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Organization**

**WHO ANNUAL MEETING  
ON BURULI ULCER**

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**ABSTRACTS**

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# **Country and NGO presentations**



## The epidemiological situation of buruli ulcer in Congo in 2010

**Presenter: Dr Damas Obvala**

Activities to control Buruli ulcer, leprosy and yaws are carried out by a combined and integrated programme which is part of the neglected tropical diseases programme.

These diseases essentially affect poor rural populations and pose a genuine public health problem.

Six out of the country's twelve departments are affected, including Kouilou, where the epicentre is Kakamoéka district, and Pointe-Noire; Niari; Bouenza; Pool (Kindamba district) and Cuvette (Mossaka district).

In 2010, the activities carried out focused on raising awareness in communities, supervision of the health workers responsible for facilities providing treatment, detection and medical and surgical treatment of patients, training health workers and systematically taking samples from cases for case confirmation by the specialized laboratory in Angers, France.

The results achieved in 2010 include the training of 50 health workers in Kouilou, Cuvette and Pool departments; completion of epidemiological surveys in Pool (along the river known as the couloir) and Lékoumou departments; detection and treatment of Buruli ulcer cases in Kouilou and in Pool; collection of samples from 81 cases, 36% of which were PCR positive, and systematic collection of iconographic data from each patient to improve case documentation.

An aggregate total of 1140 cases have been notified and 107 cases of Buruli ulcer, including 101 new cases and 6 relapsed cases have been treated by the different treatment; the geographical distribution is given below:

- Kouilou and Pointe-Noire department: 41 cases (40.5%)
- Niarie department: 44 cases (43.5%)
- Bouenza: 16 (15.8%)
- Pool department: 2 cases
- Cuvette department (Mossaka): 4 cases

Ulcers were the most common type of lesion (94%), followed by oedemas (2.9 %); children accounted for 34.5% of cases.

A total of 46% of cases were female and category II and III cases made up 73.26% of the total; the lesions were located on the lower limbs in 54.2% of cases and 25% of cases were affected by limitation of movement at the time of detection;

During 2010, 13 new communities reported notified cases and 2 new districts were affected by the disease. Case confirmation using PCR regressed on account of the shortage of suitable equipment to collect and transport samples; the most commonly used sampling method is the swab.

Surgical treatment is hard to provide in Kouilou and Niari departments, and physiotherapy/rehabilitation is still in its infancy.

The limitations faced can be essentially attributed to the lack of funds.

In order to optimize the programme's activities in 2011, we would need:

- To improve supervision of the health workers who provide case management
- To improve awareness in communities and in schools
- To improve case detection in health centres, on the basis of the operational sectors in endemic departments and involving the community intermediaries
- Systematically to take samples from all cases of Buruli ulcer for PCR examination and better to organize the laboratory network
- To improve the organization and running of the surgery and physiotherapy/rehabilitation component
- To acquire equipment for sampling and 6 digital cameras to enable the facilities responsible for control to collect photographic records of all cases and incidents that occur during field missions.

## Buruli ulcer in Australia, 2010

**Presenter: Paul Johnson**

**Authors:** Paul Johnson,<sup>1,2</sup> Caroline Lavender,<sup>2</sup> Lynne Brown<sup>3</sup>, Maria Globan,<sup>2</sup> Janet Fyfe.<sup>2</sup>

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2. Victorian Infectious Diseases Reference Laboratory, and WHO Collaborating Centre for *Mycobacterium ulcerans*, Melbourne, Victoria, Australia
3. Tuberculosis Program, Department of Health, Victoria, Australia

In 2010 thirty-three new cases of human Buruli ulcer were diagnosed in Victoria. In Queensland there were 7 cases.\* There have been no reports from other states, but a 69 year old male who presented in Victoria was probably infected in the Northern Territory based on his travel history and the VNTR profile of his isolate (Table 1). Six new cases were diagnosed in animals in Victoria--notably 3 were domestic dogs from the Bellarine Peninsula (Table 2). There have been no animal cases from outside Victoria to date. The majority of Victorian cases (Figure 1) were again linked to exposure on the Bellarine and Mornington Peninsulas (Figure 2). Age/sex and visitor/resident break-down of Victorian cases is shown in Figure 3.

**Table 1.** Buruli ulcer: laboratory-confirmed human cases – Australia

	2004	2005	2006	2007	2008	2009	2010
Victoria	25	41	61	17	35	29	32
Queensland	7	6	4	1	4	6	7*
Northern Territory	2	-	-	-	1	-	1†
New South Wales	-	-	1	-	-	-	-
South Australia	-	-	-	-	-	-	-
Western Australia	-	-	-	-	-	-	-
Tasmania	-	-	-	-	-	-	-
<b>Total</b>	<b>34</b>	<b>47</b>	<b>66</b>	<b>18</b>	<b>40</b>	<b>35</b>	<b>40*</b>

\* Queensland data is complete to October 6<sup>th</sup>, 2010.

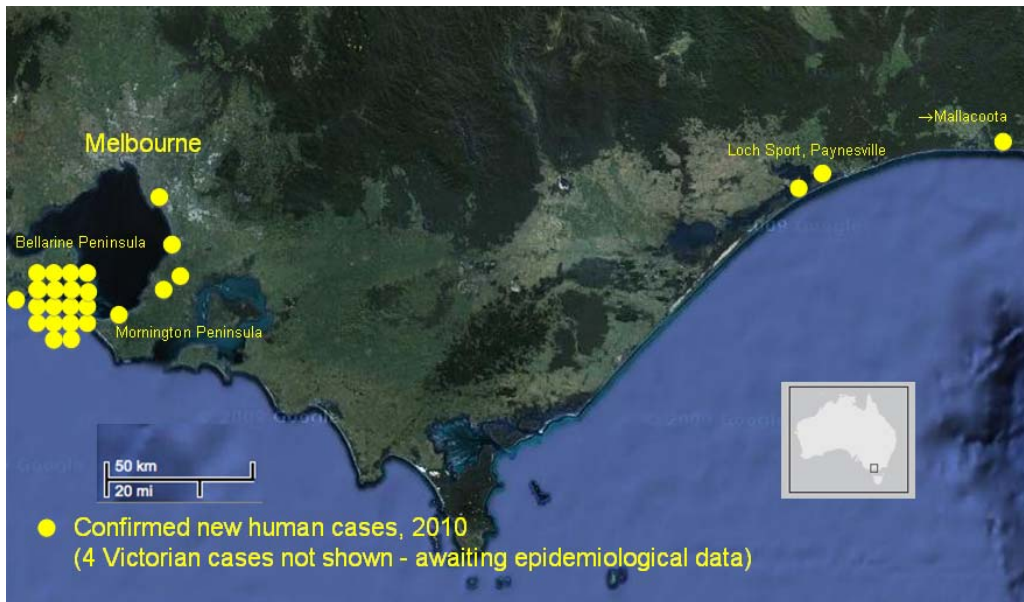
† Diagnosed in Victoria, history and VNTR profile suggests acquisition in Darwin.

**Table 2.** Buruli ulcer: laboratory-confirmed non-human cases – Victoria

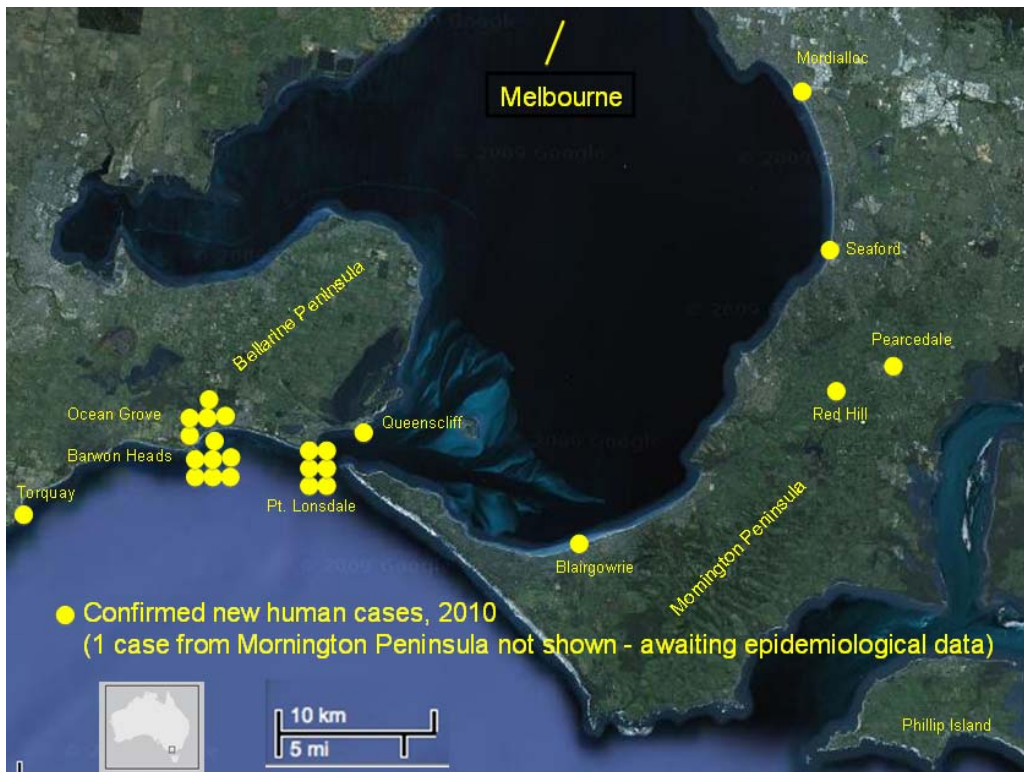
	Ringtail Possum	Brushtail Possum	Koala	Dog
Victoria 2009	7*	1*	1	1
Victoria 2010	1*	-	2	3
<b>Total last 2 years</b>	<b>8*</b>	<b>1*</b>	<b>3</b>	<b>4</b>

\* via active case finding at Point Lonsdale (there were fewer trap-nights in 2010 compared with 2009).

