of undermining of edges and of surrounding oedema. Clinical diagnosis was confirmed in 6 out of the 7 patients initially enrolled by microscopic detection of acid-fast bacilli (AFB) after Ziehl Neelsen staining, histopathological changes typical for BU and by IS2404 real-time PCR from swabs of the undermined edges and punch biopsies. In all patients undermined edges collapsed within three days. In patients with smaller ulcers (n=4) wounds healed completely without further intervention. Patients with large defects (n=2) had split skin grafting after successful heat treatment. All patients will be followed-up over 18 months for relapses. At month 9 after completion of treatment (January 2008) all patients were relapse-free. The proof of principle study was supported by the Volkswagen Foundation.
Early case-detection in the community is crucially important to achieve the objectives of Buruli ulcer control programmes, i.e. to reduce morbidity and sequelae.

In endemic zones, early detection should be at the peripheral health facility level and focuses primarily on clinical diagnosis. Laboratory case confirmation is of secondary importance.

It is important to define clinical criteria that make it possible to recognize all patients affected by Buruli ulcer.

Early detection using clinical criteria in endemic countries has been helpful in controlling advanced forms of the disease (categories II and III). The Taabo zone in Côte d'Ivoire furnishes one such example.

These results have been obtained by selecting simple yet relatively sensitive clinical criteria for detecting the disease.

Improving the sensitivity of clinical criteria is the central issue in early detection because of the importance of this intervention in controlling Buruli ulcer, the absence of biological confirmation tests at peripheral health facilities and the more extensive use of antibiotic treatment.
Taking into account the differential diagnosis is an important part of improving the quality of programmes in the treatment of infections with Mycobacterium Ulcerans. The approach vertical programmes with poorly trained staff to dermatology in remote area explains why a skin ulcer is easily labeled Buruli without really make a broader diagnostic approach.

The introduction of a therapy (streptomycin, rifampicin for two or three months) that can have side effect should encourage to detect differential diagnoses in order to give appropriate treatment.

The biological confirmation of Buruli ulcer is a rule to be applied rigorously. The development of PCR rapid diagnostic test would provide a better diagnostic sensitivity.

Some programs have problems using methods of diagnostic confirmation. Moreover sensitivity Ziehl Nielson is not very high.

The training for a better recognition of different etiologies is needed to improve the treatment of ulcers. In this regard, we have made a poster summarizing the major differential diagnoses forms ulcerated infection with Mycobacterium Ulcerans because they are the most frequently encountered.

Just like methods in the detection of tuberculosis BK negative, we have also introduced a clinical score for diagnosis of Buruli. We présent the first results of using this score. The validation of this tool would objectify characteristics for Buruli and assist clinicians in their decision.
BURULICO: Two years of experience with different diagnostic tools for Buruli Ulcer Disease (BUD) in Ghana

Karl-Heinz Herbinger1, Ohene Adjei2, Nana Yaa Awua-Boateng3, Leticia Kunau2, Jörg Nitschke1,3, Vera Siegmund1, Willemien Nienhuis4, William Thompson5, Erasmus Klutse6, Pius Aghenorku7, Paul Racz2, Bernhard Fleischner, Alexander Schipf8, Simone Reu8, Thomas Lüschert1, Gisela Bretzel1

1Department of Infectious Diseases and Tropical Medicine (DIKM), University of Munich, Germany; 2Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kwame Nkruma University of Science and Technology (KNUST), Kumasi, Ghana; 3Bernhard Nocht Institute for Tropical Medicine (BNIT), Hamburg, Germany; 4University Medical Centre Groningen (UMCG), The Netherlands; 5Agogo Presbyterian Hospital, Agogo, Ghana; 6Dunkwa Governmental Hospital, Dunkwa-on-Offin, Ghana; 7Reconstructive Plastic Surgery & Burns Unit, Department of Surgery, Komfo Anokye Teaching Hospital, School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana; 8Institute of Pathology, University of Munich, Germany

1. Baseline Data

From January 2006 until December 2007, 410 sets of specimens (550 swab specimens, 903 tissue specimens) from 354 BUD suspects (39.8% male, age 1-95 years, mean 21.1 years) with 286 ulcerative (69.8%) and 124 non-ulcerative lesions (30.2%) from nine treatment sites in Ghana (20 districts) were subjected to laboratory analysis within the BURULICO project. One hundred seventy-nine patients (50.6%) were screened for BUD within the drug trial (DT), 109 patients (30.8%) were treated by surgery only (ST), 66 patients (18.6%) received rifampicin and streptomycin (duration of treatment ranging from 3 to 120 days, mean duration 35.0 days) before surgery (ST+ antibiotics).

2. Laboratory confirmation and differential diagnosis

Out of the 354 BUD suspects 264 (74.6%) (39.8% male, age 2-80 years, mean 19.2 years, covering 19 districts) were confirmed by at least one, and 168 (47.6%) by at least two positive laboratory tests. 44.8% (139 out of 310 tested suspects) had a positive swab and/or tissue microscopy result. One hundred thirty six (97.8%) out of those microscopy results were confirmed by other tests, for 3 results other confirmatory tests are not available. 59.6% (205 out of 344 tested BUD suspects were confirmed by swab and/or tissue IS2404 PCR. 31.1% (92 out of 296 tested BUD suspects) had a positive swab and/or tissue culture (for 90 patients the cultures were confirmed by IS2404 PCR, 2 by other methods). Four of the laboratory confirmed patients had co-infections with other mycobacteria (M. mucogenicum, M. szulgai, M. phocaicum, M. gordonae). Only tissue samples from BUD suspects with negative microscopy, culture, and PCR were submitted to histopathological analysis. Histopathology confirmed 16 (30.2%) of these 53 patients as BUD (7 (13.2%) of them as “healing stages”). Analysis of 35 microscopy, PCR and culture negative samples from BUD suspects with pre-ulcerative lesions revealed 8 (22.9%) onchocercosis cases.

3. Diagnostic sensitivities of diagnostic specimens and laboratory tests among laboratory confirmed patients

Among 264 laboratory confirmed BUD cases 57.0% (139 out of 244) were confirmed by microscopy, 40.2% (92/229) by culture, 77.9% (205/263) by IS2404 PCR of swab and/or tissue specimens. Histopathology detected 16 cases which were negative in all other tests.

3.1. Ulcerative lesions
Swab versus surgically excised tissue (ST, ST+ antibiotics)

Out of 75 laboratory confirmed ulcerative lesions the swab PCR was positive in 61.1% (44/72), the tissue PCR in 44.4% (32/72). Two out of 72 lesions (2.8%) had a positive tissue PCR only, no positive swab PCR. In 14 cases (19.4%) however, only the swab PCR was positive, the tissue PCR negative. Swab microscopy was positive in 39.4% (28/71), tissue microscopy in 40.8% (29/71). Swab culture was positive in 5.9% (4/68), tissue culture in 13.2% (9/68). No significant difference between analysis of swab and tissue was detected for microscopy (p=0.86), and culture (p=0.14), however, a marginal significant difference for PCR (p=0.045). 53.3% (40/75) of these patients received antismycobacterial treatment before specimen collection. The duration of disease for ulcerative lesions before surgical excision/specimen ranged from 7-1,080 days, mean duration 101.1 days.

Swab versus 3 mm punch biopsy (DT)

Out of 59 laboratory confirmed ulcerative lesions the swab PCR was positive in 86.4% (51/59), the punch PCR in 64.4% (38/59). Two out of 59 lesions (3.4%) had a positive tissue PCR only, no positive swab PCR. In 15 cases (25.4%) however, only the swab PCR was positive, the punch PCR negative. Swab microscopy was positive in 66.1% (39/59), punch microscopy in 33.9% (20/59). Swab culture was positive in 57.4% (27/47), tissue culture in 23.4% (11/47). A significant difference between analysis of swab and tissue was detected for microscopy (p<0.01), PCR (p<0.01), and culture (p<0.01). No patient received antismycobacterial treatment before specimen collection. The duration of disease for ulcerative lesions before specimen collection ranged from 4-180 days, mean duration 53.7 days.

3.2. Non-ulcerative lesions

Surgically excised tissue versus 3 mm punch biopsy

PCR of surgically excised tissue was positive in 64.0% (16/25), PCR of punch biopsies in 91.7% (66/72). Microscopy of surgically excised tissue was positive in 56.0% (14/25), microscopy of punch biopsies in 59.1% (42/71). Culture of surgically excised tissue was positive in 25.0% (6/24), culture of punch biopsies in 63.2% (36/57). A significant difference between analysis of surgically excised tissue and punch biopsies was found for PCR (p<0.01) and culture (p<0.01), but no significant difference for microscopy (p=0.78). 28.0% (7/25) of the patients with surgically excised tissue specimens were treated with antismycobacterial drugs before specimen collection, whereas no patient with punch specimens received previous antismycobacterial treatment. The duration of disease for surgically excised non-ulcerative lesions ranged from 7-1,800 days, mean duration 126.8 days (95% CI: 6.6-247.1 days). The duration of disease for non-ulcerative lesions of which punch biopsies were taken ranged from 3-190 days, mean duration 25.9 days (95% CI: 21.3-30.5 days).

4. Conclusions

1. The laboratory confirmation of ulcerative BUD lesions can be achieved by assessment of diagnostic swabs. IS2404 PCR analysis and microscopy of swab specimens provide the highest diagnostic sensitivity.

2. The combination of a positive tissue PCR and a negative swab PCR was found to be very rare in ulcerative lesions, whereas positive swab PCR and negative tissue PCR occurred significantly more frequently.

3. Therefore, assessment of swab samples can detect the majority of ulcerative BUD cases. Positive swab IS2404 PCR and microscopy test results do not require further confirmatory tests. Additional analysis of tissue specimens does not significantly increase the diagnostic yield.
4. The lower diagnostic sensitivities of all laboratory tests in the group of ST and ST+ patients compared to DT patients are related to antimycobacterial treatment previous to specimen collection and the significantly longer duration of disease found in this group.

5. With a diagnostic sensitivity of about 90% (IS2404 PCR), and 60% (microscopy, culture) laboratory analysis of punch biopsies provides an excellent diagnostic tool for the laboratory confirmation of early non-ulcerative lesions BUD without previous antimycobacterial treatment.
Two years of histopathological activity in Benin with particular reference to the Buruli ulcer

Clemente C\textsuperscript{1}, Barogui Y\textsuperscript{6}, Bruni B\textsuperscript{2}, Dossou A\textsuperscript{5}, Ferrari AM\textsuperscript{1}, Johnson RC\textsuperscript{4}, Poggio F\textsuperscript{3}, Rao S\textsuperscript{1}, Rubino B\textsuperscript{2}, Sopoh G\textsuperscript{5}, Zavattaro E\textsuperscript{7}, Leigheb G\textsuperscript{7}

\textsuperscript{1}Dept. of Pathology and Cytopathology, S. Pio X Hospital, Milano, Italy; \textsuperscript{2}Dept. of Pathology and Cytopathology, Istituto Clinico Sant’Ambrogio, Gruppo San Donato, Milano Italy; \textsuperscript{3}Scientific Committee on Buruli Ulcer Program, Rotary Club Milano Aquileia, Rotary International, Milano, Italy; \textsuperscript{4}Programme National de Lutte contre l’Ulcère de Buruli et la Lèpre, Ministère de la Santé Publique, Cotonou, Benin; \textsuperscript{5}Centre de Dépistage et de Traitement de l’Ulcère de Buruli (CDTUB), Allada, Benin; \textsuperscript{6}Centre de Dépistage et de Traitement de l’Ulcère de Buruli (CDTUB), Lalo, Benin; \textsuperscript{7}Dermatologic Clinic, University of Piemonte Orientale “A. Avogadro”, Novara, Italy.

Histopathology in Benin is a problem. There are only a few pathologists and specialized technicians and also the laboratories, where the histopathological slides may be prepared, are scarce. Buruli ulcer may simulate, clinically and morphologically, different non neoplastic and neoplastic skin lesions; in addition, for the efficacy of the therapy and, in particular, for the HOT (Hyperbaric Oxygen Therapy) treatment, it is important to exclude false positive patients from the trials. This selection could be done only with the histological diagnosis on the surgical biopsies. Since January 2006 a pathology laboratory is operative in the CDTUB of Alladà, in the central region of Benin. The equipment was provided with the help of the Foundation Luxembourgeoise Raoul Follereau and a period of training for the laboratory technician was offered by the S. Pio X Hospital in Milan. At the end of 2007 more than 500 histological cases were locally processed, with the preparation of routine (H&E) and special histochemical stains (Ziehl Neelsen). Then all the slides were sent by mail to the Pathology Department of the S. Pio X Hospital in Milan for the final diagnoses. A telepathology system was also experimented to transmit the digital images of the slides from Alladà to Milan but, to avoid the important and frequent problems with the connection through the telephone lines, a satellite link will must be used in the future. The high cost of the satellite connection did not allow, until now, to use the virtual slide system for the routine diagnostic work. Another histopathology activity has been started in Tanguietà, in the northern region of Benin, since January 2007 and, until now, more than 100 cases were sent by mail to the S. Pio X Hospital of Milan where the material has been processed in the pathology laboratory. Different and important neoplastic and non neoplastic diseases were diagnosed and promptly referred for appropriate therapy to the medical staff of the local hospitals in Benin. The preliminary results of our activity will be discussed, with particular reference to the Buruli ulcer and to the new projects for the future histological activity, even in other different regions of the Benin.
Further studies on the confirmation of Buruli ulcer in clinical specimens sampled using fine needle aspiration

Miriam Eddyani¹, Alexandra G. Fraga², Adhemar Longatto Filho³⁷, Cécile Uwizeye¹, Krista Fissette¹, Christian Johnson³, Julia Aguiar⁴, Ghislain Sopoh⁵, Yves Barrogui⁶, Fernando C. Schmitt¹, Jorge Pedrosa² and Françoise Portaels¹

¹Mycobacteriology Unit, Department of Microbiology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium
²Life and Health Sciences Research Institute, School of Health Sciences, University of Minho, Braga, Portugal
³Programme National de Lutte contre l’Ulcère de Buruli, Cotonou, Benin
⁴Centre Sanitaire et Nutritionel Gbemoten, Zagnanado, Benin
⁵Centre de Dépistage et de Traitement de l’Ulcère de Buruli, Allada, Benin
⁶Centre de Dépistage et de Traitement de l’Ulcère de Buruli, Lalo, Benin
⁷Institute of Molecular Pathology and Immunology of the University of Porto, Porto, Portugal

Until recently the only available treatment of Buruli ulcer (BU) consisted of surgical excision of the affected tissue. The use of specific antibiotics is now, however, more and more successfully used, mainly in patients with small lesions. These antibiotics make surgery redundant in some thus making skin tissues for microbiological diagnosis less and less available. It is however very important to confirm a clinically diagnosed case of BU by laboratory techniques. Punch biopsies have been proposed to this aim. They are however invasive and require local anesthesia.

An alternative strategy would be the use of FNA also used to obtain material for cytological examinations in tissues suspected of containing tumor cells.

In November 2006 this technique was applied on BU suspected patients in parallel with excision or punch biopsies in three BU treatment centers in Benin (Centres de Dépistage et de Traitement de l’Ulcère de Buruli of Zagnanado, Allada and Lalo).

From 35 patients suspected of BU with a variety of clinical lesions, FNA were taken after which tissue fragments or punch biopsies were obtained from the same site in the lesion.

Aspirates were stored at 4°C in a sterile vial containing a liquid transport medium while tissue specimens were stored in a semi-solid transport medium (Eddyani et al., 2008).

Among the 35 patients, 25 were confirmed to have active BU, four were inactive BU cases and six were not BU. Among the 25 BU patients, 23 were on chemotherapy for 1 to 6 weeks. Cytological examination of the BU cases revealed the presence of a mixed inflammatory infiltrate with lymphocytes, macrophages, and neutrophils, as well as the presence of epithelioid cells suggesting a chronic granulomatous inflammation.

A total of 88 FNA and 88 tissue fragments were obtained. Both types of diagnostic material were subjected to the same analyses namely detection of acid fast bacilli after auramine staining, IS2404-PCR, and in vitro culture on Löwenstein-Jensen medium.

By microscopy a difference in sensitivity was observed between FNA (37.5%) and biopsies (47.7%) although not significant. Using in vitro culture a sensitivity of 20.5% was obtained for FNA while for biopsies this was 30.0%. This difference was not statistically significant though. On the other hand, the contamination rate of biopsies was significantly higher than that of FNA (20.5% vs. 5.7%; p=0.004). The relatively low sensitivity of culture compared to previous studies (45-49%) can be partly explained by the high proportion of patients on chemotherapy. The 5 patients that were on treatment for at least 4 weeks, did not render any positive culture. Among the 20 patients that were not
on treatment yet or for 3 weeks or less, the sensitivity of culture was 27.0% for FNA and 36.2% for biopsies. Using PCR as a diagnostic technique, however, we observed a statistically significant difference between FNA (52.3%) and biopsies (86.4%) (p<0.0001).

These results show that direct smear examination and in vitro culture can be used in FNA with similar sensitivities as in biopsies. The PCR technique should however be improved to obtain a better sensitivity in FNA.
Ultrasonography in Buruli ulcer: imaging and medical relevance


1Dermatologic Clinic, University of Piemonte Orientale “A. Avogadro”, Novara, Italy; 2Radiology 5, San Giovanni Battista Hospital, Torino, Italy; 3Programme National de Lutte contre l’Ulcére de Buruli et la Lèpre, Ministère de la Santé Publique, Cotonou, Benin; 4Pathology and Cytopathology Division, S. Pio X Hospital, Milano, Italy; 5Scientific Committee on Buruli Ulcer Program, Rotary Club Milano Aquileia, Rotary International, Milano, Italy; 6Centre de Dépistage et de Traitement de l’Ulcére de Buruli (CDTUB), Allada, Benin; 7Hygiene and Public Health Unit, University of Piemonte Orientale “A. Avogadro”, Novara, Italy.

Background

The infection caused by *Mycobacterium ulcerans* (M.u.) or Buruli Ulcer (BU) when untreated in its initial nodular phase, evolves towards forms which are more or less aggressive, localized or generalized, depending on immunological reactivity of the patient. Frequently it produces wide ulcerative lesions.

The pathogenesis of the cutaneous manifestations is linked to the spreading of mycolactone, a *Mycobacterium ulcerans* toxin that causes a coagulative necrosis in the subcutaneous layer with adipose and connective tissue involvement.

Aim

The extension of the subcutaneous damage and consequently of the infection is difficult to evaluate by clinical observation, also after a specific antibiotic treatment. For this reason, we thought it important to subject 20 patients, affected by BU in different clinical stages, to ultrasonography (US) to understand the possibility of monitoring the type and the extent of tissue damage. We hoped to gain important clinical information regarding the efficacy of antibiotic treatment or the need to start another therapy. In fact US could be used as a guide to establish the extent of the eventual surgical excision, avoiding the large excisions that were carried out in the past.