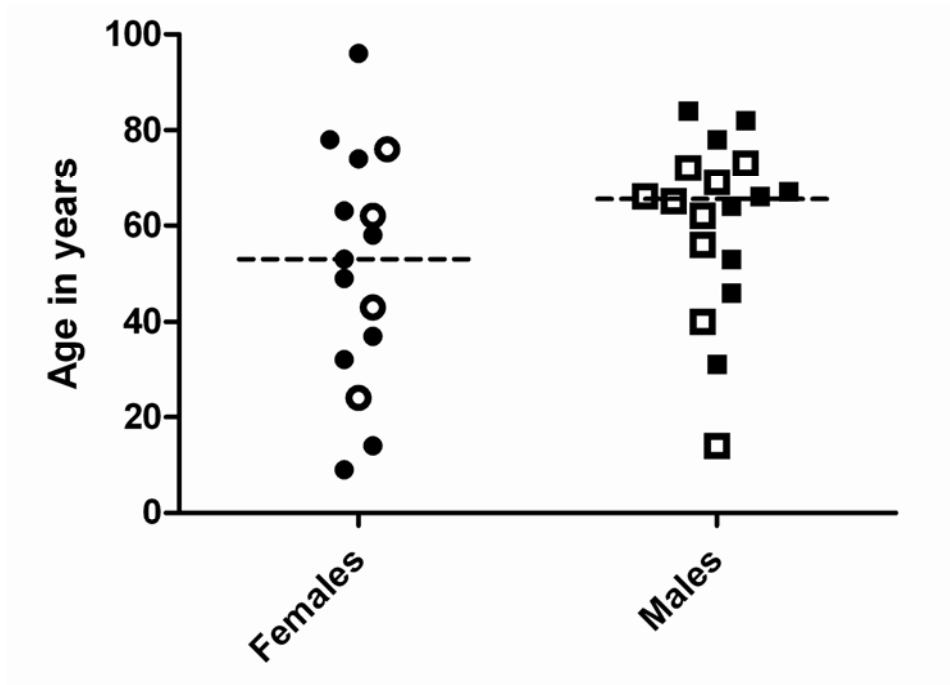
**Figure 1.** Likely location of exposure: laboratory-confirmed human cases of Buruli ulcer, Victoria, 2010 (Google Map overlay).



**Figure 2.** Likely location of exposure: laboratory-confirmed human Buruli ulcer on the Bellarine and Mornington Peninsulas, 2010 (Google Map overlay).



**Figure 3.** Buruli ulcer diagnosed in Victoria during 2010. Break-down by Age/Sex and Resident/Visitor status (visitors to endemic areas: open symbols; residents of endemic areas: closed symbols).



## ***Mycobacterium ulcerans* infection (Buruli ulcer) in French Guyana in 2010**

**Presenter: Pierre Couppié**

Dermatology Service, Centre Hospitalier de Cayenne, French Guyana

During 2010, we registered 7 new cases of *Mycobacterium ulcerans* infection in French Guyana. All 7 patients were examined in the dermatology service. Four of the patients were female and three male. Only one of them was a child aged under 15 years. The average age of the patients was 22 years. A cluster of 3 cases was from the same village (Javouhey) in western French Guyana. This area is a focus of Buruli ulcer in French Guyana. In 3 of the patients the skin lesions were plaques (2 of them ulcerative) and in 4 of them ulcers. The sites of the lesions were those usually found (on the lower limbs in 5 patients and the upper limbs in 2). In terms of category, 4 patients were in category I and 3 in category II. Ziehl-Neelsen stain was positive in 4 out of the 7 patients, PCR in 4 out of 6 and culture in one out of 5. Antibiotic treatment used a combination of rifampicin and clarithromycin for all 7 patients. None of the patients required surgery.

Since 2003, 20 patients have been treated in French Guyana by an antibiotic combination of rifampicin and clarithromycin as first-line treatment. In our experience, this combination is generally well tolerated and efficacious.

## Surveillance of Buruli ulcer in Japan

**Presenter: Rie Roselyne Yotsu**

**Authors:** Rie Roselyne Yotsu<sup>1)</sup>, Kazue Nakanaga<sup>2)</sup>, Yoshihiko Hoshino<sup>2)</sup>, Norihisa Ishii<sup>2)</sup>

<sup>1)</sup>National Center for Global Health and Medicine, Department of Dermatology, Tokyo, Japan

<sup>2)</sup>Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, Japan

The first case of Buruli ulcer in Japan was reported in 1980. This was a 19-year-old Japanese lady who had never traveled or lived outside of Japan. This case report was published in 1982 by Mikoshiba *et al.*, and later in 1989, Tsukamura advocated this novel subspecies as “*M. ulcerans* subsp. *shinshuense*” due to its microbiological proximity to *M. ulcerans*. With 23 years of absence, the second case was reported in 2003. A total of 19 cases of Buruli ulcer have been reported and registered to the World Health Organization as of today. The case counts have been increasing in the past number of years, may it be as a result of awareness and accurate diagnoses, or simply growing endemicity. Despite being able to speculate some trend, we still need to consolidate more information to discover its epidemiology and transmission. We will report the updated situation and trend of these 19 cases reported in Japan.

# **National Buruli ulcer control programme 2010, Ghana**

**Presenter: Edwin Ampadu**

## **Introduction**

In spite of the interventions, Ghana continues to be a major Buruli ulcer endemic country on the West African subcontinent. An average of 1000 cases of the disease is reported annually since 2005. Currently 62 out of the 170 districts nationwide report the disease from various regions of the country.

## **Core mandate**

To minimize the morbidity and disability associated with Buruli ulcer disease, the national programme set itself the following objectives:

- Further strengthen 6 major endemic districts in case detection and management
- Provide logistical support to all treatment centres
- Offer more quality monitoring and evaluation services to the programme
- Collaborate with research centres in Diagnosis and case management

## **Key intervention areas**

1. Community case detection and management
2. Quarterly logistical support to all treatment centres [46]
3. Close collaboration with the research works- Stop Buruli project, , WHO assisted research works, Tapa, Ashanti
4. Monitoring and supervisory visit to the treatment centres

## **Collaboration with local NGOs and research institutions**

### **Objective**

Support national programme with case confirmation and treatment. Also, human capacity development while material and specimen are available for further research works on the disease.

They included; **Health Foundation Ghana, WVI, MAP international, Rotary Club Sunyani central**

**Areas of collaboration:** Case detection and confirmation for the treatment centres, facilitating specimen collection, capacity development for field workers and motivation to the front liners in Buruli ulcer control and management. [Provision of bicycles, farm boots and training]

### **Capacity building – POD**

60 health workers were trained with skills to foresee and detect possible deformity associated with any disease lesion presented and appropriate measure instituted to predict restriction of movement or disability.

Beneficiary included Brong Ahafo, Ashanti, Central, Gt. Accra and Eastern region

This was supported by MAP international

### **Strengthening health facilities**

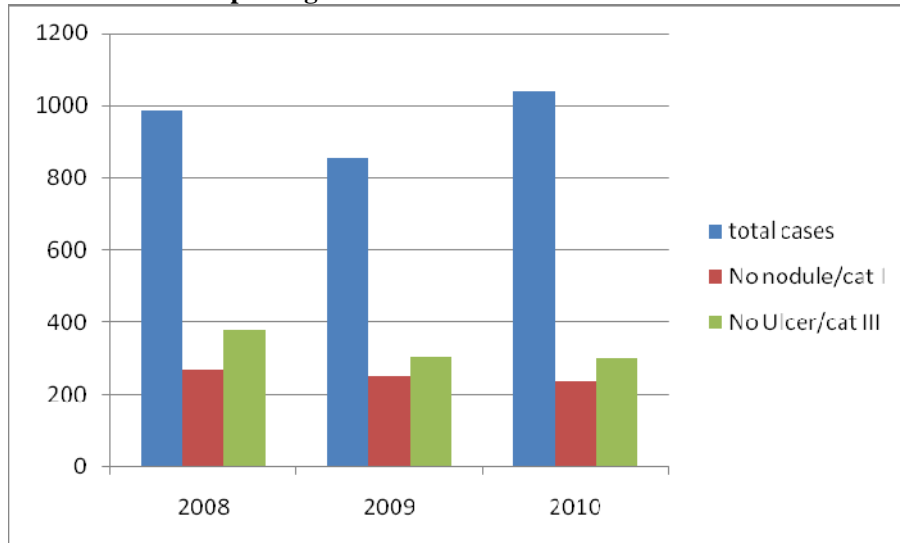
Support to most treatment centres included dressings and antibiotics,

Surgical outreach services were carried out to 4 major centres [Amasaman, Tapa, Jacobu and Bekwai hospital]

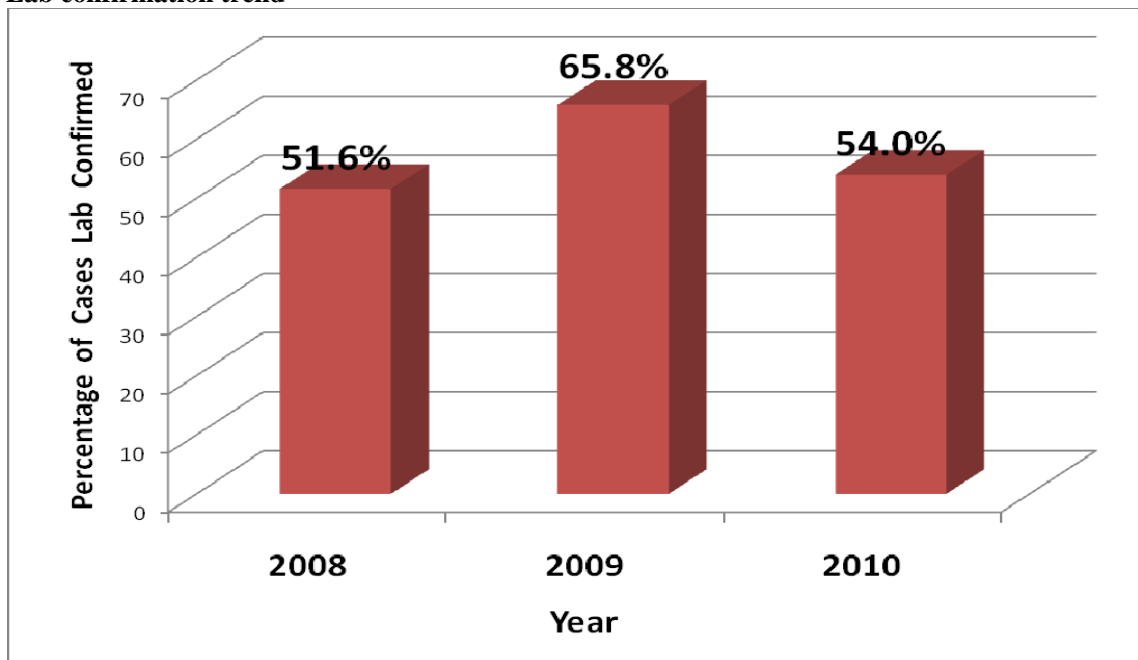
### Monitoring health facilities

5 monitoring visits were carried out at 8 major treatment centres in Ashanti, Brong Ahafo and Greater Accra regions

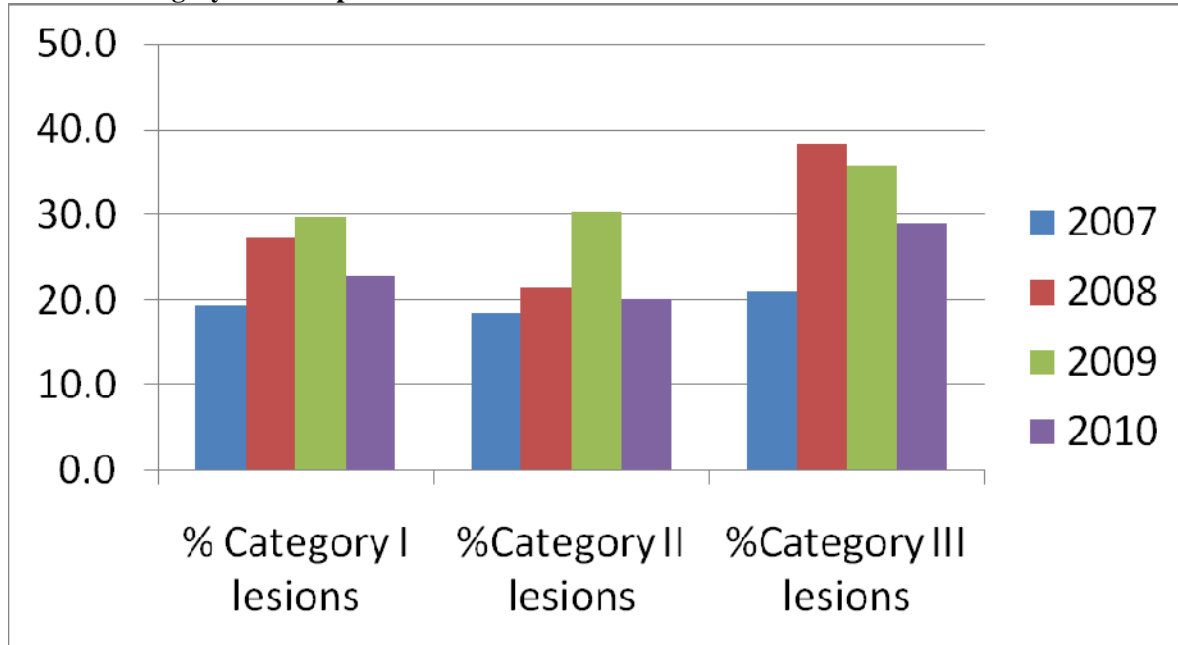
### Trend of disease reporting 2008-2010



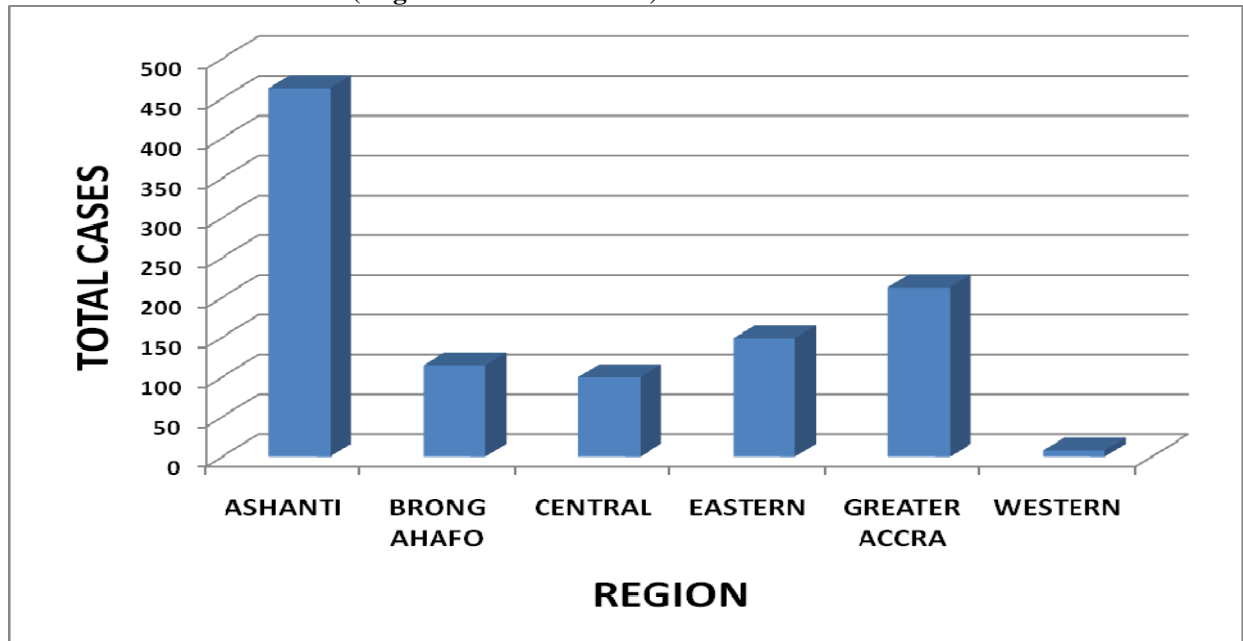
### Lab confirmation trend



**Trend of Category of lesion presented**



**Buruli Ulcer Cases in Ghana (Regional Trend for 2010)**



**Challenges**

**Logistics;** Dressing and other surgical logistics has never been enough, though Government through the programme supports the facilities [Crepe bandages, gauze and cotton wool]. In 2010 government spent GHC50, 000 [US\$45,000] on dressings.

**Personnel;** Attrition of staff at the peripheral level is very high and this is affecting some of the operation at the district levels.

We think by further strengthening the facilities with basic tools, staff will be motivated to stay on and work

**Medicine;** with the increasing number of cases particularly early lesions, the demand for antibiotics is high and for 2011, will need an increase of 27% over 2010

#### **Funds to run national office and programme**

The core mandate of the national office is to ensure effective disease control at all levels. There is the need to step up monitoring activities to cover all areas of the disease locations. Funding is urgently needed to effectively carry out this activity.

2010, the office received GHC20, 000 for all programme activities [Office running, monitoring activities and vehicular maintenance.

#### **Equipment**

Most of the basic surgical tools for the treatment of the disease is in short supply. Monitoring visits indicated that, 8 very endemic centres do not have full complement of the tools. They include; Manual and electric dermatome, Meshers, Diathermia, derma carriers, etc.

#### **2011**

Major activity: Implementation of research on all oral treatment for Buruli ulcer – comparison of SR8 and CR8. A project sponsored by the Abbott, USA; Raoul Follereau Foundation, France; ALM, USA, Sanofi Aventis France [donation of Rifampicin] and 7<sup>th</sup> Framework Programme of the European Union; Burulivac project

## **A case of cutaneous tuberculosis in a Buruli ulcer endemic area.**

**Presenter: Martin Bratschi**

**Authors:** Martin Bratschi 1, Earnest Njih Tabah 2, David Stucki 1, Sebastien Gagneux 1, Alphonse Um Boock 3, Gerd Pluschke 1

1. Swiss Tropical and Public Health Institute, Basel Switzerland and Universität Basel, Basel, Switzerland
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3. FAIRMED Africa Regional Office, Yaounde, Cameroon

For rural peripheral health care settings, microscopy after Ziehl-Neelsen (ZN) staining of acid-fast bacilli is the only available point-of-care diagnostic method for reconfirmation of clinical diagnosis of Buruli ulcer (BU). Detection of *M. ulcerans* DNA by polymerase chain reaction (PCR) is usually only performed at central reference laboratories and results can often not be awaited before treatment initiation.