Materials and Methods

Classification of the patients on the stage of evolution of the disease. US evaluation of all the patients with a US scanner with a 10 MHz linear probe (evaluation on every lesion: nodules, plaques, lymphoedema, around the ulcers). Controls in homologous, controlateral sites.

Results

The US evaluation that we performed for the first time on BU lesions allowed us to evaluate the depth and extent of the pathological process characterized by US signals of altered echogenicity in the dermal and hypodermal regions and sometimes also in muscular layer. US allows the evaluation of the amelioration of the subcutaneous damage during the antibiotic treatment (antibiotic effectiveness), moreover, in the future, it will represent a valid tool for the surgeon to plan, when necessary, a surgical excision of the necrotic tissue to encourage healing.
CONTROL SESSIONS
National Buruli Ulcer Control Programme in Congo

Dr Kossi Semenu ATTISSO, Public health medical officer

Introduction

The first cases of Buruli ulcer (BU) in Togo were described in 1996 by F. PORTAELS, M. MEYERS et coll. After their participation, in 1998, in the Conference on Buruli Ulcer Control and Research, organized by WHO at Yamoussoukro, the national health authorities set up a national Buruli ulcer control programme (NBUCP). A number of constraints prevented the Programme from regularly carrying out its activities and achieving the expected results. After October 2006, activities were effectively resumed with the appointment of a new coordinator, and 2007 was the year in which activities revived with the support and deeper commitment of partners (WHO, DAHW and Handicap International).

Overview for 2007

The Programme revival process

A formal process for the revival of activities was determined after consultation between NBUCP and its partners (WHO, DAHW and Handicap International) at the 2007 annual WHO meeting of the Global Buruli Ulcer Initiative. It was in particular decided initially to focus interventions on one region - Maritime - the most affected region in the country. A planning process was implemented in order to carry this out. Handicap International immediately offered to lend its support to the process, which was to begin with study trips to Benin and Ghana and finalize with the development of a three-year pilot project (2008-2010) followed by a situation analysis, the drafting of a policy paper and of a five-year strategic plan (2008-2012).

This process went ahead as planned:

- The study trips to Benin and Ghana took place from 21 to 25 May and from 17 to 22 June 2007 respectively;
- A sociological survey (A KAP study) on BU in Maritime region (the areas of Tsévié and Afagnan) was carried out by a national consultant;
- A situation analysis in Maritime region, in conjunction with a needs assessment, was carried out by an international consultant in July 2007;

These three importance activities received technical and financial support from Handicap International (for which we sincerely thank them);

- In August 2007, a workshop was held with the support of our usual partners to draw up the National Policy for Buruli Ulcer Control and the Strategic National Plan. Participants also came from programmes in neighbouring countries (Benin and Ghana), not to mention specialists from WHO (countries, AFRO and Geneva), to whom we reiterate our sincere gratitude.
- A three-year pilot project (2008-2010) has been drawn up thanks to Handicap International; it is currently being implemented with funding from SANOFI in Maritime region.
Support from other partners has been guaranteed for complementary and supplementary activities.

Routine surveillance and case management of cases detected in other regions is assured within the overall framework of integrated disease control, with special support from DAHW.

Other activities

- **Implementation of the Plan of action 2007 with DAHW**
  - Enhancing the case management capacity of Tsévié regional hospital (CHR) (rehabilitating and equipping an operating theatre; refurbishing and equipping hospital wards; refurbishing and fitting out a physiotherapy room; construction of a room for social and educational activities).
  - Training 12 surgeons
  - Preparation of a manual on case management
  - Provision of medicines and consumables

- **Training 20 community health workers (CHW) to identify and refer BU cases in Maritime region, with the financial support of Handicap International**

- **Training in clinical diagnosis and case management of BU for chief health post nurses (ICP) from the Lacs (Maritime Region) departmental department of health (DDS) with financial support from the EU/ADSS project.**

- **Routine activities**
  - Monitoring activities at Tsévié regional hospital
  - Technical support for the BU team at Tsévié
  - Quarterly supervisory round
  - Quarterly meeting with the Leprosy/TB/BU monitors
  - Data collection and analysis

**Statistics**

- **Data for 2007**

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*: Excluding figures for November and December, which the Programme did not receive
### Summary for 1997 to 2007

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** : From 1995 à 1998

**Comment:**

In 2004, a systematic census of Buruli ulcer cases during the national vaccination days (NVD) against poliomyelitis reported more than 1500 cases, 785 of which were clinically confirmed. This makes it possible to estimate the total number of cases for 1997 to 2007 at $664 + 785 = 1429$.

### Main difficulties encountered

- Insufficient human resources in the Coordination office;
- Inadequate case notification;
- Samples not systematically taken for confirmation;
- Circuit for processing samples taken for confirmation has not been formalized;
- Low profile assigned by the country (no budget line assigned for the operation of NBUCP and other activities).

### Prospects

Until 2010, our priority will be the implementation of the pilot project in Maritime region. During this period, we plan to conduct an exhaustive nationwide epidemiological survey of Buruli ulcer. It is very likely that after 2011, the project will be extended to one or two other regions.

### Conclusion

We are confident that thanks to the impetus given by the revival of the Programme we shall achieve increasingly encouraging results. We venture to hope that we shall always be able to count on the significant and efficacious support of our usual partners and of others, to whom we appeal for support.

We should like to thank all those who have contributed to ensuring the success of our Programme's revival process for their past and future support for our activities.
The Buruli ulcer situation in Gabon in 2007 and the control strategy

Dr Louis Bayonne Manou

Introduction

Buruli ulcer is endemic in Gabon. During 2007, a number of activities were carried out in the provinces of Moyen Ogooué and Ngounié, in conformity with the different recommendations of the World Health Organization and of Aide aux Lépreux Emmaüs Switzerland.

Activities

1. Early case detection at the community level

Almost all the cases are from central and south-central health provinces. In 2007, we registered 32 cases.

2. Distribution of new cases by sex and age

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<td></td>
<td>&lt; 15 yrs</td>
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<tr>
<td>Female</td>
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</tr>
<tr>
<td>Total</td>
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</tr>
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</table>

Girls and women were most affected (20/32).

The 15 to 49 years age group was most affected.

3. Clinical forms

<table>
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<td></td>
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<td>Non ulcerative</td>
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<tr>
<td>Ulcerative</td>
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</tr>
<tr>
<td>Total</td>
<td>7</td>
</tr>
</tbody>
</table>

Most patients were in category 2 (CAT2).
4. **Information- Education –Communication**
   - 3 information sessions were organized for the target population, who were shown a film on Buruli ulcer was shown;
   - Radio programmes were broadcast;
   - Two articles were published in the country's leading newspaper (Union);
   - A lecture-debate was organized with students at the medical faculty.

5. **Laboratory confirmation**
   Details of laboratory confirmation are given below:
   - Number of suspected cases 32
   - Number of cases for which it was possible to carry out tests 22 i.e. 68.75%
   - Number of cases confirmed by Ziehl-Neelsen 11 i.e. 50%
   - Number of cases confirmed by PCR 10 i.e. 45.45%
   - Number of cases confirmed by at least one method 12 i.e. 54.55%
   - Number of cases confirmed by Ziehl-Neelsen and PCR 09 i.e. 40.9%
   - Number of cases confirmed by histopathology (examination not performed) 0

6. **Case management**
   The table below summarizes medical and surgical case management activities

<table>
<thead>
<tr>
<th>Medical and surgical case management activities</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Total number of cases treated with topical antibiotics</td>
<td>32</td>
</tr>
<tr>
<td>Total number of cases having received a complete course of treatment</td>
<td>19</td>
</tr>
<tr>
<td>Total number of cases having received an incomplete course of</td>
<td>13</td>
</tr>
<tr>
<td>treatment (less than 8 weeks, discharged before completion of</td>
<td></td>
</tr>
<tr>
<td>treatment)</td>
<td></td>
</tr>
<tr>
<td>Number of cases cured by antibiotics alone</td>
<td>2</td>
</tr>
<tr>
<td>Total number of cases undergoing surgical treatment or a surgical</td>
<td>25</td>
</tr>
<tr>
<td>procedure</td>
<td></td>
</tr>
<tr>
<td>Total number of cases cured (complete scarring)</td>
<td>21</td>
</tr>
<tr>
<td>Total number of cases cured with no inhibition of movement</td>
<td>19</td>
</tr>
<tr>
<td>Total number of cases cured with some inhibition of movement</td>
<td>2</td>
</tr>
</tbody>
</table>

7. **Strengthening the Programme's capacity**
   - Official handing over of antibiotics (streptomycin and rifampicin) to the health authorities at Lambaréné by the WHO Representative in Gabon.
   - 27 participants (surgeons, general practitioners, nurses and nursing assistants) were trained. The participants received comprehensive information on Buruli ulcer and forms BU01 and BU02 for case notification and surveillance were distributed to the physicians;
– 57 community intermediaries and health workers from the provinces of Ngounié and Moyen Ogooué were also trained;

– A workshop was organized on the National Buruli ulcer Policy and Control Strategy in Gabon for the period 2008 to 2012;

– A senior technician from the national laboratory in Libreville took part in the M2U international microbiology course on *Mycobacterium ulcerans* at Yaoundé.

– A vehicle was purchased and staff recruited.

8. **Standardization of the system of case registration and notification**

– Forms BU01 and BU02 have been printed and made available in the different health facilities.

9. **Monitoring and evaluation of health workers**

– Just one mission was organized to monitor the activities of and evaluate health workers.
Results of an ten-year control and research effort (1998-2008) to improve case management of *Mycobacterium ulcerans* infection (Buruli ulcer) in the Democratic Republic of the Congo

*Dr Anatole Kibadi Kapay*

Collaborators: Singa Jackie (PNLUB), KayimaaAYINUA Makanzu (Nsona-Mpangu RHA), Mimuku Jean-Bedel (Nsona-Mpangu RHA), Nkuku Léontine (LNRUB/INRB), Kisile Olive (INRB), Rutumba (INRB), Muyembe-TamfumJean-Jacques (INRB), Phanzu Delphin (IME Hospital -Kimpese), Mputu-Yamba Jean-Baptiste (University of Kinshasa), Mokassa Luc (University of Kinshasa), Panda François (University of Kinshasa), Pedrosa Jorge (University of Minho/Braga), Roux Jean-Jacques (Chambéry Hospital), Meyers Wayne (AFIP/Washington), Suykerbuyk Patrick (IMT Antwerp), Boelaert Marleen (IMT Antwerp), Portaels Françoise (IMT Antwerp)

**Introduction**

In the Democratic Republic of the Congo, case-management of *Mycobacterium ulcerans* infection, also known as « Buruli ulcer » (BU) falls into three main periods. The first of them was before 1950, when in the east of the country Kleinschmids, Van Den Abbele and Lubicz treated lesions probably caused by *M. ulcerans*, although they were undocumented. The second period began with the publication on the first case by Van Oye and Ballion in 1950 and lasted until 1980; it was characterized by cases documented by Janssens et al., Meyers et al. and Portaels et al. The third period began in 2000, and was marked by the discovery of increasing numbers of new cases which were reported by Kibadi et al., Bafende et al. and Phanzu et al. In the light of the lack of information during the second period, we demonstrated, by a study carried out in 2000-2001 at the IME hospital in Kimpese in the province of Bas-Congo, that BU was definitely present in the country. As a result of this study, the National Buruli Ulcer Programme (PNLUB) was established in 2002 and the current BU control and research effort in the Democratic Republic of the Congo began to take shape.

**Part 1: The control effort**

1.1. Data reported

<table>
<thead>
<tr>
<th>Country</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RD Congo</td>
<td>17</td>
<td>119</td>
<td>487</td>
<td>51</td>
<td>74</td>
<td>1088</td>
<td>340 cases</td>
</tr>
</tbody>
</table>

* RHA Nsona-Mpangu : 94 treated (63 of them confirmed by PCR at IMT, Antwerp)
* IME Hospital, Kimpese : 243 probable cases detected (27 of them confirmed by PCR at IMT, Antwerp)
* City of Kinshasa : 3 cases (1 of them confirmed by PCR at IMT Antwerp)

N.B. Total : 340 cases

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3 National Buruli Ulcer Control Programme
4 Rural health area
5 National Buruli Ulcer Reference Laboratory/National Institute for Biological Research
6 Institute of Tropical Medicine
This total of 1088 notified cases of BU is based on the reports presented to the annual meetings at Geneva between 2002 and 2008. It does not reflect the actual number of cases, because the numerous cases that have been documented and described in the literature are not included, health personnel tend to under-report cases, in 2007, non-ulcerative forms in Nsona-Mpangu rural health area were not reported and no reports were submitted by PNLUB to the two previous WHO meetings on Buruli ulcer (2005 and 2006).

Patients treated with rifampicin and streptomycin in 2007 (DR of the Congo)

<table>
<thead>
<tr>
<th>Institution providing treatment</th>
<th>Number of patients</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nsona-Mpangu rural health zone</td>
<td>93 cases (Health centres)</td>
<td>93 cases</td>
</tr>
<tr>
<td>IME Hospital, Kimpese</td>
<td>IME Hospital : 54 cases</td>
<td>77 cases</td>
</tr>
<tr>
<td></td>
<td>outside hospital : 23 cases</td>
<td></td>
</tr>
<tr>
<td>City of Kinshasa</td>
<td>Mère Teresa CDTUB 7 case</td>
<td>3 cases</td>
</tr>
<tr>
<td></td>
<td>CM/DGI: 1 case</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CM/Dr LELO : 1 case</td>
<td></td>
</tr>
</tbody>
</table>

In all, in 2007 173 patients whose cases were notified received treatment with rifampicin-streptomycin in association with surgery in the DR of the Congo.

1.2. Essential components of the BU control strategy in the Democratic Republic of the Congo (1998-2008)

I. Strengthening the health system

- Infrastructure, equipment and logistics: IME hospital Kimpese, Nsona-Mpangu hospital, Mère-Térésa CDTUB, National Buruli Ulcer Reference Laboratory (INRB)
- Training for health workers: Leprosy-TB coordinating physicians and chief district medical officers, health-care personnel, community intermediaries (Bas-Congo province, Bandundu and city of Kinshasa)
- Standardized registration and notification using the BU 01 and BU 02 forms.

II. Community-level activities

- Early case detection at the community level in Bas-Congo province
- Information, education and communication (IEC) campaigns in communities and schools in the provinces of Bandundu, Bas-Congo and the city of Kinshasa
- Training health workers in the villages and strengthening the surveillance system in communities in the province of Bas-Congo (Nsona-Mpangu and Kimpese rural health areas).

7 Buruli ulcer treatment centre
III. Standardization of case management

- Adoption of national policy documents on control in 2004
- Case confirmation by laboratory: at the national (National Buruli ulcer reference Laboratory/INRB in Kinshasa), intermediate (IME hospital Kimpese) and peripheral (health centre) levels
- Prescription of topical antibiotics, rifampicin and streptomycin: in Bas-Congo province and the city of Kinshasa
- Surgery: Bas-Congo and Bandundu provinces and in the city of Kinshasa

Part 2: Research

2.1 Antibiotics and treatment

1)- Title: «Observational Study of the efficacy of rifampicin-streptomycin combination in association with surgery to treat Mycobacterium ulcerans (Buruli ulcer) infection in the Democratic Republic of the Congo. »

Summary: In order to evaluate the efficacy of the rifampicin-streptomycin (R+S) combination in association with surgery, recommended by the World Health Organization (WHO) to treat Mycobacterium ulcerans infection (also known as Buruli ulcer - BU), an observational study of ulcerative forms of BU (of more than 10 cm in diameter) was carried out from October 2006 to January 2008 in Nsona-Mpangu rural health area in the Democratic Republic of the Congo. Before the study began, the approval of the ethics committees (Ziekenhuis University Antwerp, Belgium and the Public Health College/Higher and University Education, DRC) was obtained, together with the free informed consent of the patients. The microbiological analyses (direct microscope examination, culture and polymerase chain reaction/PCR) of the surgical biopsies taken from the patients were carried out at IMT in Antwerp, Belgium and the histopathological analyses were performed at AFIP/USA, Minho/Braga/Portugal and at Chambery hospital in France. A total of 92 patients were recruited; 61 were PCR positive (group 1) and 31 PCR negative (group 2). The results show that at the end of week 4 of treatment with R+S but without surgery, a higher percentage of the PCR negative patients (54.8%) had a satisfactory clinical evolution than the PCR positive patients (14.8%). This difference was statistically significant (p<0.001). The final outcome after 12 weeks of treatment including surgery after four shows that a higher proportion of the PCR positive patients (70.6%) presented a satisfactory outcome than the PCR negative patients (29.4%) and the difference was statistically significant (p=0.010). There was also a significant difference between the PCR negative and the PCR positive patients in the average time taken for scarring: the average time take for scars to form was in PCR negative patients was shorter (7.48 weeks) than in PCR positive patients (10.38 weeks) (p=0.026).

Discussion: For the very first time, we have been able to observe that under the WHO protocol, the outcome for PCR negative patients is more satisfactory than for PCR positive patients. Possibly, the PCR negative patients were not BU cases. The ongoing histopathological examinations will provide us with valuable information for differential diagnosis of Buruli ulcer.

On the basis of the observations after four weeks of treatment with antibiotics alone, the treatment recommended by WHO seems inefficacious without resorting to surgery for advanced ulcerative forms (more than 10 cm in diameter), which are the commonest clinical forms of BU in our rural areas. These findings are a significant step forward in our understanding of the effectiveness of antibiotic treatment (S+R) of M. ulcerans infection. Consideration might be given to revising the
current WHO protocol as regards the best moment to carry out surgery. In the light of our results, it would be preferable to carry it out in conjunction with antibiotic treatment, at least where extensive ulcerations caused by \textit{M. ulcerans} are concerned (more than 10 cm in diameter).

**Conclusion:** Our study has shown that after four weeks of treatment with antibiotics, the evolution of extensive ulcerative forms of \textit{M. ulcerans} infection depends on the results of microbiological tests. This confirms the importance of microbiological confirmation of Buruli ulcer.

Moreover, at the present time our results do not allow us to confirm the superiority of medical treatment over surgery.

2) **Title:** «Patients' perception of repeated injections of streptomycin in treatment of \textit{Mycobacterium ulcerans} infection (Buruli ulcer): the findings of a survey in a rural health area in the Democratic Republic of the Congo»

**Summary:** This paper presents the findings of a study of treatment of \textit{Mycobacterium ulcerans} infection in a rural health area with the highest level of endemicity of Buruli ulcer in the Democratic Republic of the Congo (DRC). A survey was carried out among an initial group of 14 patients who had completed treatment with rifampicin + streptomycin in association with surgery, as recommended by WHO. Patients were questioned about the repeated injections of streptomycin (S). A second group of 14 patients of the same age, sex, level of education and socio-economic status was then interviewed at the start of their treatment. The characteristics of the patients were identical to those described in the literature: a majority of children either attending or not attending school and young adult farmers. Our results show that patients who have completed a course of treatment are more favourable towards repeated injections of streptomycin as proposed by WHO than patients starting treatment. Acceptability of surgical treatment of BU was low in both groups questioned. Our results also show that 1) patients' acceptance of repeated injections of streptomycin varies (70 % in group 1 and 57 % in group 2); 2) the proportion who have a general understanding of the side-effects of injections of streptomycin is low: 57 % among group 1 and 28 % among group 2; 3) most of the BU patients interviewed preferred injectable streptomycin to be replaced with another equally effective product administered orally.