Here we report a case of a 27 year old man which was clinically diagnosed to have BU at an integrated health centre in the Bankim health district of the Adamawa Region of Cameroon. The occurrence of mycobacteria in the cutaneous lesions of the patient was confirmed by local microscopy. However when testing samples from the patient by real-time PCR for presence of the *M. ulcerans* specific insertion sequence (IS) 2404, samples were negative. Culture of a sample from the patient resulted in mycobacterial growth after 8 weeks. The isolate was identified as belonging to the *M. tuberculosis* complex.

This case emphasises the requirement for a species-specific point-of-care diagnostic for BU. Furthermore, guidelines for the extension of chemotherapy for misdiagnosed patients with cutaneous tuberculosis who received BU treatment (rifampicin and streptomycin) should be developed.

## **Raoul Follereau Fondation (FRF) (France): 1995-2010, 15 years of partnership in Buruli ulcer control**

### **Presenter: Bénédicte de Charette**

The Raoul Follereau Foundation, faithful to the ideals of its founder, plays a major role in leprosy control, its principal sphere of activity.

In keeping with its mission to control leprosy and help all lepers, FRF has a strong field presence and is attentive to the needs expressed by health officials in those countries where it operates.

FRF received requests from Benin and Côte d'Ivoire to support national Buruli ulcer treatment projects in 1995. Given the extent of the suffering of BU patients, FRF decided to respond to this appeal and has committed itself to controlling this endemic disease in the affected countries alongside WHO.

In 1998 it helped to organize and fund the **Yamoussoukro Conference** and was one of the signatories of the final declaration, which launched the current initiatives against Buruli ulcer.

Despite strong political resolve, information about BU remains sketchy. Knowledge of this "re-emergent" disease is very limited and *Mycobacterium ulcerans* is poorly understood.

The Raoul Follereau Foundation and its scientific and medical committee have developed a clear and pragmatic strategy in three complementary thematic areas: management of BU patients, support for research, and knowledge dissemination and advocacy.

FRF focuses its efforts on three endemic countries: Benin, Côte d'Ivoire and the Republic of the Congo, where it supports both private and public facilities.

In Benin, for 15 years since 1995, FRF has supported the Gbèmontin Centre in Zou Department by providing medicines and medical consumables. This centre, run by Sister Julia, was the first to treat BU patients in Benin. It treats about 200 patients a year.

The Raoul and Madeleine Follereau Centre for the detection and treatment of Buruli ulcer was officially inaugurated at Pobè in April 2004 at the request of the Ministry of Health. The Centre is part of the strategy developed by the National BU Control Programme. Following enlargement work completed at the end of 2010, this centre now has 8 wards and 50 beds, an outpatient consultation room, a surgical unit, a physiotherapy suite and a laboratory. In addition to financing the building work, FRF has acquired all the equipment and pays all the operating costs. The Centre is managed by Dr Annick Chauty, who has been working at Pobè since the start of 2003. The Centre treats about 150 patients a year.

As part of its outreach project, FRF has funded and established a laboratory at Pobè for detecting BU using PCR assay, thus complying with WHO recommendations from previous meetings regarding case confirmation.

Since 1 January 2005, at the request of the Beninese Ministry of Health, FRF has contributed to the operating costs of the Lalo Centre for the Detection and Treatment of Buruli Ulcer situated in Mono Couffo Department. This Centre has 40 beds and treats approximately 200-250 patients a year.

In **Côte d'Ivoire**, FRF mainly assists the National Buruli Ulcer Control Programme by funding training activities and providing specific medicines (streptomycin and rifampicin), nonspecific medicines and consumables.

In 2010 it was planned to build a medical and surgical unit to treat BU patients at the Divo Regional Hospital, which is situated in a hyperendemic district. But the start of these works has been postponed owing to the crisis that has affected Côte d'Ivoire since November 2010.

In the **Congo**, FRF has been a long-time partner of the National Leprosy Control Programme and since 2005 has supported the activities of the National Buruli Ulcer Control Programme. In 2008 the Foundation upgraded an inpatient facility, the laboratory and the operating theatre for the treatment of BU patients at the Madingo Kayes Health Centre in Kouilou Department.

In total, FRF allocates a budget of about € 550 000 to support BU control efforts in the three countries where it operates.

**Support for research programmes** is the second priority area: since 1995 the aim of the selected projects has been to acquire better knowledge of *Mycobacterium ulcerans* with a view to preventing and treating the disease, for example "Studies on the ecology of *M. ulcerans*" and "Therapeutic trial of an aminoglycoside-rifampicin combination to treat clinical lesions due to *M. ulcerans* infection" by Professor Charbonnelle and Laurent Marsollier at the Angers Faculty of Medicine (2002-2003), "Analysis of the *M. ulcerans* genome" by Professor Stewart Cole at the Pasteur Institute in Paris, and "Clinical trial of a rifampicin-clarithromycin combination to treat Buruli ulcer piloted at the Pobè Centre for Detection and Treatment of Buruli Ulcer" by Professor Baohong Ji and Dr Annick Chauty. The research budgets are in the order of € 200 000 a year.

**Dissemination of information and advocacy:** this additional component of the strategy aims to enlighten the public about this "mysterious" disease and to make information available to health workers. Accordingly, FRF has funded the French-language versions of a number of publications, for example "Diagnosis of *M. ulcerans* infection", "Management of *M. ulcerans* infection", and has co-produced (with WHO) a video entitled "The mystery disease". In addition, for 10 years FRF has informed and raised public awareness in France and French-speaking countries round the world: its publications and conferences are an opportunity to disseminate messages which are then relayed by the media. The Newsletter of the Association of French-speaking Leprosy Specialists, which is funded by FRF, is a forum for publications concerning *M. ulcerans*. These combined efforts have undoubtedly helped to raise awareness nationally and internationally and contributed to better knowledge of *Mycobacterium ulcerans* infection.

In March and April 2009, the Government of Benin, in collaboration with WHO and other international organizations including FRF, organized a summit on Buruli ulcer control and research, at the conclusion of which a number of Heads of State or their representatives signed the Cotonou Declaration and thereby committed themselves to promoting Buruli ulcer control and research in their respective countries.

Thus, **having invested more than €3 500 000 since 1995, FRF** has demonstrated its commitment to controlling Buruli ulcer in Africa.

The Raoul Follereau Foundation takes this opportunity to reiterate its commitment to working alongside affected countries to research and control this major endemic disease.

## **An integrated approach to education aids in West Africa**

**Presenter: Yuki Shimomura**

**Authors:** Y. Shimomura<sup>1</sup>, T. Niiyama<sup>2</sup>, K. Fukunishi<sup>3</sup>, Hi Koeda,<sup>4</sup> S. Naruse,<sup>5</sup> T. Fujikura<sup>6</sup>

Our report will be consisted of two separate parts. I will report on the implementation of educational assistance in Benin and Togo, while I will venture a possibility of taking our 10-year-old programme into a new phase of educational aid, an educational assistance in the area of physiotherapy.

Since 2009, Kobe International University's Project SCOBU has been implementing its "in-hospital education" along with DAHW at Tsvie Central Hospital, a regional medical centre in the Maritime District, Togo. The programme itself is similar to the pilot educational programme we have continued to assist in Benin for the past 5 years. One of major functions of the Project SCOBU Educational Fund is to provide a grant for the implementation of pilot programme, providing basic education for BU children during their hospitalization to avoid discontinuation of basic education that may determine the course of their lives.

Many of the cured BU children were not able to return to school. The primary factors underlying this injustice, particularly from the perspective of the human rights, were:

- 1) hospital charges and medical fees often heavily devolve upon the entire family budget.
- 2) lack of relevant information about the disease that often result in prejudice and discrimination against the disabilities of BU patients caused by the sequelae that are resulted from surgical treatment.

All these prevent the former BU children to return to school as the former patients to resume their place in society.

Recognizing the on-going successful "in-hospital education" programmes in Benin and Togo, Project SCOBU's fieldwork in 2010 also suggests a possibility of joint venture with other NGO organizations in strengthening our educational services to children in need. In the case of Benin, major regional medical centres have an attached rehabilitation facility with sufficient basic equipment. But in some cases, provided equipment needed in the facilities often came out of the creative hands of hospital staffs and local artisans, using whatever materials available to them in the region. Lack of physiologists may also need an immediate attention. There are only a handful of specialists who are fully equipped with physiological science, while many of rehabilitation programmes often heavily depend on able but less trained assistants. The case of Togo shows the similar trend.

Project SCOBU intends to strengthen its educational programmes in the area of physiotherapy with the two-year-old school of rehabilitation at Kobe International University. As it has built a pilot educational programme in Benin and Togo in the past five years, the project is entertaining an idea of introducing an integrated educational programme in physiotherapy along with NGO organizations already present in the region.