**Conclusion:** This is the first time that patients' opinions of repeated injections of streptomycin have been analysed. Our results confirm that, as in other countries, patients prefer medical to surgical treatment, even if the medical treatment requires repeated injections.

2.2. **Operational research**

- Decentralization of antibiotic treatment and prevention of disabilities to health centres and outpatient treatment in the Democratic Republic of the Congo.

**Title:** «The «DOTBUR» strategy in outpatient treatment of patients with \textit{Mycobacterium ulcerans} (Buruli ulcer) infection: our experience in a rural health area in DRC in which tuberculosis and Buruli ulcer are endemic».

**Summary:** The purpose of this study was to assess the feasibility, efficacy and efficiency of the new «DOTBUR» strategy, which we are testing among patients receiving decentralized outpatient treatment of \textit{Mycobacterium ulcerans} infection with rifampicin + streptomycin in association with surgery in an endemic rural health area.

DOTS "directly observed treatment (short course)" for patients with tuberculosis now appears to be an effective control strategy for this disease. For the entire duration of the treatment protocol, (at least six months), patients are required to attend the clinic daily to collect their medicines. In the case of \textit{Mycobacterium ulcerans} infection, the treatment currently recommended by WHO is a combination of rifampicin + streptomycin, which may or may not be combined with surgery, for a minimum duration of 8 weeks and a maximum of 12 weeks. Streptomycin is an injectable product and needs to be
administered at a health centre. Rifampicin is administered orally and may be taken at home by the patients themselves.

Between 1 October 2006 and 15 February 2008, a total of 103 patients have already been enrolled in this «DOTBUR» strategy in the Democratic Republic of the Congo. They are receiving treatment in the following 19 health facilities that provide treatment for Buruli ulcer in Nsona-Mpangu rural health area in Bas-Congo province: de Nsona-Mpangu hospital, Nkamauna health centre (HC), Songololo referral HC, Songololo CBCO HC, Songololo Tétidio HC, Songololo Km 5 HC, Kisonga HC, Lufu-Gare CBCO HC, Km 70 or Vémadiya HC, Manzonzi HC, Lombe HC, Minkelo HC, Mbumbu HC, Mayanga HC, Mpelo HC, Lombe HC, Nkonzo HC, Mbanza-Manteke HC and Malanga-Lombe HC.

The preliminary results so far are satisfactory and very encouraging: direct treatment monitoring is excellent, the side effects of the medicines given are checked daily, adverse effects are monitored and prevented, patients receive daily moral and psychological support from the nurse and the development of resistance to rifampicin in TB patients in an endemic area is prevented.

**Conclusion:** Just as DOTS appears to be the most effective strategy for preventing the development of multi-resistant tuberculosis or MR-TB, DOTBUR seems to be the best strategy for BU patients receiving outpatient treatment in a decentralized system, as a means of preventing the emergence of resistance to this valuable first-line antimycobacterial medicine, especially in a country where prevalence of tuberculosis is high.
National Buruli ulcer control programme in Cameroon

Dr Charles Nsom Mba

Activities carried out in 2007

National level

• Coordination and monitoring of activities for the implementation of the operational plan
• Organization of the national coordinating meeting for activities in association with the leprosy coordination meeting.
• Training personnel from new disease foci in case management of BU and plastic surgery with the assistance of a team of specialists from Switzerland.
• Visit to the global Bu Focal Point at WHO headquarters at Geneva
• Distribution at endemic sites of IEC aids and medicines received from WHO
• Organization of a partners' meeting
• Preparation of a new strategic plan for BU in Cameroon for the period 2008-2012

Operational level

• Early case detection at the community level, Information, Education and Communication
  – These activities were carried out in the disease foci of Akonolinga, Mbalmayo, Bankim and Mbougué by staff from the health areas and managers from the Integrated Health Centres who received training in 2006. As a result, a large number of cases were reported in the two (sic) foci in 2007

• Compliance with treatment protocols (Antibiotics, Surgery, Prevention of disabilities and rehabilitation)
  – Since 2004, when treatment with antibiotics was adopted for treatment of Buruli ulcer, the national Programme has used this strategy. It is systematically administered in accordance with the WHO protocols, and as a result, all patients have benefited from it. It has thus been possible rapidly to cure patients and to shorten stays in hospital. All foci receive support from partners to ensure supplies of antibiotics from WHO

• Case confirmation
  – Case confirmation was provided for all patients by the Centre Pasteur in Cameroon; during the year, some 230 tests were carried out on patients, and 81 Ziehl-Neelsen stains and 69 PCR were carried out, confirming the presence of Mycobacterium ulcerans. We should also mention that the Centre Pasteur in Cameroon carries out these examinations as part of its public health activities.
• Number of cases detected and treated in 2007

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of cases</td>
<td>230</td>
<td>100</td>
</tr>
<tr>
<td>New cases</td>
<td>230</td>
<td>100</td>
</tr>
<tr>
<td>Relapses</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Children</td>
<td>106</td>
<td>45.7</td>
</tr>
<tr>
<td>Women</td>
<td>126</td>
<td>44</td>
</tr>
<tr>
<td>Disability at time of diagnosis</td>
<td>54</td>
<td>31.04</td>
</tr>
</tbody>
</table>

• Strengthening the health system
  - Improvements have been carried out at NGOANTET and are due at Mbongué.
  - A new building has been built by MSF at AKONOLINGA.
  - Equipment has been given to the new sites.
  - Two four-wheel drive vehicles have been donated by ALES and WHO to Adamaoua province and to Bankim health district.

• Training for health workers, teachers and village volunteers
  - During 2007, and with the support of ALES, the Programme provided training in case management, programme management and plastic surgery for health workers in new foci of Buruli ulcer: the training concerned 5 physicians and 15 nurses.

• Standardization of the system of case reporting and recording using the BU 01 and BU 02 forms
  - Since activities began, data collection on cases of Buruli ulcer has made use of the revised WHO forms.

• Monitoring and Evaluation.
  - The Programme's activities are regularly monitored top down from the central level to the peripheral level thanks to supervisory and monitoring missions of scheduled activities.

Prospects for 2008

• Early case detection at the community level, Information, Education and Communication
  - The Programme plans to strengthen early case detection at the community level thanks to the involvement of primary-school teachers and community intermediaries in the disease foci of Ayos, Akonolinga, Ngoantet, Bankim and Mbongué. Public education campaigns are planned.
• **Observance of case-management protocols (antibiotics, surgery, prevention of disabilities and rehabilitation)**
  - Case management protocols will be strictly observed, with an emphasis on prevention of disabilities and rehabilitation.

• **Case confirmation**
  - This will systematically be carried out by the Centre Pasteur in Cameroon. The possibility of involving other laboratories in the Programme will be considered.

**Operational level**

• **Strengthening the health system**
  - There are plans to improve infrastructure in the new disease foci, and in particular at BANKIM in Adamaoua and at MBONGUE in the South West thanks to the support of ALES and the other partners;
  - Finalization of the strategic plan 2008-2010;
  - A national coordination meeting is to be held for all partners.

• **Training for health workers, teachers and village volunteers.**
  - Training in plastic surgery is scheduled for health workers with the support of Swiss specialists as well as training for teachers and community intermediaries in existing and new Buruli ulcer foci.

• **Standardization of the case recording and reporting system using the BU 01 and BU 02 forms**
  - The new WHO Buruli ulcer forms will be used and data collection will be further computerized.

• **Monitoring evaluation.**
  - Missions to monitor and evaluate activities are to be carried out, along with a national coordination meeting for programme activities.
Trends in prevalence of laboratory confirmed Buruli ulcer cases in Adjumani district from 2004-2007

Henry Wabinga

Adjumani district has been a Buruli Ulcer (BU) endemic region since the 1960’s but however BU control activities were re-started in 2004 after being dormant for over 20 years. In addition to control activities there has been continuous monitoring and surveillance of the disease in the district and this communication provides available data on the occurrence of laboratory confirmed cases of BU since 2004. The data shows slight but steady upward trends in confirmed cases of BU with six cases reported in 2004, 8 cases in 2005, two cases in 2006 while 13 cases in 2007. One case has so far been confirmed in January 2008.

The increased number of cases in 2007 coincides with the equipping of Moyo and Adjumani district hospitals with surgical equipment donated by ANESVAD and this is probably a reflection of confidence the population is putting in the health services.
Buruli ulcer control programme, Ghana 5 years on – Achievements, challenges and way forward

Dr Edwin Ampadu

After the 1999 national survey recording over 6000 cases of suspected Buruli ulcer, a national program was set up in 2000.

The objectives;

• Develop strategic plan to guide national case control
• Accelerate and standardize case treatment and control
• Provide avenue for stakeholders to support control activities [advocacy, technical support and health system strengthening]

Initially surgery was treatment of choice in case managements. This included wide excision and split skin grafting. Treatment were not standardized and consistent. Above all they varied from facility to facility and even within a facility, varied from clinician to clinician. Treatment centres were mainly; Agogo hospital, Amasaman health centre, St Martin’s hospital Agroyesum. Recurrence rates was as high as 16-30% this was unacceptable.!

When the national program was set up in 2000, the main aim was to coordinate all activities under the disease and to inform and educate health workers and the general public about the disease.

program office was initially located under the auspices of the national TB programme.

In 2002, a substantive program manager was appointed. The Buruli ulcer program began to receive support from the WHO, ministry of health and other international stakeholders [SMHF Japan; ALM, HART, USA]. This included setting up of well furnished and equipped office, funds for capacity development

National concern at that time

• Low knowledge about the disease among health workers and the public
• Strengthening of health facilities to better manage cases
• Explore the possibility of drug treatment
• Reporting of huge ulcers with deformities among children

Major activities carried out so far

• Tissue culture trial with Buruli ulcers This was supported by the SMHF, Japan. The trail took place both in Korle Bu Accra and KATH Kumasi Ghana. In all 13 patients benefited from the project. Currently the rest of the materials are being used on other patients

• Drug trial with the use of antibiotics [Rifampicin and streptomycin]- supported by WHO
• Development of standard surveillance forms to capture all cases of Buruli ulcer in the country
Achievements

- Developed a 5 year strategic plan to guide the national program implementation
- Gradual increase in awareness of the disease among health workers and the general public
- Improving in wound care management in the treatment centres
- Antibiotics treatment showing excellent results with patients
- Attracting other stakeholders into control activities both local and international levels

Strengthening of health facilities

- Treatment centres have increased from 3 to 15
- Basic tools for wound care have increased by five folds
- Capacity development for the health workers in some of the treatment facilities has increased by 4 folds and more health workers have shown interest in case management
- Have succeeded in standardizing treatment protocol
- Currently developed national Buruli ulcer developmental agenda
- Increased the number of districts reporting on the disease by 5 folds
- Provided major surgical equipments to 3 endemic treatment centre
- Provided infrastructure for 4 centres [physiotherapy services, surgical theatre, wards]

Major areas of international collaboration

- BURULICO
- Research on Transmission [Noguchi, Ghana and Michigan state university]
- Drug treatment ; rural community approach

The future of the programme

The future is bright for the programme. The international and local stakeholders are encouraged to offer more support to various strategic activities going on in the country. Whiles the program continues to encourage local agents’ participation. We are seeing treatment revolution and we pray that it happens in our life time.

At the moment 4 treatment centres including the national office have web sites for dissemination of local information of control activities in the country and this is very laudable. They include: Upper Denkyira health, Agogo hospital, Amansie West health, Buruli Ghana.

Appreciation

The national program will like to thank MOH, Ghana, WHO, ANESVAD, Spain; ILFO, Italy, Michigan state university for the tremendous support and encouragement in the fight against the disease.
Programme against Buruli ulcer in Akonolinga, Cameroon: Presentation of the results of 2007

Dr Véronique Urbaniak, MSF Switzerland

The districts of Akonolinga Ayos and have been identified as areas with a high incidence of Buruli. Since 2002, MSF has launched a programme to combat Buruli ulcer in coordination with local health authorities and national, in the district of Akonolinga.

The overall objective of the programme is to improve the detection and treatment of patients affected by the Buruli ulcer disease in order to reduce their suffering and improve their recovery.

In 2007, we continued to improve the resources available at the hospital in Akonolinga particular through the creation of a technical ward to improve conditions for nursing care and physiotherapy.

Physiotherapy was enhanced by the provision of training in cooperation with ALES edited by Valerie Simonet. We organized a team of 3 workers who are undergoing training with the help of a professional expatriate.

We have signed a partnership with the University of Geneva Hospitals in order to benefit from their expertise in the field of HIV, infectious diseases, dermatology and rehabilitation.

The prevalence study conducted by Epicentre in March 2007 had shown that our coverage of the programme was inadequate and too limited to Akonolinga town at the expense of peripheral areas of the district. In this process we began the decentralization of care in two health centers with an objective of 4 centers opened at the end of 2008.

We try to develop finally information in community to promote early detection of cases.

Here we present the results for cohort entered the program from July 2006 to June 2007 (the last patients to have their treatment results available).
Integration of control of Buruli ulcer into the minimum package of activities: the case of the Ngoantet health centre in Cameroon

Dr Alphonse Um Boock

1. Background and justification

According to the epidemiological data available in 2000, the department of Nyong et Mfoumou, in Centre province, was the only focus of Buruli ulcer. However, the national epidemiological survey carried out in 2004 confirmed the hypothesis that the disease had gradually spread throughout both Centre province and the rest of the country.

Until 2005, knowledge of Buruli ulcer among medical personnel was still patchy. In villages in which traditional medicine was still predominant, the disease seemed to be gaining ground on the treatment available locally. The path followed by patients in search of treatment was also a long and dehumanizing one, marked by refusal that started with the village healer and concluded, for some, in the health centres at Ayos or Akonolinga after a costly and fruitless stay in one of Cameroon's large hospitals.

The problem of lack of expertise available, whether in the field of biomedicine or in traditional medicine, for a rapid response to the disease was thus compounded by the problem of accessibility. This situation called for the decentralization of case management of Buruli ulcer and deeper grassroots involvement in the strategies required to reduce its impact on individuals and their families.

On account of the level of prevalence of the disease in the village of Ngoantet, it headed the list of areas assigned priority on account of their urgent need for a response to the disease.

2. Methods

Given the situation, in 2006, a survey was conducted in order to draw up a participative and horizontal community-based strategy for case management of Buruli ulcer patients in Mbalmayo health district. The survey also fostered community mobilization in favour of an appropriate response. In addition, it made it possible to carry out a prospective study in Ngoantet health area into the mechanisms underlying the supply of health services and the path followed to obtain them.

Both health statistics and popular beliefs agree that malaria is the leading cause of morbidity in Ngoantet health area, followed by diarrhoeal and respiratory diseases.

Buruli, ulcer is perceived as being a public health problem for the community because of its devastating impact on both individuals and the community.

The survey also revealed the state of devastation that characterized the health situation in the area. The enormous shortage of infrastructure to provide treatment was compounded by the very poor quality of care, which lacked even very basic items of equipment such as microscopes. The situation was made worse by the lack of essential medicines and a huge shortage of human resources. The former health centre had only one nurse, and as a result was regularly closed whenever the nurse was doing rounds, which limited access to quality care.

In these circumstances, there was a need to envisage a global medical response to Buruli ulcer in particular and to disease in general. The following logframe was determined for the project:
3. Goal

The goal is to develop an integrated and high-quality health-care system in which beneficiaries fully participate.

4. Overall objective

To establish an integrated, viable, jointly managed and financed health centre with the full participation of beneficiaries.

5. Specific objective

To develop a horizontal approach to case management of patients with the full participation of beneficiaries;

1. To increase attendance for curative care at Ngoantet health centre to 0.4 NC / per year.
2. To increase ANC coverage in the health area to 40%.

The project for the development of the Ngoantet health centre lasted three years (2006 to 2008); the first two years were dedicated to setting up the centre and the third, 2008 will be devoted to consolidating and evaluating what has been achieved.

Strategically, technical support from ALES is organized around five lines of action:

1. Building and fitting out the health centre
2. Organizing community participation, developing a system of checks and balances in the community and social mobilization
3. Improving the technical and management skills of operators at the health centre and of the district management team
4. Transferring responsibilities to the provincial bureau and to Mbalmayo health district
5. Monitoring activities
6. Project evaluation

6. Some of the results

Mobilizing funding for activities

Fig. Level of contributions from different partners
It is noteworthy that the health centre itself generates up to 34% of the revenue needed to finance its activities. As a rule, in the Cameroonian health system, district level health facilities are entirely financed by the State. The situation at the centre reflects its success in generating funding from the community, and is a sign of the community’s acceptance of the health centre.

**Comparison of the trend in revenue between 2006 and 2007**

Despite failure to achieve the financial targets in 2007, the trend in 2007 was quite healthy in comparison with 2006, the year in which the project began.

**Evolution of revenue and expenditure in 2007**

The satisfactory trend in expenditure may be attributed to the system of community checks and balances.
Evolution of curative consultations between 2006 and 2007

Attendance at the health centre in 2007 increased significantly in comparison with 2006, although the objectives were not attained.

Evolution of ANC between 2006 and 2007

This activity really "took off" in comparison with 2006, although it was still far from achieving the objectives set.

Fig. Evolution in detection of cases of BU, 2006-2007
Despite more robust community mobilization, activities to detect cases of Buruli ulcer are still quite hesitant. In all, 41 new cases were detected. Variations in the curve may also indicate that the case-detection effort is not sustained.

Significantly, thanks to the cross-financing mechanisms introduced in the health centre, part of the revenue is used to finance certain Buruli ulcer activities, in particular food for patients, and some of the items used to treat them. In the long run, the centre should be capable of covering all the costs relating to treatment of Buruli ulcer.

7. Conclusion

This venture is novel in that it is based on a situation analysis which led to a global and integrated medical response. The response is a decentralized one and has been introduced with the full participation of the community. Buruli ulcer has served to provide leverage for strengthening the health system in Mbalmayo health district. Although it is still too early to draw conclusions, we are closely monitoring the extent to which Buruli ulcer activities are funded by the centre's own revenue thanks to the cross financing mechanisms introduced. If the experiment proves sustainable, it could serve as a model for other countries in which the disease is endemic and which are looking for ways and means of providing case management for this devastating disease.
Buruli ulcer (BU) and Skin Neglected Tropical Diseases in AFRO Member Countries

Dr Tiendrebéogo Alexandre

Introduction

In 2006, the WHO developed and launched the Global plan to combat Neglected Tropical Diseases (NTDs). These NTDs, rarely lethal, cause severe disabilities and affects poorest of the poor populations, mainly in tropical areas of the world. Among these NTDs are some skin conditions like: Buruli ulcer, Leprosy and Endemic Treponematosis (Yaws and Endemic syphilis or Bejel).

Objective

To favour the combination or integration of surveillance and control of skin NTDs in affected member countries of the WHO African Region (AFRO).

Method

Our study consisted in collecting data from Medical Officers in charge of BU, Leprosy or Endemic treponematosis in countries and distributing a questionnaire to Dermatologists attending a Francophone Dermatologist Congress in Ouagadougou, in February 2007.

Result

Out of 46 Member countries in the African Region of WHO:

- BU is afflicting 26 AFRO Member states,
- Leprosy is not yet eliminated as a public health problem in 2 countries but new cases are still reported in 40 other ones,
- Endemic treponematosis are still present in 15 countries (9 for yaws, 6 for Bejel)
- BU-LEP programmes are currently combined or run by the same programme manager at National level in 6 countries: Benin, Burkina Faso, Cameroon, Congo, Mali and Nigeria,
- Leprosy or other NTD programme staff members at intermediate and district levels are also involved in BU control and surveillance in 5 other countries: CAR, DRC, Gabon, Guinea and Togo.

Conclusion

Skin NTDs are overlapping in 24 AFRO Member countries. Surveillance and control activities to fight these diseases could be efficiently combined or integrated. This would allow a better use of scarce available resources and to increase health service coverage for skin NTDs.
Improvements in wound and scar management and enhanced care for BU patients.

Statement on behalf of the Médecins Sans Frontières (MSF) Geneva group, Buruli ulcer control programme, Akonolinga (Cameroon).


As part of MSF Geneva's programme to revitalize Buruli ulcer control efforts at Akonolinga in Cameroon, the overall objective is to improve case detection and patient management in order to reduce suffering and facilitate healing.

A number of activities have already been enhanced, but progress still needs to be made in wound management. At last year's session, the importance of dressings was emphasized in several programmes, in the context of antibiotic treatment and surgery alike.

During the last 10 years or so, skin wound dressings have been revolutionized through better understanding of the physiological processes of scarring. Conventional approaches based on disinfection with antiseptics and drying out of the wound have changed profoundly in specialist practice. There is an international consensus in favour of developing protocols for treating wounds and scar management, which will thereby ensure quicker treatment and pain reduction.

- Controlled scar management in humid settings is specified and defined by the following four steps:
  - Cleansing;
  - Disinfection;
  - Budding;
  - Epidermization.

Wound assessment is crucially important.

"Modern" dressings such as alginate or hydrogel are available, if they can be afforded, and some have already been tested at the Akonolinga Buruli ulcer control centre.

The above remarks merely underline the message that health workers must receive appropriate training to enable them to play a full part in improving the quality of wound care.

Under a partnership agreement operating since 1998, the nursing sector of the Haute Ecole de Santé in Geneva has worked with the Yaoundé Private Catholic Nursing School and the Nkolndongo Health Centre in Yaoundé to provide continuing training for teachers. It has also developed expertise in the field of wound care and scar management, as evidenced by its postgraduate course entitled "Interdisciplinary management of wounds and scarring" and its numerous continuing training sessions.

Acute or chronic wounds are commonplace in Africa and modern protocols are virtually ignored. A continuing training module on wound management and scarring aimed at teaching nurses at the Yaoundé Private Catholic Nursing School has been developed in partnership with Médecins Sans Frontières (MSF) Switzerland.
Qualified nurses from the Buruli ulcer treatment unit at Akonolinga hospital will be allowed to register for this training, in addition to professionals from the other partner health centre of the Haute École de Santé.

This training, which develops general skills and competencies in wound care, will feature modules focusing specifically on Buruli ulcers and other more specific wounds occurring in Africa. It is envisaged that this project will last 2 years because we wish to profit from the wound care experience of the Cameroonian professionals and the joint working practices developed in the field to adapt the proposed protocols to the specific features of the various diseases, conditions at the various local centres, and the local culture.

The goal of this two-year continuing education project is to enable nurses to develop regional expertise in improving wound and scar management in Yaoundé and the surrounding area. At the end of the project, these nurses will have developed and passed on their skills locally. They will also benefit from ongoing contact with the centre of expertise at the Haute École de Santé, and they will form part of the network of international experts.

The presentation at the 2008 Annual Meeting will include a summary of the initial phase of the project, scheduled for 10-21 March 2008.

The training module will include a course on the interdisciplinary management of wounds and scars. The technical and theoretical aspects of the course will be taught by Caroline Wyndham-White, a part-time lecturer at the Haute École de Santé and the contact point for the Certificate of Advanced Studies in wound care and scar management. There will be input from Cameroonian technical experts.

Practical exercises may also be offered in the pilot units during the first semester of 2008.

We shall focus on work performed in cooperation with Buruli ulcer referral staff at Akonolinga hospital. This service, which specializes in the treatment of BU-type ulcerative wounds, already has experience of working with modern dressings. By then we shall have considered various factors such as identifying needs, adapting the protocols and introducing possible methods of treatment at this specialized site.

We thus hope to report on future prospects and expected follow-up action.
Evaluation form sores and the use of modern dressings for the treatment of Buruli – Presentation of the first results

Carine Adib, MSF Switzerland.

Médecins Sans Frontières (MSF) Switzerland participates in cooperation with the Ministry of Health programme against Buruli ulcer in the district of Akonolinga in Cameroon since 2002. Dressings used in Africa are mostly based compresses saturated with iodine or dakin covered by a single secondary dressing.

For 10 years in Europe, there is a consensus to say that the moist wound healing (concept TIME) enables more effective treatments. MSF, in cooperation with the Groupe Plaies et Cicatrisation des Hôpitaux Universitaires de Genève, introduced protocols based on the principles of moist wound healing’.

Two of the pillars of this approach are the systematic description of the wounds with an evaluation sheet and the gradual introduction of modern dressings (alginate, hydrogel, hydrocellulaire ...).

Since September 2007, the staff has been trained, the evaluation sheet wound was introduced and the modern dressings were used to Akonolinga.

Our experience already shows that the comfort of patients and the work of caregivers improved by these protocols. The pain is lower, detersion of the wound is facilitated and the proliferation of tissues. The fact that we need to renew the dressing every 4 or 5 days make the ambulatory treatment more easy.

This experience appears promising, but there is a need to strengthen the training of caregivers, to continue adapting protocols and monitor objectively our observations.

The limited distribution of these drugs in Africa and the high price of modern dressings remain obstacles. In situations of limited resources, a list of essential equipment has been defined. Its use must be codified. Similarly, exchanges of experience in tropical countries will be promoted.
Key messages health workers would like patients and their families to know about preventing disability in Buruli ulcer

*Linda Faye Lehman*

This presentation will summarize key messages which health workers feel other health workers need to know to teach persons affected by Buruli ulcer (BU) to prevent disability. It will also include what persons affected by BU and their families consider important. Comments have been collected from workers and persons affected by BU in Cameroon, Cote d’Ivoire DR Congo and Ghana.

The key areas addressed are:

1. How to identify a nodule, swelling or open wound for early detection of BU
2. When BU is suspected, who should be shown and where can treatment be received
3. How can antibiotic treatment be taken without interruption
4. When is surgery necessary and what care will be needed before and after surgery
5. What activities require the active participation of the persons affected by BU and their family to prevent or minimize disabilities. These activities include:
   - Care of wounds, skin and scars
   - Positioning to prevent edema and deformities
   - Movement needed to prevent joint stiffness/limitations
   - Daily Self-care and participation in self-help groups
   - When to go for help and where to go for help if there are bigger problems
6. What the importance of good nutrition and continuing daily activities with family, school, work and community contributes to caring for and curing BU.

Not only must these messages be heard but opportunities to develop the skills to do the activities will develop confidence and lessen the feelings of helplessness. Awareness and daily practice will strengthen participation, understanding and compliance.
The best way to prevent and minimize disability is to include specific activities within Buruli Ulcer control programs in the field. This presentation will look at how prevention of disability activities can be integrated into field visits. The most frequent issues that are addressed are 1) wound and skin care; 2) positioning to control edema and prevent contractures/deformity; 3) mobility to prevent soft tissue shortening and joint stiffness; 4) self-care training and 5) simple record keeping which allows impairments and disability to be monitored.

Adequate planning prior to community field visits is important for preparing equipment and materials that may be useful for teaching and treating problems which can cause disability. Frequently mobility aides are needed. On the job training during field visits can help the field health workers and the community to learn how to use local resources within the community.

Simple records can be kept which allows for impairment, activity limitations and/or participation restrictions to be monitored. The monitoring is important to help make decisions if the person affected by BU needs to be referred to a hospital or specialty center. It also helps to evaluate if health workers and/or persons affected by BU are effective in their efforts to prevent and minimize disability.
Basic rehabilitation for Buruli ulcer patients: A practical field guide

Valérie Simonet, occupational therapist, Aide aux Lépreux Emmaüs-Suisse

In every country affected by Buruli ulcer, the issues of decentralization of case management for BU and community participation are more topical than ever.

The practical field guide on basic rehabilitation for BU, which WHO plans to publish this spring, directly addresses these concerns. It is intended mainly for health professionals in peripheral health centres who we would like to encourage to take responsibility for managing ordinary, uncomplicated BU cases.

Aims

- To assist in training in prevention of disabilities from BU in peripheral health centres.
- To help to educate persons affected by BU and their families about treatment.
- To improve links between health centres, families and community intermediaries.
- To harmonize practice in the different centres and in the community, thereby facilitating referral and counter-referral.
- To provide step-by-step guidance for professionals working in the field, from identification of the problem to the appropriate essential actions.

This guide is the fruit of more than three years' work in contact with people affected by Buruli ulcer, their families, community intermediaries, carers in peripheral health centres and professionals specialized in the prevention of disabilities from BU.

Its primary ambition is as closely as possible to meet the needs of people working in the field, in terms not only of the issues addressed, but the language employed.

It is a practical tool that seeks to emulate the simplicity of a recipe book, enabling the reader to pass directly from observation to action, without the need for a complicated analysis that would require deeper knowledge of anatomy and physiology. Writing the guide was comparable to walking a tightrope: it was necessary to provide just enough information for readers to understand what they are doing and why they are doing it, but no more than that so as to avoid confusion. The 130 drawings and photographs that illustrate the guide are a substitute for words and make it easy rapidly to put the guide into practice.

Particular emphasis has been placed on encouraging community and/or family participation, which is why the guide is clearly adapted towards case management by patients themselves.

The Practical Field Guide has been developed and tested by Aide aux Lépreux Emmaüs-Suisse, principally in Cameroon, where it has been in use since 2006, and has served as the basis for short training courses in 2006 and in 2007. At the request of WHO, it has been made available for consultation in several health centres in Côte d’Ivoire in 2007. It has also been re-read by people working the field of BU in the Democratic Republic of the Congo and in Togo. Criticism from these sources, together with numerous comments and suggestions by Health professionals and humanitarian workers in the Americas has made it possible to complete the work.
Buruli ulcer control initiatives at the Kimpese Evangelical Medical Institute hospital in 2007

Phanzu M.D., Diengidi M.B., Imposo B.B.D., Nsiangana S.Z., Lukanu N.P., Suykerbuyk P., Portaels F.

Kimpese Evangelical Medical Institute, Bas-Congo, Democratic Republic of the Congo

I. Introduction

Buruli ulcer is still endemic in the province of Bas-Congo in the Democratic Republic of the Congo, particularly in the vicinity of Songololo, which is one of the most significant foci of the disease nationally.

The Kimpese Evangelical Medical Institute hospital, which houses the UB control programme, is located at Songololo, 220 km south-west of Kinshasa. It regularly treats patients presenting with Mycobacterium ulcerans infection. It is the general referral hospital for the rural health zone of Kimpese, which in 2007 had area of 3,900 km² and a population of 143,382.

Impressed by the hospital's BU control efforts and its progress in recent years, the WHO Technical Advisory Group designated the Kimpese Evangelical Medical Institute hospital a centre of excellence and a sentinel site for epidemiological surveillance of BU in the Bas-Congo region.

In 2007, as in previous years, commendable efforts have been made in the various spheres of intervention recommended by WHO, in partnership with institutions and organizations at the national level (National BU Control Programme, National Institute for Biomedical Research) and internationally (American Leprosy Mission, Antwerp Institute of Tropical Medicine, the European Union, the World Health Organization, Fondation Damien).

II. Activities in the various spheres of intervention

2.1 Early detection at community level. Information, education and communication

- 34 awareness-raising sessions have been organized in communities and schools involving films and lectures on BU;
- 24 active detection campaigns have been carried out in the Songololo area that comprises two rural health zones, namely Nsona-Mpangu health zone and Kimpese health zone;
- 10 supervised training sessions have been organized for physicians and nurses at peripheral health centres.

14 of the 20 health facilities in the Kimpese health zone were visited by our team in 2007.

2.2 Adherence to treatment protocols (treatment with antibiotics, surgery and prevention of disability/rehabilitation)

- Rifampicin-streptomycin treatment is now gradually being entrusted to peripheral health facilities, in accordance with the WHO provisional guidelines;
• Organization of community-based POD training at workplaces of physicians and nurses in peripheral health facilities.

2.3 Laboratory confirmation of cases

• Strengthening capacity of human resources: participation of a physician and laboratory technician from the Kimpese Evangelical Medical Institute hospital at the international M2U course from 17 to 26 September 2007 at the Centre Pasteur in Cameroon (Yaoundé).

• Locally, almost 359 samples have been examined using the Ziehl-Neelsen staining procedure and 50 samples have been cultured on Lowenstein-Jenssen medium since 14 September 2007. About 20 samples have been subjected to histopathological analysis;

• In addition, 369 samples have been sent to the Antwerp Institute of Tropical Medicine for supplementary analysis.

2.4 Strengthening the health system

• Lobbying of the political, administrative and health authorities to ensure that BU control efforts are incorporated into primary health care in Bas-Congo province;

• Standardization of the system for recording and notifying cases in the Kimpese rural health district;

• Designation of 7 local treatment centres, including 4 in the north of the Kimpese rural health zone (Mukimbungu health centre, Kasi regional health centre, Nkuanza health centre, and Matundulu regional health centre) and 3 in the south (Lovo health centre, Songa regional health centre and Viaza health centre).

2.5 Training of health workers, teachers and village volunteers

• Training in detection of BU and treatment of BU patients for 3 physicians working in the endemic zone, 3 supervisors and 30 health centre nurses in January 2007;

• Training in BU detection for 35 community intermediaries in the Kimpese health zone in January 2007.

III. Results

3.1 Outside the Kimpese Evangelical Medical Institute hospital

Awareness-raising and active BU case detection campaigns have been restricted mainly to the Songololo area and Kimpese health zone in particular.

• BU was detected in 184 patients, including 109 new cases, 5 relapses and 70 inactive forms of the disease;

• Among the new patients, 23 were given rifampicin-streptomycin combination treatment in their communities;

• 35 of these 184 patients were classified as complex cases according to WHO criteria and were referred to the Kimpese Evangelical Medical Institute hospital. Only 16 of these patients were able to get to the hospital;
One year or more later, 38 former patients treated at the hospital had benefited from care in their community.

Table 1: **Classification and origin of patients**

<table>
<thead>
<tr>
<th>HZ Kimpese</th>
<th>New cases</th>
<th>Relapses</th>
<th>Inactive forms</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HZ Nsona Mpangu</td>
<td>26</td>
<td>4</td>
<td>21</td>
<td>51</td>
</tr>
<tr>
<td>HZ Tshela</td>
<td>10</td>
<td>0</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>HZ Sekebanza</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>109</strong></td>
<td><strong>5</strong></td>
<td><strong>70</strong></td>
<td><strong>184</strong></td>
</tr>
</tbody>
</table>

Figure 1: **Origin of patients in Bas-Congo province**

3.2 At the Kimpese Evangelical Medical Institute hospital

The general surgical unit at Kimpese Evangelical Medical Institute hospital admitted 75 suspected UB patients, of whom 53 were from the Kimpese health zone and 22 from outside it. The number of admissions has increased steadily since 2002 (Figure 2).
Age and sex

Both sexes were equally affected and no one age bracket predominated.

Table 2. Age and sex profile of cases detected

<table>
<thead>
<tr>
<th></th>
<th>&lt; 15</th>
<th>15-49</th>
<th>&gt; 49</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td>13</td>
<td>15</td>
<td>9</td>
<td>37</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>17</td>
<td>15</td>
<td>6</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
<td>15</td>
<td>75</td>
</tr>
</tbody>
</table>

Classification of patients

Most patients (90.7%) were new cases with relapses accounting for just 9.3% of cases. Disabilities among new cases were observed in 32.4% of patients on admission.

Among the 68 new patients, 49 originated in the Kimpese health zone and 19 from outside it.

Table 3. Number of BU cases detected during the year

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Of which children under 15</th>
<th>Of which female</th>
<th>Of which ulcerative form, simple or mixed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New cases</strong></td>
<td>68</td>
<td>27</td>
<td>34</td>
<td>55</td>
</tr>
<tr>
<td>Including disabled at detection</td>
<td>22</td>
<td>14</td>
<td>12</td>
<td>19</td>
</tr>
<tr>
<td><strong>Relapses</strong></td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
Site of lesions at detection

Lesions were present on the lower limbs in 61.3% of cases, on the upper limbs in 36% of cases, on the head in 10.6% of cases, on the thorax in 4% of cases, on the back in 2.6% of cases, on the abdomen in 1.3% of cases, and on the perineum and buttocks in 1.3% of cases.

Table 4. Site of lesions

<table>
<thead>
<tr>
<th>Site</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head and neck</td>
<td>8</td>
</tr>
<tr>
<td>Upper limbs</td>
<td>27</td>
</tr>
<tr>
<td>Lower limbs</td>
<td>46</td>
</tr>
<tr>
<td>Thorax</td>
<td>3</td>
</tr>
<tr>
<td>Back</td>
<td>2</td>
</tr>
<tr>
<td>Abdomen</td>
<td>1</td>
</tr>
<tr>
<td>Perineum and buttocks</td>
<td>1</td>
</tr>
</tbody>
</table>

Clinical forms at detection

80% of patients were detected at the ulcerative stage.

Table 5. Clinical forms and categories of lesions in all cases detected

<table>
<thead>
<tr>
<th>Non-ulcerative</th>
<th>CAT I</th>
<th>CAT II</th>
<th>CAT III</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcerative</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>17</td>
<td>34</td>
<td>75</td>
</tr>
</tbody>
</table>

Laboratory confirmation of cases (new cases and relapses)

Of the total number admitted to hospital:

Table 6. Case confirmation

<table>
<thead>
<tr>
<th>Confirmation method</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total confirmed by ZN</td>
<td>42</td>
</tr>
<tr>
<td>Total confirmed by molecular biology (PCR)</td>
<td>27</td>
</tr>
<tr>
<td>Total confirmed by histopathology</td>
<td>3</td>
</tr>
<tr>
<td>Total where no confirmation possible</td>
<td>32</td>
</tr>
<tr>
<td>Total confirmed by at least one method</td>
<td>43</td>
</tr>
</tbody>
</table>

Treatment

54 patients (72%) were treated with specific antibiotics (rifampicin-streptomycin); 54 patients (72%) also underwent surgery.