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The projected aims will be:

- 3) to transmit a substantial techniques and technologies in the field through a short-term on-the-job training programme with the local staff
- 4) to enhance existing rehabilitation programme to produce more specialists in the field of physiotherapy
- 5) to provide continuing guidance for various treatments and assist facilitating the hospital in the field

The fieldwork in 2010 and the primary research in Togo and Benin in 2011 with two physiotherapists were intended to be the first step toward the realization of the planned programme. After 10 years of activities for BU children in West Africa, the project welcomes this new development. We hope to materialize this idea with close cooperation with the existing NGO organizations present in the region.

Finally, Project SCOBUR recognizes an indispensable part of health workers in implementing BU programmes in the endemic regions. In 2010, the project, in addition to the “in-hospital education” programme, restored its auxiliary fund to assist the crucial functions of field operators in Togo. The fund had once supported an early detection programme at Wewak General Hospital in Papua New Guinea for three years between 2004-2007. The project hopes to join our colleagues in being loud in their praises.

## Stop Buruli – progress, lessons learned, and outlook

**Presenter: Susanna Hausmann-Muela**

Almost four years ago, the UBS Optimus Foundation has invited a group of researchers to develop a proposal following an overall research agenda with the aim to leverage Buruli Ulcer Research and pave the way towards a breakthrough of controlling the disease.

A research consortium, consisting of eight main partner institutions in four continents, has, over the past two and a half years, carried out coordinated research on transmission, diagnostic, clinical, and social aspects. Substantial progress has been achieved in the development of platform technologies, molecular epidemiology, and in diagnostics. Formative research, leading to evidence-based implementation studies, is currently being carried out.

The Stop Buruli consortium will soon enter into its second funding cycle. The experiences over the past two and a half years have been highly satisfactory. Important lessons learned will support and guide future planning:

1. The transdisciplinary approach of the consortium is most enriching, counteracting fragmentation of biomedical research and responding to the need of understanding diseases in their real world. At the same time, the specialized concepts, approaches and languages in each field of expertise require time and respect to dialogue with each other. The fruits of this process only begin to fully mature now, after more than two years.
2. From the beginning, the alignment of the Stop Buruli research agenda with WHO disease control priorities has been our firm intention. Also here time and efforts were required to optimize communication and to come to mutual understanding. For the second phase, alignment of overall research goals with requirements for disease control is implemented by close consultation with WHO from the very start of research portfolio development.
3. The combined expertise in various fields of biomedical sciences available in the consortium is impressive and in a first phase research within the consortium has greatly benefited from this. In the second phase, use of this concentrated expertise as a resource centre for other research groups will be strongly encouraged.
4. Capacity building is an integral part of the research consortium. New concepts are required to promote education and career development of young talented African researchers and technical staff. In the second phase the consortium will intensify its capacity building efforts by networking and trans-disciplinary teaching to counteract fragmentation of health research, brain drain and disconnection from the needs of endemic countries.

The Stop Buruli consortium is now in the process of defining the new research portfolio for the second phase. Based on the developments in research and on the lessons learned, it will propose a new series of innovative, transdisciplinary and interlinked collaborative projects for funding to the UBS Optimus Foundation.



# **Antibiotics treatment**



# **Clinical and bacteriological response of Buruli ulcer patients in Ghana to treatment with rifampicin-streptomycin for 7 days per week for 8 weeks compared with rifampicin-streptomycin for 2 weeks followed by rifampicin and clarithromycin for 6 weeks**

**Presenter: Richard Phillips**

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## **Introduction**

As part of a programme to develop an oral antibiotic regimen for treatment of Buruli ulcer, a small non-blinded randomised controlled trial is in progress to compare the clinical and microbiological response to standard rifampicin/streptomycin for 8 weeks (RS8) with rifampicin/streptomycin for 2 weeks followed by rifampicin/clarithromycin for 6 weeks (RS2RC6) in patients with small Buruli lesions. This study will further determine if mycolactone reduction in Buruli lesions correlates with development of the immune response. This is an interim analysis of 83 patients recruited to the study.

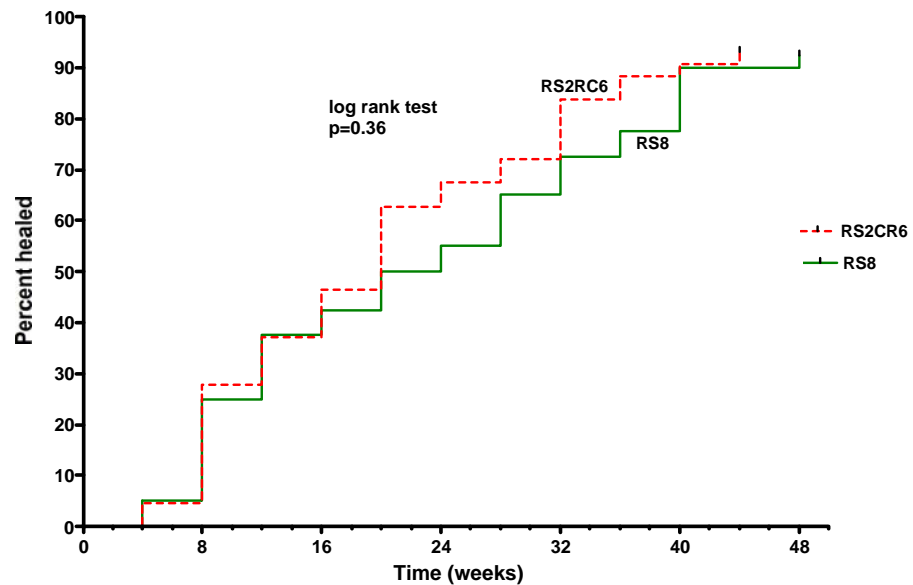
## **Methods**

Between July 2009 and January 2010 patients were recruited from the Ahafo Ano North, Atwima and Asante Akim North districts of Ghana and active Mu disease was diagnosed by microscopy for AFB, culture for *M. ulcerans* (Mu) and PCR on 4 mm punch biopsies, fine needle aspirates or swabs. Subjects with Buruli lesions that were <15 cm in maximum diameter were randomised to receive RS8 or RS2RC6. Two further biopsies were obtained both at week 6 and week 12 if the lesion was still ulcerated. One biopsy was cultured for Mu by a semi-quantitative method and the other was stored for measurement of mycolactone concentration. Whole blood was obtained at the same time points for stimulation with Mu antigens and quantification of cytokine IFN $\gamma$  release. 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**

Out of 83 patients recruited all of whom had a positive PCR for Mu, 40 were randomised to receive RS8 and 43 to receive RS2RC6. The two groups were well matched demographically. All lesions were <15cm in diameter but 4 in the RS2RC6 group and 1 in the RS only group had multiple lesions, classified as category III. Currently all 83 patients have completed antibiotic treatment and follow-up for 12 months?. There was no difference in the proportion healed in each group after 4, 8, 12, 16, 20, 24 and up to 48 weeks. There have been no recurrences to date but 4 (3 enlargements, 1 new lesion) developed paradoxical reactions in the RSRC6 group compared to 3 (enlargements) in the RS8 group. Two were lost to follow up in each treatment arm. The success rate was 93% in each group.

Eleven of 16 (69%) cultures from the RS only group were positive after 6 weeks and 3 of 5 (60%) after 12 weeks compared with 10 of 16 (63%) and 3 of 7 (43%) at 6 and 12 weeks respectively in the RS2RC6 group. There was no difference in the number of bacteria cultured at the different time points.



% healed on RS8	5	25	38	43	50	55	65	73	78	90	90	93
% healed on RS2RC6	5	28	37	47	63	67	72	84	88	91	93	93

### Conclusions

There was no difference in healing or viability of *M. ulcerans* of small Buruli lesions using RS8 compared with RS2RC6. No recurrences were noted after 12 months follow up.

**Acknowledgement: Support from the EFINTD is gratefully acknowledged**

# Successful outcomes using oral fluoroquinolone antibiotics in the treatment of *Mycobacterium ulcerans*.

**Presenter: Daniel O'Brien**

**Authors:** O'Brien DP, McDonald A, Callan P, Robson M, Hughes A, Friedman ND, Walton A, Athan E.

## Introduction

The management of *Mycobacterium ulcerans* infection has rapidly evolved with combination antibiotics now forming the mainstay of treatment. Despite evidence of in vitro effectiveness against *Mycobacterium ulcerans*, clinical evidence for the use of fluoroquinolone antibiotics is lacking. The Bellarine Peninsula, in coastal south-eastern Australia, has been experiencing an epidemic of *Mycobacterium ulcerans* since 1998.

## Methods

A retrospective review was performed of the treatment of initial *Mycobacterium ulcerans* infections from the Bellarine Peninsula over a 12-year period between 1998 and 2010. Medical treatment was defined as the use of antibiotics for more than 7 days, surgical treatment as the surgical excision of a lesion. Treatment success was defined as complete healing of the lesion without recurrence at 12 months of follow-up. A complication was defined as an adverse event attributed to an antibiotic that required its cessation.

## Results

A total of 132 patients with 136 lesions were studied. Median age was 61.5 years (range 3-94 years) and 67 (52%) were males. 84% of the lesions were ulcers, 8% nodules and 7% oedematous. 46 (34%) had surgical treatment alone, and 90 (66%) had combined surgical and medical treatment. 64 (48%) required a split thickness skin graft and 16 (12%) required a vascularized tissue flap. Ciprofloxacin and rifampicin comprised 60%, rifampicin and clarithromycin 23%, and ciprofloxacin and clarithromycin 4% of first-line antibiotic regimens respectively.