Table 7. Treatments

| Specific antibiotics (rifampicin-streptomycin) | 54 |
| Other antimycobacterial drugs (specify)      | 0  |
| Other therapies alone (specify)              | 0  |
| Surgery (excision, suture, graft, amputation)| 54 |
| Other associated therapies (specify)         | 75 |
| POD, dressings                               | 75 |
Discharged cases

At the end of 2007, 56 patients (74.7%) had been discharged, of whom 40 were cured without complications, 8 were cured with complications (accounting for 14.3% of discharged patients), 3 discharged themselves against medical advice, 2 died from causes other than BU, 2 were transferred and 1 was a mistaken diagnosis.

In all, 243 suspected cases were detected, of which 173 were active forms and 70 inactive forms of the disease. Among developing cases, 75 patients were hospitalized at the Kimpese Evangelical Medical Institute and 98 at peripheral health facilities.

Indicators

Table 8: Indicators

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>New case detection rate, Kimpese health zone</td>
<td>34.2 per 100 000</td>
</tr>
<tr>
<td>Percentage of new cases with limitation of articulatory movement</td>
<td>32.4%</td>
</tr>
<tr>
<td>Percentage of persons under 15 among new patients</td>
<td>39.7%</td>
</tr>
<tr>
<td>Percentage of females among new patients</td>
<td>50%</td>
</tr>
<tr>
<td>Percentage of all confirmed cases</td>
<td>57.3%</td>
</tr>
<tr>
<td>Percentage of ulcerative cases among detected cases</td>
<td>80%</td>
</tr>
</tbody>
</table>
Ten years' experience of the Capuchin Friars Minor in Buruli ulcer control in Côte d'Ivoire

Dr Marie Constance Kadio

The Capuchin Friars Minor have been involved in the management of Buruli ulcer patients in Côte d'Ivoire for more than 10 years.

Initially, during their pastoral visits to local villages, the Friars noted that many children and some adults had large and apparently incurable wounds. They observed that these wretched patients continued to increase in number, and that they often lived in isolation owing to the malodorousness of their wounds. Many affected persons, quite disheartened and having tried numerous remedies without any result, attributed their affliction to sorcery. The Friars felt a duty to assist these people and resolved to learn more about this disease. They garnered abundant information from fact-finding visits to a leper colony at Manikro, a village in central Côte d'Ivoire, where patients were already receiving treatment for BU. Following their investigations and considering the serious nature of the disease, they decided to found a Buruli ulcer centre in 1996 at Zouan Hounien, a town in western Côte d'Ivoire.

In April 2003 the centre has moved to Abidjan because of the war.

The objectives of the centre are:

- To treat patients with Buruli ulcer;
- To raise community awareness of the disease with a view to its early treatment;
- To limit disability through rehabilitation;
- To correct sequelae;
- To promote social rehabilitation.

The number of patients admitted has risen from 4 in 1996 to 175 in 2007. Between 1996 and 2007, the centre has treated 1703 patients of whom 1377 have been cured.

The relapse rate has fallen from 10% in 1996 to 1.15% in 2007.

As a result of its awareness-raising efforts, the centre now admits more patients with nonulcerative forms of the disease.

Early rehabilitation has led to a reduction in the number of cured patients leaving treatment with sequelae.

A number of cured patients have been reintegrated into everyday life and helped to establish income-generating microprojects.

Over the last decade, the following trends have become apparent: increasing numbers of patients have attended the centre, time spent in hospital has been reduced, the number of relapses has decreased, and the number of outpatients has risen thanks to antibiotic treatment initiated by WHO. The war has not lessened the Capuchin Friars' enthusiasm for their mission.
Buruli ulcer in Togo: Review of BU referral centre activities in 2007

A. Assiobo, K.S. Attisso

Introduction

Buruli ulcer is a serious skin infection caused by *Mycobacterium ulcerans*. In Togo, incidence of the disease is highest in the maritime region. The purpose of our review of activities in 2007 is to identify the epidemiological profile of patients at the Togo referral centre and to examine the treatment protocols that were used to improve care of BU patients at the Tsévie referral centre.

Material and methods

63 patients were admitted to the Tsévie referral centre between 1 January and 31 December 2007. The majority were admitted at the ulcerative stage and all were subject to direct bacteriological examination. Only 6% of patients had a PCR test. Radiography was used in cases of bone involvement. A 4-week rifampicin/streptomycin treatment protocol was followed where necessary by excision and skin grafts.

Results

Of the 63 patients admitted, 57% were male. The average age was 29 (age range 3-70). 34% of the patients were under 15 years of age. The majority of lesions were present on the limbs (93% of cases); 76% of cases presented with ulcers. All patients underwent treatment for the minimum period of 4 weeks. 55% underwent surgery (excision and skin grafts). Surgical outcomes were satisfactory with very few complications. The average time spent in hospital was 3.5 months. 2% of patients were discharged with functional sequelae.

Discussion and conclusion

The patient sample does not reflect the true incidence of Buruli ulcer in Togo, which is much higher. The disease is serious because of its disabling sequelae, especially in young people. It therefore has far-reaching social and economic consequences. Recourse to surgery is still frequent despite systematic medical treatment for all patients. The lengthy period of hospitalization may explain why patients are reluctant to seek hospital treatment. These findings confirm the need for better case management, early detection, outpatient treatment and BU awareness-raising and prevention campaigns.
RESEARCH SESSIONS
Deciphering the genetic basis for polyketide variation among mycobacteria producing mycolactones

Sacha A Pidot¹, Jessica L Porter¹, Grant A Jenkin¹, Hui Hong², Peter F Leadlay², Caroline Demangel³, Tim Stinear¹

¹ Department of Microbiology, Monash University, Clayton, VIC, Australia
² Department of Biochemistry, University of Cambridge, Cambridge, UK
³ Pathogénomique Mycobactérienne Intégrée, Institut Pasteur, Paris, France

Since the discovery of mycolactone A/B ten years ago, studies have revealed that different strains of M. ulcerans produce several distinct structural variants of mycolactone that have been named mycolactones C, D, E and F. All mycolactones maintain a conserved 12-membered lactone core with variation occurring in the length and oxidation state of the acyl side chain. We have identified the specific genetic changes in the polyketide synthases among strains of M. ulcerans that have led to the production of these natural variants and by introduction of a gene encoding a specific P450 monooxygenase into a M. ulcerans strain producing mycolactone F we have created the first non-natural mycolactone, mycolactone G. We have then utilized these variants to explore the relationship between mycolactone structure and its activity and demonstrated that mycolactone A/B appears to be the most potent form of the molecule with respect to both immune suppression and cytotoxicity and that its activity is related to the structure of the acyl-side chain.
Gene deletions in *M. ulcerans* haplotypes

*M. ulcerans* is regarded crucial for the pathogenicity of *M. ulcerans*. Our recent genetic analyses identified genomic variations across a world-wide *M. ulcerans* strain collection and identified six haplotypes that have evolved in two major lineages. Variations in prevalence and incidence of Buruli ulcer suggest that the classical lineage found in Africa and Australia is more virulent than the ancestral lineage present in Asia and America. These epidemiological data speak for the modulation of virulence of mycolactone producing *M. ulcerans* by additional genetic factors.

A detailed comparative analysis of regions of difference representing 7% of the genome across the six haplotypes identified 227 genes that were subject to either deletions or functional disruptions. Genes belonging to the functional groups “virulence, detoxification and adaptation” and “PE/PPE proteins” are overrepresented among the coding sequences that were deleted or inactivated by different mechanisms in more than one haplotype. Loss of functionality of these genes may be important for the adaptation of the species to a new ecological niche. Genes exclusively disrupted or deleted in strains belonging to the classical lineage may represent anti-virulence factors. The encoded proteins represent a selection of candidates for experimental investigation of virulence and host-pathogen interaction.
A comparison of DNA extraction procedures for the detection of *M. ulcerans* in environmental samples

**Lies Durnez**¹,²*, Pieter Stragier², Karen Roebben², Anthony Ablordey²,³, Herwig Leirs¹,⁴, Françoise Portaels²

¹ Evolutionary Ecology Group, Department of Biology, University of Antwerp, Antwerp, Belgium; ² Mycobacteriology Unit, Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium; ³ Bacteriology Department, Noguchi Memorial Institute for Medical Research, Accra, Ghana; ⁴ Danish Pest Infestation Laboratory, University of Aarhus, Faculty of Agricultural Sciences, Department of Integrated Pest Management, Kongens Lyngby, Denmark

*Mycobacterium ulcerans* is the causative agent of Buruli ulcer (BU), the third most common mycobacterial disease in humans after tuberculosis and leprosy. This disease is mainly endemic in Central and West Africa, where it affects mostly poor communities. Epidemiological evidence strongly associates BU with aquatic ecosystems and *M. ulcerans* is considered an environmental pathogen. Cultivation of the bacillus is however difficult to achieve. Therefore, at the moment, detection is based on demonstrating by PCR the presence of the insertion sequence IS2404 present in *M. ulcerans* and few other closely related *Mycobacterium* species namely *M. liflandii*, *M. pseudoshottii* and the mycolactone producing *M. marinum* species. This DNA sequence has been identified in water, fish, aquatic insects, detritus, leeches, crustaceans and mollusks.

Before PCR detection of the IS2404 sequence, DNA extraction is needed to concentrate the DNA and eliminate inhibitory substances in the environmental samples. Since several DNA extraction methods are available, three DNA extraction methods were compared for their sensitivity, reproducibility, reliability and rapidity: The one tube cell lysis and DNA extraction procedure (OT), the FastPrep procedure (FP), and the modified Boom procedure (MB). The detection limit of the IS2404 specific PCR following the DNA extraction methods was determined using serial dilutions of *M. ulcerans*. The three methods were also applied to 63 water related environmental samples collected in Buruli ulcer endemic areas in Uganda.

This study shows that the MB, although more lengthy, is more sensitive and reliable than the OT and the FP. Other DNA extraction methods, e.g. the Maxwell 16 System (M16) and the sequence capture procedure (SC), should be tested as well to ensure an adequate detection of *M. ulcerans*-DNA in environmental samples. More research is ongoing.
Mycolactone suppresses T cells by a mechanism involving the Src-family kinase Lck

Sheerazed Boulkroun1, Maria-Isabel Thoulouze2, Marc Monot1, Hui Hong3, Anaïs Merckx4, Gordon Langsley4, Tim Stinear5, Peter F. Leadlay3, Georges Bismuth6, Vincenzo di Bartolo2 & Caroline Demangel1

1Department of Genomes and Genetics, Institut Pasteur, Paris, France, 2Department of Immunology, Institut Pasteur, Paris, France, 3Departments of Chemistry and Biochemistry, University of Cambridge, Cambridge, UK, 4Department of Infectious Diseases, Institut Cochin, Paris, France, 5Department of Microbiology, Monash University, Clayton, Australia, 6Institut Cochin, Université Paris Descartes, CNRS (UMR 8104), Paris, France.

Since BU patients with active lesions show defective cellular immunity, we examined whether mycolactone exerts immunomodulatory properties on human T cells. Using a radiolabeled form of the toxin, we found that mycolactone penetrates lymphocytes rapidly, and initiates a spatial redistribution of the Src-family kinase Lck. Within minutes, the lipid-rafts of mycolactone-exposed lymphocytes were markedly enriched in activated Lck. Stimulation of Lck basal activity by mycolactone induced a dysregulation of intracellular signaling pathways coupled to TCR activation, leading to impaired production of interleukin-2 in response to stimulation. Interestingly, the immunosuppressive potency of mycolactone structural variants correlated with their capacity to activate Lck. Moreover, subcutaneous injection of mycolactone in mice resulted in comparable activation of Lck and functional anergy. Our results therefore demonstrate that mycolactone is able to suppress T cell functions in vivo. Moreover, they define a novel mechanism of T cell immunosuppression involving the redistribution and hyper-activation of Lck.
BCG Vaccination has clear-cut preventive activity in two mouse models of *Mycobacterium ulcerans* Disease. A study of *M. ulcerans* containment, histopathological features, and cytokine expression.


**Background**

BCG vaccine efficacy in preventing Buruli ulcer (BU) is controversial with clinical studies suggesting a delay in disease development and/or a reduction in *M. ulcerans* (*Mu*) dissemination, e.g., osteomyelitis. Mouse model studies have also failed to provide clear-cut answers.

**Methods**

C57BL/6 and BALB/c mice were subcutaneously BCG vaccinated with \( \sim 10^7 \) cfu and challenged 6 weeks later with \( \sim 10^6 \) *Mu* in the right hind footpad. Time to footpad swelling, cytokine responses at vaccination and challenge sites (RT-PCR), and tissue histopathology were assessed. As negative controls, mice were injected with the vehicle without vaccine 6 weeks before *Mu* challenge. Unvaccinated positive control mice were treated daily with 10 mg/kg rifampicin (Rif).

**Results**

BCG was detectable in spleen the day after vaccination. In 3 weeks, BCG multiplied to \( \sim 4 \log_{10} \) cfu in BALB/c, reaching a plateau that persisted until \( \geq 6 \) wks after *Mu* challenge; in C57BL/6, BCG cfu counts were similar at day 1 and wk 3 but were at least 1 \( \log_{10} \) lower at subsequent time points (Fig. 1). Histopathological analysis revealed enlarged lymph nodes in the flanks of BCG-vaccinated mice. One day after challenge, \( \sim 3 \log_{10} *Mu* cfu were isolated from all footpads. There was a small increase of *Mu* to nearly \( 4 \log_{10} *Mu* in negative control mice as assessed at weeks 2 and 4 after challenge. In BCG-vaccinated BALB/c there was a decline in *Mu* counts at week 2 and sharp reductions thereafter, whereas in C57BL/6 mice there was a plateau during the first 4 weeks by which time unvaccinated C57BL/6 mice were beginning to show footpad swelling and thus could no more be assessed for *Mu* growth before swelling (Fig. 2). Differences in mouse footpads after *Mu* challenge were not readily apparent in terms of infiltrating cells. The *Mu* cfu counts in the footpads decreased markedly in Rif-treated positive controls, with a less pronounced effect in the C57BL/6 than in BALB/c mice. In C57BL/6 mice, the median time to footpad swelling was 7 weeks and 13 weeks in unvaccinated and vaccinated mice, respectively. In BALB/c mice, the median time to footpad swelling was 15 weeks in unvaccinated controls whereas only 3 of 34 vaccinated mice developed swelling by 17 weeks (Fig. 3). Lymphokine and histopathology results are pending.
Conclusions

(i) BCG vaccination delays the development of symptomatic *Mu* infection in both C57BL/6 and BALB/c mice. (ii) However the delay was longer in BALB/c than in C57BL/6 mice, the former allowing greater multiplication of BCG but limiting the growth of *Mu* and/or the development of *Mu* footpad swelling more effectively than did the classically strong Th1, mycobacteria-resistant C57BL/6 mice. In addition the response to treatment may be more pronounced in BALB/c mice. Analysis of histopathological changes and cytokine responses may provide important insights into the pathogenesis of BU.
Phagocytosis of *Mycobacterium ulcerans* in the course of rifampicin and streptomycin chemotherapy

*Daniela Schütte *, Alphonse Um-Boock †, Gerd Pluschke *

* Swiss Tropical Institute, Molecular Immunology, Basel, Switzerland  
† Leprosy Relief Emmaus-Switzerland (ALES) Cameroon, Yaounde, Cameroon

To elucidate the processes taking place in Buruli ulcer lesions in the course of chemotherapy we performed an in-depth histological analysis of lesions excised from two patients after four weeks of rifampicin and streptomycin (R/S) treatment. Results are compared to findings in untreated and eight weeks treated lesions, respectively.

Clinically confirmed BU patients admitted to the Buruli ward in Ayos, Cameroon, were enrolled in this study. Tissue excision samples were analysed by HE and ZN staining as well as immunohistochemistry. The main features evaluated were local immune responses, histopathological alterations and fate of extracellular bacterial clusters symptomatic for *M. ulcerans* infections.

After four weeks of R/S treatment we observed large numbers of intracellular rod-shaped mycobacteria internalized by macrophages but not neutrophils. Occasionally internalized mycobacteria formed globus-like aggregations. While distinct bands of inflammatory leukocytes surrounded the necrotic core in an early ulcer, acute cellular infiltration covering the whole section had developed in a nodular lesion. Early granuloma formation was apparent in the ulcer’s healthy appearing margins. In contrast, lesions after eight weeks chemotherapy show only bacterial debris inside macrophages and Langhans’ giant cells and extensive chronic infiltrates are present forming huge granulomas.

R/S treatment of BU lesions leads to a rapid onset of local cellular immune responses associated with phagocytosis of the extracellular *M. ulcerans* by macrophages. This might be due to declining levels of the macrolide toxin mycolactone in the tissue permitting survival of infiltrating leukocytes thus leading to an enhanced chemotherapy-induced clearance of the infection.
Pathological findings of Buruli ulcer lesions during antibiotic treatment

Junichiro En,1,2 Stephen Sarfo,3 Richard O. Phillips,3,4 Mark Wansbrough-Jones,5 Masamichi Goto1

1Department of Human Pathology, Kagoshima University, Kagoshima, Japan
2National Sanatorium Hoshizuka-Keiaien, Kagoshima, Japan
3Komfo Anokye Teaching Hospital, Kumasi, Ghana
4School of Medical Sciences, KNUST, Kumasi, Ghana
5Centre for Infection, Department of Cellular and Molecular Medicine, St. George’s, University of London, U.K.

In order to evaluate changes in the immunological response to M. ulcerans during antibiotic therapy aimed at killing M. ulcerans and thus preventing further secretion of mycolactone, we compared the histopathology of skin lesions of Buruli ulcer. Our hypothesis is that when mycolactone production is reduced, macrophages will be able to phagocytose M. ulcerans and that it will be possible to observe organisms within macrophages using electron microscopy (EM).

Skin biopsies (4mm) were taken from 7 Ghanaian patients with proven M. ulcerans infection just before treatment (week 0) and 6 weeks after starting daily streptomycin (15mg/kg) - rifampicin (10mg/kg) according to WHO guidelines. Samples were obtained from patients with nodules (2), ulcers (3) and oedema (2). The tissue samples were divided into two and fixed with formalin for light microscopy (LM) or glutaraldehyde for EM. In formalin-fixed hematoxylin and eosin stained LM slides, at week 0 most cases showed necrosis and mild neutrophilic infiltration, but at week 6 the majority of specimens showed fibrosis with lymphocytic infiltration and/or epithelioid cell granuloma formation. Fite's acid-fast staining was focally positive in only one oedema case on week 0, and became negative after treatment. One μm thick plastic sections for EM stained with toluidine blue of this case did not show the bacilli before or after treatment. Immunohistochemistry using an anti-BCG polyclonal antibody cross-reactive with mycobacteria was positive only in Fite positive areas.