13/46 (28%) of those treated with surgery alone failed treatment compared to 0/90 (0%) of those treated with a combination of medical and surgical treatment ( $p < 0.0001$ ). There was no difference in treatment success rate for antibiotic combinations containing a fluoroquinolone (61/61 cases; 100%) compared with those not containing a fluoroquinolone (29/29 cases; 100%). Complication rates were similar between ciprofloxacin and rifampicin (31%) and rifampicin and clarithromycin (33%) regimens (OR 0.89, 95% CI 0.27-2.99).

## Conclusions

Medical therapy significantly reduces treatment failure for *Mycobacterium ulcerans* infections and fluoroquinolone containing antibiotic regimens can provide an effective and safe oral treatment option.

# **Antibiotic treatment: 12 months assessment of follow-up after treatment of a cohort of patients in Cameroun.**

**Presenter: Alphonse Um Boock**

**Authors:** Dr Alphonse Um Boock , Dr Earnest Njih Tabba

## **Background**

Recently the WHO has introduced new provisional antibiotic treatment guidelines for BU following a successful pilot study from Ghana, which confirmed that human lesions can be sterilised with antibiotics (Etuafu *et al*, 2005). The WHO protocol has led to a new approach to treatment with the potential to reduce cost, to allow delivery of care closer to the homes of patients, and to encourage patients to present earlier as the fear of major surgery is lessened. These excellent results demonstrate that healing can continue for many months after the completion of therapy. This observation needs to be taken into account when assessing the overall success of antibiotic treatment and the definition of “treatment failure”.

## **Objective**

We have designed a prospective cohort study, to confirm these excellent results, to build confidence in this new treatment strategy at the local level and to investigate the reason(s) why occasional patients may fail treatment.

## **Method and material**

Routinely the diagnosis of Buruli ulcer at the DTC (AFB) and the PCR .

Patients confirmed as having Buruli ulcer for the first time and without any history of previous Buruli ulcer infection is considered new case. “Local recurrence” -- a new lesion after apparent healing, confirmed as BU by histology, PCR.

lesion after apparent history of Buruli ulcer in the same place is considered a recurrent case.

All new patients are submitted to streptomycine/ rifampicin combination antibiotics treatment in the DTC for 8 weeks.

On discharge these patients are then follow up on out-patient basis during 12 month.

## **Case recruitment**

150 clinical cases of Buruli ulcer were screened: 72 were positive for AFB with Ziehl Neelsen (ZN) staining and PCR , 104 were confirmed positive by PCR , 46 patients had typical clinical Buruli ulcer lesions but were negative both for ZN stain and PCR. There were however treated as Buruli ulcer cases

**HIV serology status:** 4 PCR positive patients were also HIV positive.

## **Treatment**

### **At 4 weeks:**

Are eliminated from this analysis 16 patients: 10 patients have decided after their confirmation to the PCR to follow the heat treatment instead of antibiotic treatment . 6 lost cases.

The evolution of the treatment at the end of 4 weeks, shows a significant association with the type of lesion and the category. This evolution of the treatment does not differ significantly according to the demographic characteristics of the patients (sex and old).

There is no difference for the treatment centers where the patients were treated, that means that the care are standardized.

For the comparison of the evolution of the treatment at 4 months between positive cases PCR and negative PCR, the evolution of the treatment does not show significant difference according to the result of the test ( $P = 0.3$ )

***At 8 weeks:***

The evolution of the treatment at 8 weeks shows a significant variation according to the center of treatment. This difference is especially marked between Ayos and Bankim. Indeed, the cicatrization is observed to 72.6% of patients of Ayos, whereas it is raised only at 46.8% of those of Bankim.

On the other hand more than half patients of Bakim presents the « ready site for grafting » against only 27.4% of those who are followed in Ayos. This tendency does not confirm the result of the fourth week, treatment and suggests the need for leading more thorough analyses multi type varied for better controlling the factors of confusion and this on a larger sample.

**Follow-up after treatment**

All patients healed after 8 weeks of treatment. No new lesion appeared during 12 months of follow-up after 8 weeks of treatment, even in HIV positives patients.

However, we observed the occurrence of leprosy in one of the patients at the 12th month of follow-up. He presented with clawed fingers, indicating that he has had leprosy for a long while but unknown. The particular patient was negative for HIV. He is currently on treatment for leprosy and will be followed-up till completion of his leprosy treatment.

The second was to discover one paradoxyal reaction (to be shown).

The prevalence of HIV in our sample was 2% (n=4). All of the patients had a CD4 count above 200 and responded very well to the treatment for Buruli ulcer. No reaction after 12 month.

# **The influence of the immunological status of co-infected HIV/Buruli patients on the evolution of their Buruli treatment**

**Presenter: Vanessa Christinet**

## **1- Implementation**

During the first consultation for inclusion in the program - after confirmation of Buruli diagnosis – the patient goes through a complete medical consultation where classification of the wound(s) is defined according to WHO's categories, photographic and anthropometric data are recorded, wound protocol are established as well as a physiotherapy's functional evaluation.

During the medical consultation 2 HIV tests are proposed (Determine & UniGold). In case of a positive result their WHO status is established and blood samples are taken in order to measure their CD4.

Then patients are sent to Yaoundé for X-Rays of the Chest and the affected area(s).

## **2- Results**

In MSF's Buruli Project in Akonolinga Cameroon 100 patients have exited, who have completed their full treatment (CFT), and had the CD4 count done (in the case of HIV+) in the year 2010. Out of those 100 patients 86 were non co-infected HIV/BU patients with an average length of stay (ALS) of 150 days while 14 co-infected had an ALS of 266.

Taking into account WHO's classification of CD4, 7 patients had <500 CD4 with an ALS of 402 days, while the other 7 patients had >500CD4 with an ALS of 131 days. It is interesting to note that out of the group <500 CD4 86% (6/7) came with a wound Category 3 while out the group >500 CD4 71% (5/7) presented a wound Category 1 and 28% (2/7) Category 2 and 3.

In general, patient co-infected HIV/BU stay longer than the non co-infected. However, co-infected patients non immunosuppressed (>500CD4) stay 19 days less than the non co-infected patients (131 days vs. 150 days).

Therefore patients with immunosuppression (<500 CD4) their length of stay is around 3 times more than patients >500CD4 and 2.7 times more than the non co-infected ones.

It is interesting to notice that the majority of immunosuppressed patients present complicated lesions at the moment of their inclusion, while the ones >500 CD4 comes with a Category 1

**In total, during 2010, important differences have been noticed regarding the evolution of the wounds of the Co-Infected patients (Buruli + HIV/AIDS) and the non Co-Infected ones, with a longer period of treatment for the first ones linked to their immunological status influencing the degree of complication of their wounds at the moment of their inclusion.**

## **3- Challenges**

The main challenge is to have patients who can meet selection criteria. Indeed, several patients are not eligible for the analysis of data due to abandon, death or refusal of HIV status (what percentage of patients refused testing and age/sex? I guess there is no problem for kids).

Moreover, MSF not being the direct implementer of HIV activities complicates the gathering and completion of all data.

## **4- Solutions**

Several strategies have been designed in order to confront those issues. An increase of resources have been decided so more emphasis is put on PSEC, medical management of co-infected patients and collaboration with the partner in charge of HIV+ patients in the area.

# Microbiological, histological, immunological, and toxin response to antibiotic treatment in the mouse model of *M. ulcerans* disease

**Presenter: Paul J. Converse**

**Authors:** Paul J. Converse, Deepak V. Almeida, Opokua Amoabeng, and Jacques H. Grosset  
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## Background

*Mycobacterium ulcerans* (*Mu*) infection slowly leads to swelling in humans and in experimental animals. Part of the pathogenic process is the production of the immunosuppressive mycolactone (ML) toxin by *Mu*. How soon this begins after infection is unknown (1) as is how soon it stops due to antibiotic treatment. We evaluated the evolution of *Mu* (Mu1615, Malaysian strain) infection in the mouse footpad model by assessing not only the CFU number, but also ML production, immune responses, and histopathology at different times after infection and after the onset of antibiotic therapy.

## Materials and methods

BALB/c mice were challenged with  $10^5$  *Mu*/ right hind footpad, resulting in the detection of  $\sim 10^3$  organisms on day 3. Footpads were harvested from 9 mice (3 for CFU, 3 for ML detection, 3 for histopathology) at different time points after infection up to  $\geq$  grade 3 swelling. After the onset of swelling, treatment with rifampin (R, 10 mg/kg) and streptomycin (S, 150 mg/kg) began 5 days/week for 8 weeks. The response to treatment was monitored with histopathological, bacteriological, and toxicological analyses. CFU were enumerated on Middlebrook 7H11 agar plates. ML detection was attempted by the Kishi-Jackson boronic acid method (2, 3). Histopathology involved hematoxylin and eosin (H&E) as well as acid-fast (AF) staining. Spleens were harvested to assess responses to mycobacterial culture filtrate proteins (CFP) by multiplex ELISA.

## Results

Footpad swelling first appeared in some mice 3 weeks after infection and averaged grade 1 soon after the initiation of treatment, 4 weeks after infection at which time there were approximately  $6 \log_{10}$  CFU per footpad. Swelling continued to increase over the following 3 weeks in untreated mice whereas CFU remained unchanged. In contrast, both swelling and CFU declined markedly in the RS-treated mice. Histologically, increased numbers of AFB correlated with increased swelling in untreated mice, being evident initially in the dermis and eventually in sub-epidermal zones and the epidermis with the onset of ulceration. Inflammatory cell infiltrates appeared to be maintained in RS-treated mice but were disrupted and progressively disorganized in untreated, control mice. AFB were still detectable but acquired a beaded appearance during treatment and even after treatment completion. Three months after treatment completion, bacilli were detectable but not cultivable. In general, cytokine and chemokine production by splenocytes in response to CFP were reduced in treated compared to untreated mice. Results for toxin production before and after treatment are pending.

## Conclusions

Our results show that RS treatment of experimental *Mu* disease results not only in the reduction or elimination of viable bacteria but also in clinical and histopathological improvement of lesions. The inflammatory response is also downregulated with reduced production of cytokines such as TNF alpha.

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## **Light as adjunct treatment in the healing of Buruli Ulcers**

**Presenter: Alvar Grönberg**

**Authors:** Alvar Grönberg, Desiree Wiegleb Edström, Richard E Lee, Lydia Mosi, Fred Stephen Sarfo, Pamela Small and Sven Britton  
Dept Medicine, Karolinska Institutet, Stockholm, Sweden, Health Sciences, University of Tennessee, Memphis, USA, Noguchi Institute for medical Research, Accra, Ghana, Komfo Anokye Teaching Hospital, Kumasi, Ghana.

Our studies on the effects of mycolactone on human keratinocytes – one of the key cells in wound healing - have shown that it is highly cytotoxic to these cells and that generation of reactive oxygen metabolites is a central mechanism in this process. Cytotoxicity can be completely inhibited by addition of antioxidants such as deferoxamine but also by exposure of mycolactone to light before adding it to the cells. A short exposure of mycolactone to UVA or UVB light corresponding to 30 min in sunlight was sufficient to inactivate its cytotoxic effect. Furthermore, exposure to sunlight from which all UVA and UVB had been filtered away, also inactivated mycolactone. As short exposure as 15 minutes to filtered sunlight was sufficient to reduce activity by more than 50% whereas 30 minutes exposure completely ablated cytotoxicity. Analysis of light treated mycolactone by mass spectroscopy, revealed that the original molecular weight had been reduced. These results show that mycolactone is extremely light sensitive and a short exposure to visible light will cause its degradation and inactivation.

We intend to investigate the implications of the above latter findings in the treatment of BU. Wounds will be randomized to receive standard treatment with or without light exposure at the time of dressing during the eight weeks of antibiotic regimen when rate of healing normally is slow, and comparing time to complete healing.

# **Antibiotic treatment using a combination of rifampicin and clarithromycin (R/C) by the MSF– Akonolinga project, Cameroon**

**Presenter : Dr Serge M. Kaboré**

**Authors :** Dr Serge M. Kaboré, Christine Kabanda, Dr Eric Comte, Abanda Meva'a et Dr Pablo Diaz Badial

## **1. Implementation:**

Patients were recruited using precise medical criteria:

- Category 1 lesions without any complications (HIV, TB, or any other co-infection and patients not requiring physiotherapy),
- Children accompanied by a responsible adult in a health area within Akonolinga district. The live close to an integrated health centre in which MSF has implemented its decentralization programme. If the patients are minors, they must be accompanied by an adult (parent of legal guardian).
- In hospitals, the rifampicin + clarithromycin (R/C) protocol is used for patients for whom streptomycin is contraindicated.

The decision to treat a patient with the R/C regimen for 12 weeks is taken at the inclusion examination carried out by the physician and the physiotherapist, as soon as the patient has been confirmed as a Buruli ulcer case (ZN, PCR, Culture, strong clinical suspicion).

Patient monitoring:

- In hospitals, patients receiving R/C are monitored daily.
- In decentralized facilities, they are monitored twice weekly by an MOH nurse on secondment to MSF.

The nurse makes a weekly round to each of the 3 decentralized centres. R/C is first provided for four days, and then for one week if the patient is compliant.

The following are examined while the patient is being monitored: number of tablets remaining, any complications or side effects of the treatment, and lastly assessment of the lesion and its evolution under dressing.

## **2. Results :**

Since June 2008, 50 patients (26 during 2010) have been given R/C treatment and 2 have dropped out. 30 (60 %) were women and 43 (86%) were in the 2 to 38 years age group.

Of the 50 patients, 33 (66%) had category I and 12 (24%) category III lesions.

26 (79%) patients out of 33 were at the decentralized level. The proportion with HIV coinfection was 29% (10).

Of the 50 patients given R/C treatment, 42 had positive samples (an 84% confirmation rate).

Of the 48 patients who completed their treatment with R/C, 41 were discharged and 7 had unhealed lesions.

At the end of 2010, 37 patients had been discharged cured, a cure rate of 90%; one of the 37 had sequelae (retraction).

Nine (22%) were discharged within three months, while 27 (73%) were discharged within three months to one year, 43% of them between 3 and 6 months.

One female patient relapsed (a nodule on the same limb) 8 months after completion of the R/C treatment and one month after being discharged from the programme (2009).

The number of patients who complied correctly with the antibiotic treatment (no more than three days without treatment) was 92% (44/48).

### **3. Constraints:**

- The need to monitor patients' compliance with the antibiotic treatment
- The need to transfer skills to peripheral health centres.
- Withdrawal of treatment with clarithromycin since August 2010 in peripheral health centres

### **4. Solutions:**

- Introduction of an individual tablet distributor to improve compliance.
- Regular training for the CSI nurses
- International partners working to control this disease must cooperate to carry out clinical trials.

# **Early detection, surveillance and decentralization**



# Scaling up early detection and treatment to reduce Buruli ulcer morbidity in the Asante- Akim north district of Ghana

**Presenter: Anthony Ablordey**

**Authors: Ablordey, A<sup>1</sup>, D. Amissah<sup>1</sup>, W. Thompson<sup>2</sup>, A. Gyabaah<sup>2</sup>, K. Abass<sup>2</sup> and F. Portaels<sup>3</sup>.**

<sup>1</sup>Noguchi Memorial Institute for Medical Research, <sup>2</sup>Agogo Presbyterian Hospital, <sup>3</sup>Institute of Tropical Medicine, Antwerp.

## Background

Early case detection and initiation of chemotherapy is an important component in the control of Buruli ulcer (BU). This strategy reduces or prevents disabling complications associated with late stages of the disease and result in a better integration of BU control activities into the primary health care system, as well as lowering of the direct and indirect cost of treatment. The three-year project, funded by the European Foundation initiative for Neglected Tropical Diseases (EFINTD) and the International Association of National Public Health Institutes (IANPHI) is being implemented in 28 endemic communities of the Asante Akim North district which is the second most endemic district in Ghana.

## Objective

The overall objective of this project is to implement strategies for the promotion of active community participation in early case detection and treatment seeking, and to support health centres in confirmation and management of cases.

## Activities carried out

Strategies employed to strengthen community and health care facilities activities include:

- (i) Training community based surveillance volunteers (CBSVs) and health workers to identify early BU lesions and link victims and families to treatment centres.
- (ii) Active case search (by CBSVs, community sensitization and screening by health workers).
- (iii) Set up of laboratory for BU diagnosis in district hospital (PCR and smear microscopy).
- (iv) Quality control: Performance of district hospital laboratory assessed by NMIMR reference laboratory.

## Results

- (i) Forty CBSVs have been trained to suspect and refer cases and another 40 health workers trained in the management of the disease. Sensitization and screening activities have been carried out in 4 communities.
- (ii) The district hospital laboratory has been equipped and two technicians trained to diagnose BU by PCR. Training in smear microscopy is underway.
- (iii) From September 2010 - January 2011 a total of 72 cases were reported of which 54 were positive and 18 were negative. Sixty nine (96%) of the cases were referred by community volunteers and three self reported. There were 50 pre-ulcers and 22 ulcers (cat.I-10, Cat.II-8 and Cat.III-4).
- (iv) PCR positivity rate of all suspected cases was 75%. .

### **Conclusion and perspectives**

Majority of the suspected cases referred were confirmed as BU- a reflection of the important role CBSVs play in active case finding as well as their good skills in case suspicion.

The establishment of a diagnostic centre enable cases to be confirmed in two days and consequently waiting time to treatment initiation has been reduced from two weeks to two days.

It is expected that 80% of cases will be identified in the pre ulcerative stages leading to significant reduction in disease morbidity in the district.

We also plan to extend the early detection campaign and diagnostic services to endemic communities in neighbouring districts.

# The contribution of health workers and community intermediaries in Moyen Ogooué (Lambaréné) province to raising awareness of Buruli ulcer

**Presenter: Louis Bayonne Manou**

## Introduction

Buruli ulcer has been a public health problem in Gabon since 2005, when a large number of cases were detected in Moyen Ogooué province, which remains the only focus of the disease and where there are 50 cases each year on average.

As a result of this discovery, training sessions were organized to enable health workers to provide proper treatment and for community intermediaries to enable them to provide information to the populations concerned.

## Goal

Early case detection in the community and referral to the district health facility.

## Methods and material

The management team of the National Buruli Ulcer Programme visited Moyen Ogooué province once each quarter to monitor activities.

The following measures were used to ensure that the community intermediaries were involved in the Buruli ulcer control effort:

- Training, to acquaint them with the clinical signs and epidemiological background to Buruli ulcer;
- Use of Information, Education and Communication (IEC) aids (image box, leaflets and posters).

The intermediaries who had been trained organized information sessions in the villages; at the sessions, people with skin lesions not caused by an injury were told to consult the nearest health facility.