Careful examination of peripheral nerve bundles in these specimens revealed degeneration with vacuolar changes both before and after treatment. This was in keeping with the findings of nerve damage in mice inoculated with M. ulcerans observed in our previous study (Goto M et al., Am J Pathol 2006).

As the number of M. ulcerans in the sampled specimens was low and killing of bacilli was successful on week 6, we could not demonstrate phagocytosis in this human Buruli ulcer study. However, nerve degeneration with vacuolar changes was observed irrespective of treatment. These findings help to explain the painless nature of Buruli ulcer.
Cytotoxicity of mycolactone from *Mycobacterium ulcerans* infected human tissue during antibiotic treatment with Streptomycin-Rifampicin

**Sarfo FS**, Phillips R\(^1\), Phillips W\(^2\), Boateng A\(^4\), Adentwe E\(^4\), M. Phillip\(^5\), Terelli E.T\(^5\), Waddel S\(^5\), Rangers B\(^6\), Small P\(^6\), Wansbrough-Jones M\(^5\)

\(^1\)Komfo Anokye Teaching Hospital, Kumasi, Ghana.
\(^2\)School of Medical Sciences, KNUST, Kumasi, Ghana
\(^3\)National Buruli ulcer Control Programme, MOH, Ghana
\(^4\)Tepa Government Hospital, Ahafo Ano North District, Tepa, Ghana
\(^5\)St George’s University of London, London
\(^6\)University of Tennessee, USA

**Introduction**

*Mycobacterium ulcerans* (Mu) secretes toxic macrolides called mycolactone which are cytotoxic to immune and non-immune cell lines in-vitro and cause local immunosuppression. Purified mycolactone injected into the skin of guinea pigs causes ulcerative lesions similar to those observed in human subjects. The aim of this study was to extract mycolactone from Mu infected human skin and to determine whether tissue levels diminish during treatment with rifampicin and streptomycin for 8 weeks.

**Methods**

32 patients with active Mu disease were recruited at Tepa Government Hospital. 4 mm punch biopsies were obtained to establish the diagnosis of Mu disease by microscopy, culture and PCR. Further biopsies obtained at 0, 4, 6, 8 and 12 weeks for detection and quantification of mycolactone were snap frozen in liquid nitrogen. Acetone soluble lipids (ASL) were extracted by Folch’s procedure followed by precipitation in ice-cold acetone. Analysis for the presence of mycolactone was performed using thin layer chromatography (TLC) and mass spectrometry under electron spray conditions for the presence of an ion with an m/z of 765.5 with fragmentation to produce the conserved core lactone with m/z of 429. Purified mycolactone supplied by Professor Pam Small was used for comparison. Biological activity of ASL was measured as cytotoxicity for human embryonic lung fibroblasts after overnight culture; percent cytotoxicity was determined in a dimethylthiazolyl diphenyl tetrazolium bromide (MTT) assay. Antibiotic therapy was standard rifampicin 10mg/kg orally and streptomycin 15mg/kg I/M for 8 weeks.

**Results**

Among 32 patients with confirmed Mu disease, 17 had ulcerative lesions, 8 nodules, 4 oedema and 3 plaques. There was a steady decline in surface area of lesions during treatment with 26 lesions healing within 8 weeks of treatment while the rest healed later. Mycolactone was detected in infected tissue on TLC as a UV active band with a refractive index of 0.25. This band was not visible in specimens taken during antibiotic treatment suggesting a decline in levels of mycolactone. On mass spectrometry mycolactone was detected as an ion with m/z 765.5 and characteristic fragmentation to produce the core lactone ring with m/z 429. Signals were detected in ASL from all lesions before during and 4 weeks after completion of antibiotic treatment. Cytotoxicity was 56.0± 3.9 (percentage mean ± SEM).
at 0 weeks, 54.3±3.9 at 4 weeks, 36.0±5.4 at 6 weeks, 20.6±6.4 at 8 weeks and 19.2±5.3 at 12 weeks suggesting a decline with antibiotic treatment.

**Conclusion**

These results showed that mycolactone A/B was detectable in *M. ulcerans* infected human skin and its bioactivity declined during antibiotic treatment in association with clinical healing of the lesions.
Dynamics of the cytokine response to *M. ulcerans* during successful antibiotic treatment for human *M. ulcerans* disease (Buruli ulcer)

Sarfo FS¹, Phillips R¹,², Ampadu E³, Boateng A⁴, Adentwe E⁴, Wansbrough-Jones M⁵

¹Komfo Anokye Teaching Hospital, Kumasi, Ghana.
²School of Medical Sciences, KNUST, Kumasi, Ghana
³National Buruli ulcer Control Programme, MOH, Ghana
⁴Tepa Government Hospital, Ahafo Ano North District, Tepa, Ghana
⁵St George’s University of London, London

Introduction

We have shown previously that after stimulation with Mu sonicate of whole blood from patients with Mu disease, there was significant IFN-γ production which was higher in patients with established ulcers than in those with early lesions, compatible with slow development of a TH1 type immune response. IL-10 production from Mu stimulated blood was sustained from an early stage and somewhat non-specific, being similar in patients with active tuberculosis. If development of the immune response is inhibited by mycolactone, antibiotic treatment which kills Mu and inhibits production of mycolactone would be expected to enhance the immune response. Therefore we have studied the evolution of the IFN-γ and IL-10 response after Mu sonicate stimulation of whole blood from patients with early Mu lesions during antibiotic treatment.

Methods

Twenty six patients with *Mu* disease were recruited from the Ahafo Ano North District in Ghana. Using a whole blood assay, IFN-γ and IL-10 responses to Mu sonicate were measured at 0, 4, 8 and 12 weeks of treatment with the combination rifampicin 10mg/kg orally and streptomycin 15mg/kg I/M (RS). The diagnosis of Mu disease was confirmed by PCR for IS 2404 in all 26 patients, 13 with pre-ulcerative lesions (4 nodules, 4 plaques and 5 oedematous) and 13 with ulcers. Size of lesions was assessed before treatment and again at 8 and 12 weeks. Lesions were grouped into small (<10cm in diameter) and large ≥10cm in diameter. Patients were reviewed 2 weekly up to 12 weeks and monthly thereafter up to 52 weeks.

Results

19 lesions healed in the first 12 weeks of treatment and the remainder within 24 weeks. There were no recurrences after follow up for 12 months.

There was a significant increase in IFN-γ secretion from a baseline median concentration of 463 pg/ml (range 37-1780) to 1128 pg/ml (range 271-2228) after 4 weeks treatment with RS (p=0.004) and a further increase after 8 weeks to 1330 pg/ml (185-3339) (p=0.0002 compared to baseline). At 12 weeks IFN-γ secretion remained higher than at baseline at 1115 pg/ml (66-3279) but not significantly so.
10 patients with large lesions including 4 with oedema exhibited a dramatic increase in median IFN-γ secretion from a baseline of 395 pg/ml (37-1461) to 1217 pg/ml (557-2178) at 4 weeks, 1745 (930-3329 pg/ml) at 8 weeks and 1233 (944-2615 pg/ml) at 12 weeks (p = 0.0015, 0.0002 and 0.0075 at 4, 8 and 12 weeks respectively compared with baseline).

There was a gradual decline in the median IL-10 response during antibiotic therapy but there were no significant differences between the time points [median 699 pg/ml at 0 weeks, 545 at 4 weeks, 513 at 8 weeks and 497 at 12 weeks]. There was no inverse correlation between IFN-γ and IL-10 responses.

**Conclusions**

Treatment with RS for 8 weeks resulted in healing without recurrence in all 26 patients. IFN-γ secretion in response to Mu antigen stimulation recovered during antibiotic therapy in patients with Mu disease, particularly in patients with oedematous or large lesions. This response occurred independently of IL-10 secretion. The increase in IFN-γ secretion observed during antibiotic treatment suggests that the demise of *M. ulcerans* and suppression of mycolactone production encourages development of a Th1 response.
The immunosuppressive activity of mycolactone in primary human monocytes and macrophages depends on a post-transcriptional inhibition of cytokine and chemokine production.

Rachel E. Simmonds, Ferdinand Lali, Tim Smallie, Sandra Sacre, Pam Small and Brian Foxwell

The virulence and immunosuppressive activity of *Mycobacterium ulcerans* (*M. ulcerans*) is attributed to mycolactone, a polyketide toxin synthesised by the bacteria. We have explored the consequence and mechanism of mycolactone treatment of primary human monocytes and macrophages in response to a wide range of Toll-like receptor (TLR) ligands. We found that the production of cytokines, chemokines and other intracellular effector molecules are strongly and dose-dependently inhibited by mycolactone irrespective of the stimulus. In contrast, mycolactone had no effect on activation of signalling pathways including MAP kinases and NF-κB or the steady state levels of mRNA transcripts for TNF, IL-6 or Cox-2. Furthermore, mycolactone was capable of preventing further cytokine production by pre-activated monocytes and spontaneous cytokine release by RA synovial membrane cultures. Levels of IL-6 protein were found to be reduced in cell lysates as well as supernatants. Taken together, this suggests that mycolactone may exert its effect by translational control. Indeed, mycolactone was found to increase the phosphorylation of eukaryotic-initiation factor 2α (eIF2α) in macrophages. This likely represents one of several mechanisms by which mycolactone acts to paralyse innate immune responses in Buruli ulcer.
Environmental Predictors of Buruli ulcer cases and *Mycobacterium ulcerans*: a division between human disease and pathogen distribution in nature


¹Department of Entomology, Michigan State University, East Lansing, MI 48824, USA.
²Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA.
³Center for Global Change and Earth Observations, Michigan State University, East Lansing, MI 48824, USA.
⁴Parasitology Department, Noguchi Memorial Institute of Medical Research, Legon, Ghana.

Although epidemiological studies and reviews of Buruli ulcer often associate disease outbreaks with disturbed aquatic habitats, there have been no studies that have, 1) successfully quantified this relationship; 2) sought to identify specific biological or ecological mechanisms that would explain these associations; and 3) undertaken research that would provide data to inform future experimental studies for understanding these potential mechanisms. Recent studies in Benin have shown that land use/cover is related to both disease presence and prevalence at large spatial scales (20 km), but local-scale processes still have not been identified. The first goal of this study was to evaluate water quality variables that partition waterbodies into groups according to disease and pathogen occurrence. The second goal was to use this analysis to inform site selection for future studies into ecological interactions of human disease, pathogen distribution and environmental variables related to focal outbreaks.

Water quality variables were measured from a total of 98 sites with most located in three major regions of southern Ghana: Volta, Greater Accra, Ashanti. *In situ* physical water variables were measured using a YSI Data Sonde deployed in each waterbody for at least one hour, and 1L water samples were analyzed for other physical, chemical and nutrient criteria that collectively represented 23 variables. Sites were considered endemic for the disease if at least one case was reported between 2003 and 2006, and non-endemic if otherwise. *M. ulcerans* was detected from waterbodies using a serial testing PCR approach using primers first targeting ER (enoyl reductase), and subsequently for VNTR loci unique to *M. ulcerans* of the ER positives. A site was considered to be present with *M. ulcerans* if at least one of the following samples tested positive: 5 – 8 nitrocellulose or fiberglass filters with filtrate from the water column, or biofilm scraped from any one of three dominant aquatic plant taxa. These environmental samples (i.e., filters or biofilm) have been found in companion studies to be the best indicators of *M. ulcerans* in aquatic habitats.

Classification tree (CT) analysis was used to develop a stepwise decision tree process for identifying water quality variables related to aquatic habitats associated with human disease, and to evaluate decision congruence for independent pathogen (*M. ulcerans*) detection. Classification tree analysis was ideal for this process, as it is commonly used to predict water quality variables impacting habitat for biological communities, and it is also used in disease epidemiology and other scientific disciplines where large, multivariate datasets are analyzed for detecting predictable associations. Furthermore, this procedure does not require the traditional assumptions of parametric statistics, and CT can include both categorical and continuous predictor variables together for decision tree development, lending itself to easy and practical interpretation of large multivariate datasets. Further, it is possible to identify specific sites for more focused study in the future. The CT analysis for this study included the following predictor variables: region, waterbody classification type (e.g., pond, river, wetland), waterbody flow type (i.e., lentic, lotic), waterbody history (i.e., natural, human made), and 23 water quality variables (e.g., dissolved oxygen, nitrates, ions).
There was severe division in the decision trees constructed for human disease presence/absence and pathogen presence/absence, and the exclusion of some water quality variables led to slightly different decision trees. For all trees, region was the most important variable that initially partitioned sites, where no human disease or pathogen was detected from sites in the Volta region. After accounting for region, sites within the Greater Accra and Ashanti regions were partitioned by several water quality variables, with differentiation occurring between human disease and pathogen decision trees.

For human disease, sites in the Greater Accra and Ashanti region were partitioned by dissolved oxygen (DO), chloride, magnesium and nitrites. Sites with high DO (> 87%) were rare, but were always nonendemic. Sites with lower DO and high chloride concentrations (> 4mg/l) were mostly endemic, and within this group of sites, those with high magnesium (> 11.6mg/l) were all endemic. Those sites with low DO, high chloride, but low magnesium and low nitrites (< 0.011 mg/l) were most often endemic. Although speculative, these results suggest that human disease may be primarily related to associations with underlying geological conditions that are represented by chloride and magnesium ions, perhaps as indicators of human mining or agricultural activities.

The pathogen was rarely detected at sites in the Ashanti and Greater Accra regions with relatively low concentrations of suspended solids (< 10 mg/l). Of those sites with higher suspended solids the number of sites with and without pathogen detection was variable, but those that also had low nitrate concentrations (< 0.57 mg/l) were more often without *M. ulcerans*. These results indicate that *M. ulcerans* presence/absence may be primarily driven by ecological factors occurring within a particular waterbody of the Greater Accra and Ashanti regions. On-going analyses are addressing the differential role of large-scale geological factors and local-scale ecological conditions that drive focal human disease emergence. We hypothesize that focal human disease emergence is a function of dynamic biological processes within waterbodies that are mediated by complex human landscape use associations within a large matrix of agriculture.
Unusual members of the *M. marinum/M. ulcerans* complex causing disease in Australian reptiles

Janet Fyfe¹, Jessica Porter², George Reppas³, Richard Malik⁴ and Tim Stinear².

¹ Victorian Infectious Diseases Reference Laboratory, North Melbourne, Australia
² Department of Microbiology, Monash University, Clayton, Australia.
³ Symbion Health Vetnostics, Sydney, Australia
⁴ Post Graduate Foundation in Veterinary Science and Faculty of Veterinary Science, The University of Sydney, Australia

Paraffin-embedded tissue sections from a range of captive reptile species with clinical mycobacterial disease were submitted for molecular identification of the associated mycobacterial species. All the reptiles had been domiciled in New South Wales, Australia, a state where only a single case of infection with *Mycobacterium ulcerans* has so far been reported.

The internal transcribed spacer (ITS) regions were successfully amplified from three of the DNA extracts prepared from the fixed tissues, and sequence analysis identified the presence of a member of the *Mycobacterium marinum/Mycobacterium ulcerans* complex.

Subsequent molecular analysis, including VNTR typing and sequencing of a region of the IS2404-like element, showed that one of the cases, a carpet snake with multifocal disseminated granulomatous disease, was infected with a strain closely related to the “missing link” previously described from a human patient in France (Chemlal *et al.*, 2002, J Clin Microbiol 40: 2370-80).

A painted turtle, which had died at Taronga Zoo (Sydney, Australia), was shown by real-time PCR, to be infected with a member of the *M. marinum/M. ulcerans* complex, which contained IS2404 and KR, but not IS2606. This would indicate that this turtle was infected with a member of the mycolactone-producing mycobacteria (MPM), phylogenetically related to an isolate (*M. marinum* CC240299) previously reported to have caused disease in Israeli fish (Ucko & Colorni, 2005, J Clin Microbiol 43: 892-895).

A second snake (blotched python), which had presented with anorexia and unusual coiling was found to be systemically infected with a mycobacterium which proved to be a novel member of the *M. marinum/M. ulcerans* complex, with copies of IS2404, but lacking both IS2606 and pMUM. Sequence analysis of several MLST and VNTR loci was performed in order to determine the phylogenetic status of this novel strain within the complex.
Investigations into the possible role of Ringtail and Brushtail possums as wildlife reservoirs for *Mycobacterium ulcerans* in south-eastern Australia.

Carolyn O’Brien¹, Janet Fyfe², Caroline Lavender², Kath Handasyde³, Christina McCowan¹, Ian Beveridge¹, Joseph Azuolas⁴, Timothy Stinear⁵, John Hayman², Paul Johnson⁶

1. Faculty of Veterinary Science, The University of Melbourne, Werribee, Victoria, Australia
2. Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria, Australia
3. Department of Zoology, The University of Melbourne, Parkville, Victoria, Australia
4. Department of Primary Industries, Attwood, Victoria, Australia
5. Department of Microbiology, Monash University, Clayton, Victoria, Australia
6. Department of Infectious Diseases, Austin Hospital, Heidelberg, Victoria Australia

Natural infections caused by *Mycobacterium ulcerans* have been observed in a variety of native Australian marsupials, including common ringtail (*Pseudocheirus peregrinus*) and brushtail possums (*Trichosurus vulpecular*) domiciled in human endemic areas of south-eastern Australia. Indeed, possums are considered to be particularly prone to infections caused by mycobacterial species and in New Zealand where Australian brushtail possums are a significant pest, large numbers are infected with *Mycobacterium bovis*, where they act as a reservoir species for bovine tuberculosis but are also susceptible to developing fatal disseminated tuberculosis.

Field surveys of one particular endemic coastal area in south-eastern Australia have identified large numbers of ringtail and brushtail possum excreta positive for IS2404, IS2606 and KR DNA by real-time PCR. VNTR analysis shows this to be the same organism responsible for human disease in this area. The signals from some samples indicate the presence of at least $10^7$ organisms/faecal pellet. Similar surveys in locations not currently associated with Buruli ulcer cases indicate that the presence of *M. ulcerans* in possum excreta is not geographically widespread. These findings, together with the culture of *M. ulcerans* from the liver of a dead brushtail possum, have lead us to hypothesise that possums may play a role as a wildlife reservoir of *M. ulcerans*.

To test the theory that possums act as a reservoir, rather than spill-over host, we plan to follow a rolling cohort of animals naturally domiciled in Point Lonsdale, Victoria, the site of a significant and ongoing outbreak of Buruli ulcer in people. The possums will be trapped on a tri-annual basis. They will be permanently marked for ongoing identification (micro-chipped and tattooed) and morphologic data and samples such as blood and fresh faeces will be collected for analysis. The results of this study should lead us to a clearer understanding of the ecology and mode of transmission of *M. ulcerans* in Australia.
Modeling transmission of Mycobacterium ulcerans through local food webs

Benjamin Roche1, M. Erik Benbow2, Richard Merrit2, Ryan Kimbirauskas2, Mollie McIntosh2 and Jean-François Guégan1,3

1 Génétique et Evolution des Maladies Infectieuses, UMR GEMI IRD-CNRS 2724, Montpellier, France.
2 Michigan State University, Department of Entomology, 243 Natural Science Building, East Lansing, MI, USA.
3 The French School of Public Health, Hôtel-Dieu Central Hospital, Paris, France.

One puzzling aspect of Mycobacterium ulcerans transmission is to know how it can be transmitted from natural and/or man-made ecosystems to human. Even if the transmission of the mycobacteria through the bite of an aquatic bug is likely to happen, we do not know anything, or so few, on disease agent transmission within aquatic food webs. In this oral communication, using a mathematical model supported by quantitative data from 27 different localities in western Africa, we elaborated an optimal “disease web”, which represents how disease agent transmission can happen within local aquatic host communities. We will show what are the more likely patterns of disease transmission across the different aquatic communities we studied, and we will compare those patterns of disease transmission with natural food-webs occurring in these types of West African standing water ecosystems. Then, within the framework of food web theory, we will discuss main routes of Mycobacterium ulcerans transmission within the environment. We are considering classical indicators like connectance and nestedness to understanding the stability and predictability of Mycobacterium ulcerans transmission within and across different localities.
**Mycobacterium ulcerans** detection with different molecular techniques in skin biopsies and environmental samples from Benin

Leigheb G¹, Zanetti S², Cannas S³, Molicotti P², Dossou A³, Poggio F⁴, Clemente C⁵, Zavattaro E¹, Johnson RC⁶, Sopoh G³, Leigheb F⁷

¹Dermatologic Clinic, University of Piemonte Orientale “A. Avogadro”, Novara, Italy; ²Mycobacteriology Laboratory, University of Sassari, Italy; ³Centre de Dépistage et de Traitement de l’Ulcère de Buruli (CDTUB), Allada, Benin; ⁴Scientific Committee on Buruli Ulcer Program, Rotary Club Milano Aquileia, Rotary International, Milano, Italy; ⁵Pathology and Cytopathology Division, S. Pio X Hospital, Milano, Italy; ⁶Programme National de Lutte contre l’Ulcère de Buruli et la Lèpre, Ministère de la Santé Publique, Cotonou, Benin; ⁷Hygiene and Public Health Unit, University of Piemonte Orientale “A. Avogadro”, Novara, Italy.

The diagnosis of Buruli Ulcer (BU) is based on the employment of different techniques, with the gold standard being represented by PCR, which is able to specifically identify various nucleotide sequences in the **Mycobacterium ulcerans** genome.

IS2404 was the first sequence used for diagnostic confirmation in biopsies from BU patients; consequently it was used to identify **Mycobacterium ulcerans** in environmental samples. More recently IS2606 and Nested-PCR techniques were introduced to obtain more specificity and to reduce the number of false positives that have come to light.

With this in mind, we performed the two PCR techniques in a collection of 319 biological samples from the BU endemic areas of Zé and Lalo, Benin, collected during the years 2006-2007. They were composed of 100 cutaneous specimens from patients affected by lesions suspected to be BU or with uncertain Ziehl-Nielsen, 208 aquatic animals and 11 samples from water sources and algae.

The samples were stored in 70% ethanol, and then subjected to PCR for IS2404 and IS2606 in the Mycobacteriology Laboratory of the University of Sassari, Italy. For the identification of IS2404 the nested-PCR technique was used.

Among the cutaneous specimens only 18% resulted positive for IS2404 compared to 29% with IS2606. The subsequent sequencing of all the obtained positive templates confirmed the nested-PCR data, while IS2606 was confirmed in only 24 cases.

In the environmental samples, comprised of insects, crustaceans, molluscs, amphibians, fish, algae and mud, only one Naucoride gave a positive result and this was confirmed by sequencing; whereas 3 apparent positives in mosquitoes were found to be non-specific by nested-PCR.

The small number of specific positive PCR results in environmental samples detected by our investigation puts into doubt that numerous positives for **Mycobacterium ulcerans** previously reported in animals are a confirmation of their role as vectors in BU.
Role of the extracellular matrix of *Mycobacterium ulcerans*

Laurent Marsollier,1 Mary Jackson,2 Geneviève Milon,3 Jacques Aubry,4 Céline Leroy,1 Priscille Brodin5

1 Host Pathogen Interactions Study Group, Angers Faculty of Medicine and University Teaching Hospital, Angers, France; 2 Mycobacteria Research Laboratories Department of Microbiology, Immunology and Pathology, Colorado State University, USA; 3 Laboratory for immunophysiology of intracellular parasitism, Institut Pasteur, Paris, France; 4 University of Nantes, U601 Inserm, Nantes, France; 5 INSERM Avenir Team, Institut Pasteur of Korea, Seoul, Republic of Korea.

*Mycobacterium ulcerans* is a mycobacterium whose reservoir is in water and soil. Like very many bacteria and mycobacteria, *M. ulcerans* is able to form biofilms characterized, inter alia, by coating with an extracellular matrix of lipids, carbohydrates and proteins. This structure, which has been observed in bacilli directly isolated from human samples, is the reservoir of mycolactone. The mycolactone in the extracellular matrix is not free; it is contained in vesicles that ensure better diffusion of the toxin, which is a very hydrophobic molecule. The extracellular matrix that boosts the resistance of bacteria to microbicides also plays a preponderant role in the ecology of the bacillus. Bacilli not coated with an extracellular matrix are unable to colonize water bugs. Moreover, the synthesis of the extracellular matrix is subject to environmental factors. It is not present, for example, in populations of *M. ulcerans* cultured in media that have been enriched in aquatic plant extracts. It is therefore important to understand how this extracellular matrix can be regulated in order to develop inhibitors that specifically prevent the formation of the matrix, concomitantly ensuring greater effectiveness of antibiotics in patient therapy and better immune response.

Programme supported by the French Raoul Follereau Foundation, the International Network of Pasteur Institutes (PTR 212) and the National Institute for Health and Medical Research (INSERM).
Buruli ulcer is an emerging disease in tropical regions, particularly in equatorial Africa. It is caused by *Mycobacterium ulcerans*, a mycobacterium present in aquatic environments. The disease affects populations that live or work near wet or marshy areas. Although the study of the mode of transmission of the bacillus is a priority for the Global Buruli Ulcer Initiative, this is still poorly understood. Studies carried out 10 years ago first indicated that water bugs are responsible for transmitting the disease. Additional findings have provided further evidence of the dual role of water bugs as vectors and hosts of the bacillus. However, knowledge of the biology of these carnivorous flying insects is still limited. A study designed to shed more light on the life cycle of the bugs was initiated in Cameroon several months ago. Thus, the distribution of species and their rate of infection by *M. ulcerans* are being studied from a seasonal perspective. The study should help to increase knowledge of the bugs' biology and determine environmental and seasonal risk factors. To date, seven families of water bugs have already been inventoried. Initial findings suggest significant variation in the monthly and seasonal distribution of these families and their rate of infection.

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Relationships of *Mycobacterium ulcerans* with aquatic plants: An intensive survey of 80 waterbodies in Ghana, Africa

Mollie D. McIntosh\(^1\), Heather Williamson\(^2\), M. Eric Benbow\(^1\), Ryan Kimbirauskas\(^1\), Rebecca Kolar\(^3\), Richard W. Merritt\(^1\), Pamela L.C. Small\(^2\), Daniel Boakye\(^3\), Charles Quaye\(^3\), Charles Yeboah\(^3\), Felix Akpabey\(^4\)

\(^1\)Department of Entomology, Michigan State University, East Lansing, MI 48824, USA.
\(^2\)Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA.
\(^3\)Parasitology Department, Noguchi Memorial Institute of Medical Research, Legon, Ghana.
\(^4\)Water Research Institute, Accra, Ghana.

Numerous studies have associated Buruli ulcer disease with disturbed aquatic habitats; however, the natural reservoir, distribution and mode of transmission of the bacterial pathogen, *Mycobacterium ulcerans*, remain unknown. To better understand the ecology of this disease a large-scale survey of aquatic habitats was initiated to identify potential ecological (abiotic and biotic) relationships that influence the extent and distribution of *M. ulcerans* in the environment and human disease outbreaks. Aquatic plants have been identified as a potential biotic community that could influence *M. ulcerans* distribution. One goal of this large-scale survey was to assess any potential relationships between *M. ulcerans* and aquatic plants. Specifically, our objectives were: (1) to evaluate differences in the aquatic plant community between Buruli ulcer endemic and non-endemic waterbodies; and (2) identify potential interactions between *M. ulcerans* and aquatic plants.

As part of a large-scale standardized assessment of aquatic habitats associated with Buruli ulcer, plant communities were surveyed from 80 waterbodies of Ghana, Africa from 2006-2007. The waterbodies were characterized by disease endemicity (endemic and non-endemic based on case histories), location (e.g., Volta, Ashanti, or Accra region) and type (e.g., stream, river, pond, fetch, wetland). Within each waterbody, two transects were established in areas of dominant aquatic vegetation. Three randomly selected 1m\(^2\) area quadrats along each transect were selected and the three dominant plant types were identified and estimated for percent cover. This sampling protocol provided data on plant taxa presence/absence, relative plant abundance, percent plant cover and plant diversity. Non-metric multi-dimensional scaling (NMDS) was also used to explain overall plant community structure within and among waterbodies. To detect the presence of *M. ulcerans*, plant and associated biofilm samples were collected from 2-3 dominant plants from each waterbody and analyzed using a serial testing PCR approach using primers first targeting ER (enoyl reductase), and subsequently for VNTR loci unique to *M. ulcerans* of the ER positives. A site was considered to be present with *M. ulcerans* if at least one of the following samples tested positive: 5 – 8 nitrocellulose or fiberglass filters with filtrate from the water column, or biofilm scraped from any one of three dominant aquatic plant taxa.

In general, overall plant community structure did not differ between endemic and non-endemic waterbodies; however, grasses and terrestrial plants were relatively more common in endemic sites. Further, there was no evident plant community structure separation among regions, yet a difference in specific plant taxa was detected between waterbody types. Plant communities were dominated by emergent plant taxa (~80%) compared to floating or submergent plants. Of all plant/biofilm samples, 8% were positive for *M. ulcerans* and these were collected from 35% of endemic and 10% of non-endemic sites; in addition, the positive samples came from 10 different plant taxa, either emergent plants or detritus. These results indicate no clear relationships between specific plant taxa and *M. ulcerans* presence and/or site endemicity; however, other attributes such as plant structure, location and interactions with the surrounding environment could also be important in the ecology of this disease. Further analyses of these data, especially within endemic regions, in combination with other biotic and abiotic variables measured in the large-scale survey should provide more information of these potentially complex ecological relationships.
Identification of the environmental reservoir of *Mycobacterium ulcerans* within the aquatic ecosystem has been a salient research area within the last five years. Based on extensive environmental sampling and elegant laboratory models, associations have been made between the bacterial DNA and aquatic invertebrates, biofilms, plants and detritus material captured on 0.2µm pore filters. Recent results from our laboratory suggest a possible association between the bacteria and chitinous surfaces of the predaceous aquatic insect belostomatidae. Chitin is an important polysaccharide and source for nutrients and energy in aquatic environments. Bacteria that encode chitinase genes ultimately convert the polymer to acetate, ammonia and fructose-6-phosphate for use in various metabolic processes. The annotated genome of both *M. ulcerans Agy99* and *M. marinum M strain* indicate the presence of three unlinked chitinase/cellulase genes with the same transcriptional direction. Protein prediction models reveal that the first two genes encode for three extracellular carbohydrate binding domains (CBD_II) with hydrolase activity for O-glycosyl compounds and one intracellular polysaccharide deacetylase domain (Polysac_deac_1) with hydrolase activity on carbon-nitrogen bonds. We have employed a series of assays to determine the catabolism of chitin by *M. ulcerans* 1615 and *M. marinum* 1218. We show that neither bacterium is able to degrade colloidal chitin but can adhere to chitin beads in media. These results suggest possible chitin binding but no chitinase activity. It is possible that the substrate specificity for the polysaccharide deacetylase in mycobacteria may vary from other known bacterial chitinases. In insects however, there is partial digestion of the chitinous exoskeleton and gut lining during molting by the action of insect secreted proteases and chitinases. Products arising from this process may be used by *M. ulcerans* as energy sources and hence elucidating the apparent association between the bacteria and the insects. On the other hand, *M. ulcerans* within the matrix of biofilms and detritus may be binding to carbohydrates, which are abundant in such matrices, and hence the high percentage of *M. ulcerans* DNA detection. More work is being done to further understand the role of *M. ulcerans* chitinases in the persistent colonization of aquatic insects as well other polysaccharide rich matrices within the aquatic environment.
A standardized environmental assessment of *Mycobacterium ulcerans* distribution among waterbodies and macroinvertebrates of southern Victoria, Australia

**Presenter:** John R. Wallace

**Co-authors:** M. Eric Benbow, Janet Fyfe, Caroline Lavender, Jenni van Ravensway, Jiaguo Qi, Richard W. Merritt, Tim Stinear, Paul Johnson

1. Department of Biology, Millersville University, Millersville, PA 17551
2. Department of Entomology, 243 Natural Science, Michigan State University, East Lansing, MI 48824-1115
3. Mycobacterium Reference Laboratory, Victorian Infectious Diseases Reference Laboratory (VIDRL), 10 Wrecklyn Street, North Melbourne, Victoria 3051, Australia
4. Center for Global Change and Earth Observations, Michigan State University, East Lansing, MI 48824
5. Department of Microbiology, Monash University, Wellington Road, Clayton 3800, Australia
6. Infectious Disease Department, Austin Health, Heidelberg 3084, Melbourne, Australia

The rationale for this study was to test a methodology currently employed in Buruli ulcer projects in western Africa to sites identified in southeastern Australia involving a standardized approach to sampling abiotic and biotic components of waterbodies for *Mycobacterium ulcerans*. Our objectives were to: 1) determine the distribution and relative concentration of *M. ulcerans* among abiotic and biotic samples from waterbodies associated with past and present Bairnsdale ulcer outbreaks; 2) identify potential water chemistry variables associated with *M. ulcerans* positivity, and; 3) evaluate landscape topography among sites where *M. ulcerans* was detected compared to sites where it was not using GIS.

In order to assess potential environmental reservoirs of *M. ulcerans* among aquatic microhabitats and macroinvertebrates, we employed a standardized field sampling approach in waterbodies adjacent to disease outbreaks as well as sites where no cases had been reported in southeastern Australia. Thirteen sites were selected based on past (within last 20 years, e.g., Phillip Island) and current outbreaks of BU (Pt. Lonsdale and Ocean Grove) as well as a few sites where no outbreaks or cases had been historically reported.

Landscape topography analyses used a standard geographic wetness index to evaluate topographic complexity and overall landscape drainage in successive buffers around site waterbodies. Water chemistry data were collected *in situ* using a YSI 6600 data sonde placed into each site waterbody. Abiotic samples included hydric soils and suspended matter filtered from the water column. Biotic samples included plant stems and attached biofilm as well as aquatic stages of macroinvertebrates and adult mosquitoes. Macroinvertebrates (e.g., several trophic levels e.g., midges, crustaceans, snails, dragonfly larvae, aquatic beetle larvae) were collected using D-frame nets. Larval and adult mosquitoes were collected with 250ml dippers oviposition traps respectively. All samples were assayed for the concentration of *M. ulcerans* using rtPCR.

We found that abiotic and biotic samples from past BU outbreak sites were PCR negative for IS2404. One site representing a current outbreak area (Site 7, Pt. Lonsdale) was positive for IS2404, IS2606 and KR, by real-time PCR, in soil, plant stem and suspended sediment samples. A soil sample from a second site ((Bonny Small site in Ocean Grove) was weakly positive for IS2404 . In addition to the small number of adult mosquitoes sampled, all other aquatic macroinvertebrate samples were negative for IS2404 from all sites.

Initial analysis of landscape topography found that the landscape wetness was lower and more variable at greater distances from sites. Among site variation in the wetness index was substantial, necessitating the inclusion of additional sites where there has never been reported human disease. On-going analyses will include these additional sites and landscape – water chemistry interactions among sampled sites.
Spatial analysis linking ecologic field observations and BU surveillance in Ghana

Lance A. Waller, Department of Biostatistics, Rollins School of Public Health, Emory University, Atlanta, GA 30322

Ellen Whitney; Department of Epidemiology and Center for Public Health Preparedness and Research, Rollins School of Public Health, Emory University, Atlanta GA 30322

Eric Benbow, Department of Entomology, Michigan State University, East Lansing, MI 48824

We present an overview of a new research program focused on developing accurate and reliable spatial statistical methods for linking field ecology of M. ulcerans with surveillance data for Buruli ulcer (BU). The program takes a systems view to bridge the local ecology of disease with the surveillance system in order to track risk, incidence, and prevalence of, and local risk factors for, BU in Ghana. Analysis involves local spatial analyses at field sites and the initiation of a detailed look at the “ecology” of surveillance: that is, exploring how incident cases move from an individual health event to a data record. Specifically, we are interested in how cases are identified, reported, and diagnosed, as well as how data enter and progress through the surveillance system, in order to provide more accurate local estimates of incidence and prevalence and to identify aspects and locations where the system might be improved.
Environmental Interactions with *Mycobacterium ulcerans*

HR Williamson¹, M Rodriguez¹, PLC Small¹

¹University of Tennessee, Knoxville, Knoxville, Tennessee, USA

The epidemiological association of Buruli ulcer with slow moving water sources has recently led to a broad survey of aquatic environments in Ghana, Africa. From this survey, *M. ulcerans* DNA was found to be wide-spread in water bodies both endemic and non-endemic for Buruli ulcer. Indeed, samples positive for *M. ulcerans* DNA included broad taxa of invertebrates, plants, and water filtrate. The highest percentage of *M. ulcerans* DNA was identified from biofilm samples collected on glass slides which were submerged within the water bodies. *M. ulcerans* has also been experimentally shown to form biofilms. The ability of environmental mycobacteria to form biofilms has been shown previously in such species as *M. avium*, *M. bovis*, *M. smegmatis*, and *M. marinum*. Bacterial population dynamics within these biofilms are largely due to factors including nutrient availability and the presence of bacterial and eukaryotic species. It has been shown that one such important bacterial regulator within biofilms is free-living amoeba. Amoeba, as well as other protozoa obtain nutrition by grazing on bacteria. Therefore, some bacteria have adapted in order to survive this predation. It has been found that *M. avium*, *M. fortuitum*, and *M. marinum* are able to infect and survive within protozoa. Parallels exist between protozoan interactions and those of intracellular pathogens within mammalian hosts. This is thought to be due to mechanistic similarities such as phagocytosis and the presence of surface receptors between amoebae and macrophages. Like other environmental bacteria that can survive within amoebae, these mycobacteria are also intracellular within the human host. *M. ulcerans* has recently been found to also possess an intramacrophage growth phase. Because of this finding, as well as the association with water sources, we sought to examine whether *M. ulcerans* could survive and replicate within the protozoan host, *Acanthamoeba polyphaga*.

Cytotoxic activity of mycolactone on *A. polyphaga* was first studied by the addition of serially diluted mycolactone to *A. polyphaga* in a 96-well plate. Next, amoebae were infected with *M. ulcerans* followed by the addition of amikacin to inhibit growth of extracellular bacteria. The amoebae were observed for signs of lysis by *M. ulcerans*. Seven days, 14 days, and 30 days after *M. ulcerans* infection, amoebae were collected where they were mechanically and chemically lysed, stained for acid-fast bacilli, and plated onto M7H10 media. *A. polyphaga* were found to survive following admistration of mycolactone even at the highest concentration. *M. ulcerans* was also able to survive within the amoeba throughout the course of the study. *M. ulcerans* bacilli were found within metabolically active amoeba trophozoites as well as within amoeba cysts. These data suggest these protozoa could be potential environmental hosts for *M. ulcerans*. Work is in progress to determine survival and replication within other protozoan hosts both in a laboratory setting and field-collected samples. Increases in virulence as well as resistance to bactericides as a result of growth in protozoa are also being determined in order to assess the potential role these protozoa could play in human infection by *M. ulcerans*
Free-living amoebae in the environment of regions in Benin with low and high endemicity of Buruli ulcer

Miriam Eddyani 1, Johan F. De Jonckheere 2, Lies Durnez 1,3, Patrick Suykerbuyk 1, Herwig Leirs 3, Françoise Portaels 1

1Mycobacteriology Unit, Department of Microbiology, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen, Belgium
2Research Unit for Tropical Diseases, Christian de Duve Institute of Cellular Pathology, Catholic University of Louvain (UCL), Brussels, Belgium
3Department of Biology, University of Antwerp, Antwerpen, Belgium

Objectives

Buruli ulcer (BU) occurs most commonly in defined areas related to rivers, swampy terrain or lacustrine systems, and habitats in which free-living amoebae occur in high numbers as well. Moreover, populations that use unprotected sources of water for domestic purposes have higher prevalence rates of BU than those who use protected sources. For this reason a study on a possible link between the habitat of Mycobacterium ulcerans and free-living amoebae was carried out.

Methods

From 2005 until 2006, 132 water and biofilm specimens were taken from lakes, rivers, swamps, rain tanks, boreholes, wells and water towers in villages with high and low endemicity of BU in Benin. From these specimens the isolation of free-living amoebae as well as mycobacteria was attempted.

Results

In 104 out of 132 (78.8%) samples amoebae were isolated. There was no significant difference noted in the amoebal contamination rates between protected and unprotected sources of water (67.6% vs. 82.7% respectively).

When comparing the amoebal contamination rate in regions with high and low BU endemicity, a significant difference was observed with water bodies in highly endemic areas being more contaminated by amoebae than those in low endemic areas (83.3% vs. 66.7% respectively; p=0.037). This difference was only significant for protected sources of water, with those in highly endemic areas showing 88.0% amoebal contamination while those in low endemic areas only showed 11.1% amoebal contamination (p<0.0001). The unprotected sources of water were equally contaminated by amoebae in areas with low and high BU endemicity (85.2% vs. 81.7% respectively).

Several free-living amoebae and mycobacteria, ubiquitous in nature were isolated. Some of them have pathogenic properties. Mycobacterium ulcerans was not isolated.

Conclusion

Our results demonstrate that the studied area has a problem in relation to its water supply since both sources of water that are unprotected and supposedly 'protected' are heavily contaminated with free-living amoebae. This is even more so in regions with high BU endemicity. Our observations indicate that additional studies are required to explore the possible link between free-living amoebae and mycobacteria.
Are biting water bugs likely vectors of Buruli ulcer disease? A large-scale field study in Ghana suggests this is unlikely.

Richard W. Merritt1, M. Eric Benbow1, Heather Williamson2, Ryan Kimbirauskas1, Mollie D. McIntosh1, Rebecca Kolar1, Charles Quaye3, D. Boakye3, and Pam Small2.

1 Department of Entomology, Michigan State University, E. Lansing, MI 48824-1115, USA.
2 Department of Microbiology, University of Tennessee, Knoxville, TN, 37996, USA.
3 Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Ghana.

Studies suggest that possible transmission by Mycobacterium ulcerans via aquatic biting insects is possible in the laboratory; however, no field studies have ever tested this hypothesis. Our study represents the first field examination of biting water bugs (Hemiptera: Naucoridae, Belostomatidae, Nepidae) in disease endemic and non-endemic areas of Ghana, Africa. From collections of 22,832 invertebrates, we compared composition, abundance and associated M. ulcerans positivity between 15 endemic and 12 non-endemic sites. Biting hemipterans were rare and represented a small percentage (usually < 2%) of invertebrate communities. There were no significant differences in hemipteran abundance or pathogen positivity between endemic and non-endemic sites, and there were no correlations between biting hemipteran abundance and M. ulcerans positivity. Therefore, while it is possible for infection through insect bites in the laboratory, there is little field evidence to support biting hemipterans as primary vectors of M. ulcerans in nature.
OTHER ABSTRACTS
Follow-up of a cohort of patients with an infection with *Mycobacterium ulcerans* and carriers of HIV

*Dr Agnès Sobry MSF Cameroon*

Doctor Without Borders participates in cooperation with the Ministry of Health programme against Buruli ulcer in the district of Akonolinga in Cameroon since 2002.

In 2006 we realized that a number of patients with ulcers were important carriers of the HIV virus. Since June 2007, we propose a systematic and voluntary testing for HIV patients with Buruli. In cooperation with specialists HIV University Hospital of Geneva, we have defined indicators that will enable us to better understand the relationship between HIV and Buruli.

Due to the small number of patients that we have followed so far it is not possible to identify HIV as a risk factor for infection or not with *Mycobacterium Ulcerans*. Our aim is to estimate the importance of HIV infection among patients Buruli, describe any clinical or paraclinical characteristics and better understand the value of ARV treatment in the evolution of the lesions.
Contagiousness and natural defence in endemic zones

*Dr. Désiré Imoso*

In the Songololo endemic zone in the Democratic Republic of the Congo, not all villages exhibit the same degree of contagiousness. It has been noted that in an environment with a practically identical ecosystem, some villages are more affected than others. Some are not affected at all.

Our attention was drawn in particular to a village where 7 inhabitants have contracted BU. All these persons are descendants, going back four generations, of a man who had BU (his daughter, his 5 granddaughters and 2 great grandchildren: a boy and a girl). Most of these patients were treated in our hospital. Other, similar cases were noted in other villages.

These observations compel us to reconsider the role of family or genetic factors already been referred to by F. Portaels, Dousou-Yovo et al., aside and apart from environmental factors.

It seems to us that there exists a natural defence mechanism; where this mechanism is not present, individuals are more susceptible to BU. This characteristic is transmitted to a person's descendants.
A simple method of bacteriological diagnosis of osteomyelitis due to *Mycobacterium ulcerans*

*Dr Désiré Imposo*

So far, diagnosis of osteomyelitis from *Mycobacterium ulcerans* (MU) in our hospital has been based on samples from a bone biopsy, which were sent abroad for histopathology and PCR.

At the present time, as we are unable to send samples abroad, we have decided to take per operative samples from the bone marrow.

The procedure first of all involves extracting the intra-osseous sequestrum or necrosed bonelet. The sequestrum or bonelet is then split and a sterile swab is used to collect the bone-marrow secretions from the centre of the pièce.

The swab is then used for a slide smear which is sent to the laboratory for a Ziehl-Neelsen stain. In the case of a long bone with no sequestrum, a trephination is carried out in the medullary canal.

Out of 7 samples for which the technique was used, 4 were Ziehl-Neelsen (+). Culture and PCR are being carried out.

This method may be recommended for use in hospitals in which there is no pathology department.
Planning the work area to do the essential prevention of disability activities in the community, at the peripheral health service and at the referral health service

*Linda Faye Lehman, OTR MPH C.PED, POD Consultant for American Leprosy Missions for Latin American and Africa living in Brazil* lehman@uaigiga.com.br

This presentation is to stimulate small group discussion by those working to implement Prevention of Disability (POD) within Buruli Ulcer management program in the Community and at peripheral, district and national referral health services.

Careful identification and focus on priority activities are important to preventing and managing disability. It is the foundation from which planning is done for developing the work areas. Good organization of the patient waiting area, the records and scheduling area, the patient evaluation and treatment areas, supply storage and worker administrative and rest areas are needed to facilitate patient flow, efficient treatment sessions and documentation. The areas should have adequate lighting, ventilation, water, electricity work surfaces and storage for regularly used materials and supplies. Space which permits privacy and individual treatment as well as group activities should be included.

Equipment and consumable materials are planned for both administrative and treatment areas. Space equipment and materials are needed for wound care, simple plaster casting and/or splinting/orthotics, adaptive devices, gross motor and fine motor activities which promote joint mobility, coordination and strengthening and self-care training.

Care needs to be given to not use equipment which requires high maintenance and skill. Much of this equipment has not been found to be more effective or efficient in preventing disability and can be unsafe if not properly used. Most of the equipment can be substituted for simpler, locally available resources however training continues to be essential for its most effective use. These alternatives may not only be less costly, but can be easily maintained. Ideally equipment and materials should be used that can be acquired within the country or locally so that persons affected can continue doing at home what they learned at the health service and/or hospital. Training and supervision are needed to help health workers identify local resources, organize and use properly the space, equipment and materials they have available.
Preliminary results on response to treatment with the combination rifampicin-streptomycin for 5 days per week for 8 weeks for Buruli ulcer

Phillips R¹,², Sarfo FS¹, Nsiah R¹, Dinko B¹, Opare W³, Ampadu E³, Boateng A⁴, Adentwe E⁴, Asiedu K⁶, Wansbrough-Jones M⁵

¹Komfo Anokye Teaching Hospital, Kumasi, Ghana.
²School of Medical Sciences, KNUST, Kumasi, Ghana
³National Buruli ulcer Control Programme, MOH, Ghana
⁴Tepa Government Hospital, Ahafo Ano North District, Tepa, Ghana
⁵St George’s University of London, London
⁶WHO Geneva Switzerland

Introduction

The combination of Rifampicin and streptomycin is the drug regimen currently recommended by the WHO for treatment for humans with Buruli ulcer caused by Mycobacterium ulcerans (Mu) infection. In African countries where Buruli ulcer is endemic, ambulatory treatment with daily intramuscular injections of Streptomycin may be difficult to maintain for 8 weeks especially on Saturdays and Sundays when small clinics are closed. The aim of this study is to determine healing, viability of M. ulcerans and recurrence of M. ulcerans disease in subjects treated with Streptomycin (STR) and Rifampicin (RIF) for 5 days per week for 8 weeks.

Methods

From October 2007 to December 2007 in a prospective study patients with active Mu disease recruited from the Ahafo Ano North district of Ghana were administered STR-RIF combination daily or 5 days a week for 8 weeks after swabs, 4 mm punch biopsies and fine needle aspiration (FNA) were obtained to establish the diagnosis of Mu disease by microscopy, culture and PCR. Further biopsies or swabs where possible were obtained at 6 weeks for culture. Clinical response to antibiotic therapy was assessed 2 weekly by measuring the surface area of lesions serially until complete healing. Photographs of lesions were also taken at review. Subjects are being followed up after completion of treatment for 12 months to determine recurrence.

Results

Among the 29 patients with PCR/clinically confirmed MU disease: 5 (21%) subjects had category 1 (<5cm diameter), 14 (48%) Category II (5-15cm diameter) and 10 (34%) had Category III lesions. After 4 weeks of treatment, 6 (21.3%) lesions (4 Cat I, 1 Cat II and 1 Cat III) were completely healed. After completion of antibiotic therapy at 8 weeks, 5 more lesions (3 Cat II and 2 Cat III) were healed bringing the cumulative total number of healed cases to 11 out of 29 (38%). At 12 week assessment, 6 (Category 1 and II lesions) out of 12 remaining unhealed lesions had significant (>50%) reduction in lesion size. Five Category III lesions whose surface area could not be determined by tracing on acetate paper because of large lesion size are still having wound dressings and would need skin grafting. One patient who defaulted treatment after 2 weeks had relocated from study area.
Culture for *M. ulcerans* in these patients before and during treatment is still ongoing and no histopathology results are available yet. So far, no recurrence has been demonstrated yet and patients are being followed up to 12 months.

**Conclusion**

Preliminary observations suggest that STR-RIF combination administered 5 day a week may be effective in treating Buruli ulcer and further studies are needed to confirm these findings.

Support is gratefully acknowledged from the World health Organization.
Buruli ulcer is an imported and exported disease

Portaels F¹, Debacker M¹, Anyo G¹, Meyers WM²

¹ Institute of Tropical Medicine, Antwerp, Belgium
² Armed Forces Institute of Pathology, Washington DC, USA

Buruli ulcer (BU) is an infection caused by *Mycobacterium ulcerans*. The disease prevails primarily in impoverished, humid, rural areas of tropical Africa, where incidence is increasing, surpassing tuberculosis and leprosy in some regions. Endemicity is highest in Africa; however, smaller numbers of cases come from the Americas, Asia and Australia. A comparatively few cases have been reported from non-tropical zones of China, Japan and southern Australia.

Most publications on Buruli ulcer (BU) concern patients diagnosed in well known endemic regions. There are, however, several reports of patients diagnosed in industrialized countries where BU is not endemic. Some of them may be considered as imported cases since the patients originated from BU non-endemic countries and developed the disease during or following a visit to, or residence in a BU endemic area.

There is a second category of individuals who were born and lived in BU endemic countries who developed BU in a non-endemic country. We designate these patients as exported cases of BU.

We are aware of a combined total of approximately 20 imported and exported cases of BU that have been reported. The first published patient was an American boy of six and one-half years, the son of a missionary family living in the Bandundu Province of the Belgian Congo who presented with a large undermined ulcer on the left foot (Van Oye & Ballion, 1950). The boy was evacuated to a hospital in New York. The lesion was Ziehl-Neelsen positive for acid-fast bacilli and positive in culture for *M. ulcerans* (Meleney and Johnson, 1950).

Other imported cases have resided in or travelled to endemic areas (China, Papua New Guinea, Peru, South-East Asia, West and Central Africa) and have been diagnosed and treated in European, American, Canadian and Australian medical centres.

The first exported case was published in 1974 by Lindo and Daniels. The patient was a Nigerian physician who developed BU while he was working in New York City. He came to the USA in 1970 and accidentally scraped his elbow on a bathroom mirror. The elbow became edematous a few days later and subsequently ulcerated. The clinical diagnosis was confirmed bacteriologically and histologically. We believe that the patient acquired the infection in Nigeria. This case is of particular interest because it suggests, for the first time, that patients may have an asymptomatic infection (latent BU) that subsequently is reactivated, producing BU disease. In this instance, activation or reactivation was probably due to the local trauma described above.

All the other exported cases from Australia, Central and West Africa, and Suriname were diagnosed and treated in Europe (Belgium, France, Germany, Switzerland and the United Kingdom).

A third category of exported/imported cases is comprised of individuals who moved from an endemic country to another endemic country (e.g. from Côte d’Ivoire, to Ghana, or from Nigeria or Togo to Benin) (Debacker et al., 2002).

All imported and exported BU cases have been confirmed by at least two positive laboratory tests as recommended by WHO (2001). From these cases some new foci of the disease have been discovered. For example, the first case from China was diagnosed in Holland. The lesion of BU was in a Chinese woman who had been living in Europe for 9 years but traveled to China for 2 months. Three months
after returning directly to Holland from China, a swelling developed on her lower leg which later ulcerated (Faber et al., 2000).

Travel to and from parts of the world where BU is endemic is becoming more and more frequent. As is true for other tropical diseases, health professionals in countries where BU is non-endemic are not usually familiar with the disease. Thus, many BU patients are diagnosed late, if at all. Delayed diagnosis often leads to severe disabilities, sometimes requiring amputation. The patient’s recent travel history, and the clinician’s awareness of the cutaneous and bone lesions of BU are important for accurate diagnosis and treatment of the disease.

The study of imported and exported cases has enabled us to acquire more knowledge regarding the pathogenesis of the disease (latency and reactivation), and the existence of new foci. It also teaches us that BU has no racial or socioeconomic preference.

Correct laboratory confirmation remains essential to confirm the discovery of new foci and the use of fingerprinting techniques can provide additional information on the precise location where patients were infected. (Stagier et al., 2006; Hilty et al., 2006; Ablordey et al., 2007).

References


Correlation between clinical diagnoses and laboratory analyses in the management of buruli ulcer patients

Pius Agbenorku¹, Ohene Adjei², Jörg Nitschke³, Gisela Bretzel³, Margaret Agbenorku⁵, Pawson Kuadzi⁶

¹Reconstructive Plastic Surgery & Burns Unit, Department of Surgery, Komfo Anokye Teaching Hospital, School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana;  
²Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana;  
³Department of Infectious Diseases and Tropical Medicine (DITM), University of Munich, Germany;  
⁴Bernhard Nocht Institute for Tropical Medicine (BNI), Hamburg, Germany;  
⁵Health Education Unit, Global Evangelical Mission Hospital, Apromase-Ashanti, Ghana;  
⁶Department of Mathematics, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana

In the past, diagnosis of Buruli Ulcer (BU) was largely based on clinical examination only. This was because in many circumstances, the simple means of diagnosis for example, Ziel Nelson (ZN) test for AFB’s, was simply unavailable. Of course this has been largely criticized. The evolution of Polymerase Chain Reaction (PCR) for confirming BU has become a routine now in addition to the ZN stain, Histopathology and Culture studies of the tissues.

In a series of 46 patients over the period of one year (January - December 2007) treated at the Global Evangelical Mission Hospital, Apromase, Ashanti Region of Ghana 20 of these patients had their specimens sent for various laboratory analyses. For swab Ziel Nelson (ZN) test for Acid Fast Bacilli (AFBs) there was 5% positive, 60% negative and 35% of the specimens were not subjected to this test. The tissue ZN stain was positive in 10% cases and negative in 75% results. Tissue PCR was 35% positive and 65% negative whilst swab PCR was 45% positive and 35% negative. The ages of these 20 patients ranged from 8 to 72 years with the mean age as 25.25. The male: female ratio was 9: 11 (45%: 55%).

In this study it was clear that even though clinically these cases were all diagnosed as Buruli Ulcers, the majority of them were not confirmed as such by the laboratory investigations. The study therefore was designed to find out what correlation between the laboratory results and the clinical diagnoses.

The laboratory investigations were done at the Department of Infectious Diseases and Tropical Medicine (DITM), University of Munich, Germany; Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana and Bernhard Nocht Institute for Tropical Medicine (BNI), Hamburg, Germany and were sponsored by BURULICO.