## Indicators

- The large number of new cases recorded (see table n°1)
- The drop in the number of lesions classified as category 3 ( $\geq 15$  centimetres)( see tableau n° 2)

## Results

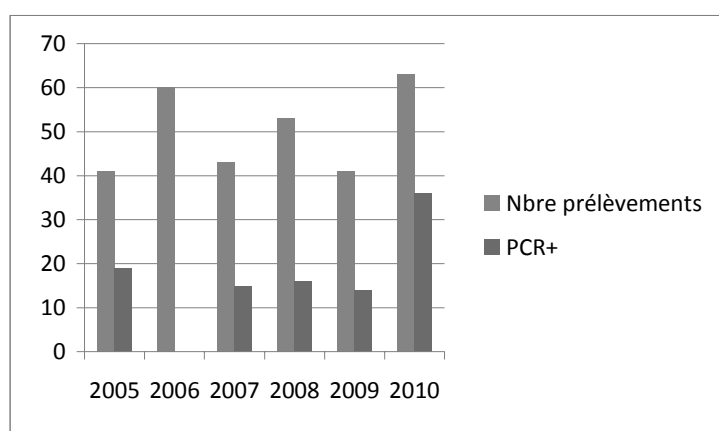
Table n°1

Provinces	Number of cases reported	PCR +	ZN+	Percentage PCR
Ogooué Ivindo	2	0	0	0
Ogooué LoLo	9	0	0	0
Ogooué Maritime	3	0	0	0
Moyen Ogooué( Lambarené)	<b>48</b>	<b>35</b>	<b>37</b>	<b>72,91</b>
Nyanga	1	0	0	0
Libreville	2	0	0	0
<b>Total</b>	<b>65</b>	<b>35</b>	<b>37</b>	<b>53 ,84</b>

Tableau n°2

Provinces	Category 1	Category 2	Category 3
Ogooué Ivindo	0	2	0
Ogooué LoLo	2	5	2
Ogooué Maritime	0	3	0
Moyen Ogooué( Lambarené	25	23	0
Nyanga	0	0	1
Libreville	0	2	0
<b>Total</b>	<b>27</b>	<b>35</b>	<b>3</b>

Table n°3 Number of cases notified since 2005



### Weaknesses

- Delays sending samples to Libreville,
- Difficulties moving around, especially along the river,
- Absence of intermediaries in some groups of villages,
- Lack of a financial incentive for the local elected officials.
- Belief in witchcraft in some communities.

### Strengths

- The village community is familiar with all the signs of the disease and as a result people consult at an early stage and there are fewer very large lesions,
- Traditional healers have become involved,
- A small facility has been set up in an urban health centre in Lambaréné at which treatment for Buruli ulcer is provided by a health worker.

**Conclusion**

Pending the availability of a vaccine, mobilization of the community intermediaries by campaigns to raise awareness among the populations concerned is still the only way to detect new cases. Ensuring continuity of this activity will make it possible both to reduce very extensive lesions and to limit the social and economic consequences of the endemic.

# **Contribution of Frontline Workers in Early Detection of Buruli ulcer- Amansie West District Experience**

**Presenter: Joseph Adomako**

## **Background**

Buruli ulcer management and control had been one of the major public health intervention challenges in the Amansie West District before 2003. The district used to be the worst affected district in Ghana with a crude prevalence rate of 150.8/100,000 as compared to the national average of 20.7/100,000 (1999 national case search).

Community intervention strategies implemented between 1993 and 2000 had been very successful and well documented (Amofa et al 1993 and Phillips et al 2000). Yet those strategies were not sustainable. That was because the strategies were "health-worker" centred whereby, health workers moved from facilities to communities to search for cases and treat. There was minimal involvement of community-based health workers and volunteers whose role was delegated to only mobilising the community for the screening exercise.

Nonetheless, since 2003 the Amansie West District Health Directorate, with support from a local and an international NGOs (Health Foundation of Ghana and ANESVAD Foundation of Spain respectively) has been working with community-based agents (CBAs) on a project to improve early detection of the disease with the aim of reducing the severity and complications of the disease, particularly among children in the district.

## **Project Design**

The project was designed to focus mainly on the community, working closely with community-based surveillance volunteers (CBSVs), traditional birth attendants (TBAs or "village midwives"), chemical sellers ("village pharmacists"), and teachers. This group of people together formed community-based agents (CBAs) for detection of not only BU but other skin conditions including yaws.

The project implementation involved the training of CBAs to screen, detect, document and refer cases to health facilities, as well as dress small ulcers and do follow ups of cases. The CBSVs move from house to house within the community with picture cards, educating people about the disease, and screening people with suspected lesions. With respect to the chemical sellers and TBAs, they look out for suspected lesions when people report to them. The teachers also screen school children to detect, document and refer suspicious lesions. The involvement of the teachers also aims at improving their knowledge about the disease and allaying their fears about healed lesions being infectious as well as eroding stigmatization of the disease among the population.

The health system which has to receive and appropriately manage the cases is nonetheless not left behind in the project. The focus, however, has been on the peripheral facilities. This involves improving knowledge among health workers at the peripheral health facilities (private and public) to receive referred cases from the CBAs and managing the simple ones and referring the complicated ones to the district hospital. They also support and supervise the school and community level activities in their respect areas.

**Results**

There has been increased awareness of the disease among the general population which has led to early detection and reporting for treatment and also minimised the use of herbs for treatment. Additionally, there has been remarkable improvement in the knowledge and skill of health workers at the peripheral facilities in the management of the disease. Consequently there has been, not only an increase in early case detection (from as low as 20% in 2003 to 63% in 2010) but a significant reduction in number of cases being detected (from 230 cases in 2000 to 32 cases in 2010).

**Conclusion**

Improving Buruli ulcer management and control, like all public health interventions, should not be the preserve of only health workers; it requires the concerted efforts of all - the health worker and the Community-based Agent.

# **Lessons drawn from experience of decentralization of Buruli ulcer control in Côte d'Ivoire: the case of the Irish-One project.**

**Presenter: Julien Aké**

**Authors:** Julien AKE, Aubin YAO, Konan NGUESSAN  
MAP International – Côte d'Ivoire office

## **Background**

Buruli ulcer is a public health problem in most countries in wet tropical regions. The first control measures were carried out by specialized organizations, most of them private. As a result, it was possible neither to provide satisfactory geographical coverage of care nor to determine the actual scale of the disease. Most patients received treatment in facilities when the disease was at an advanced stage and were left with scars and disabling sequelae.

Integration of Buruli ulcer control into the public health system in order to improve coverage of control measures represents both a solution and a challenge.

Several organizations have decided to test decentralization in support of national Buruli ulcer control programmes. One of them is MAP International, Côte d'Ivoire, which since 2007 has been carrying out a project to integrate Buruli ulcer control measures into the public health system in collaboration with the National Buruli Ulcer Control Programme (PNLUB).

The project's objective is to improve Buruli ulcer control measures in eight health districts. The project has focused on the following three main lines of action:

- Strengthening organizational skills in the health districts;
- Training health professionals and community intermediaries;
- Providing vehicles, computers and medical equipment for health facilities;
- After three years' experience, the lessons learned are described in this paper.

## **Lessons drawn from the Irish One project**

These illustrate the strengths and weaknesses of, opportunities for and threats to decentralization of Buruli ulcer control.

### **1. Decentralization: the strengths**

- Better coordination of control activities: introduction of coordination at each level of the health pyramid (district, regional and central levels).
- **Better coverage by control measures:**
  - i. 656 health professionals and 2145 community intermediaries have been trained for a population of approximately 2 million people, i.e. 1 health professional per 3 000 inhabitants and 1 community intermediary per 900 inhabitants
  - ii. An increase in the number of cases detected in the districts (the number of cases before the health professionals and community intermediaries were trained has increased four-fold)

- **A better cost/benefit ratio:** the State's human, material and logistic resources are made available to the project.

## **2. Decentralization: the weaknesses**

- The no-fee policy announced by the heads of State when the Global Buruli Ulcer Initiative came into being conflicts with the policy of cost recovery practised by public facilities.
- Failure to disseminate clear policies and instructions

## **3. Decentralization: the opportunities**

- Existence of decentralized state facilities which are capable of providing financial and material support for the effort to control Buruli ulcer,
- Existence of local NGOs and community-based organizations capable of actively participating in Buruli ulcer control.

## **4. Decentralization: the threats**

- Frequent outages of drug supplies which undermines the motivation of those involved on the periphery,
- Heavy dependence on the public health system: any upsets affecting the system have a considerable impact on control measures at all levels.

## **Conclusion**

Decentralization of Buruli ulcer control in Côte d'Ivoire is now a fact of life in several health districts. The Irish One project has made an important contribution to this approach by enabling the health districts to take ownership of the detection and medical treatment of Buruli ulcer cases. To ensure the sustainability of this initiative, the National Buruli Ulcer Control Programme and MAP International are carrying out supervision of the principal actors of the control effort.

## **The position and role of community guides and intermediaries in health promotion to control Buruli ulcer in Allada/Zè/Toffo (ZS AZT) health area in southern Benin:**

**Activities by volunteers during the January 2011 prevalence surveys carried out under the “Blue Hope”.**

**Presenter: Yolande Goudoté**

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### **Summary**

The "Blue Hope" study is a community action and participation project which is being carried out as part of a PhD on health promotion. Its purpose is to improve early case management and to reduce the prevalence of Buruli ulcer in the villages of Houédota and Azonmém in southern Benin.

Health promotion allows individuals to take responsibility for and to improve their health by acting on the social determinants of health.

It assigns a significant role to volunteers because in order to ensure continuity, it relies on voluntary participation, ownership and empowerment of communities affected by Buruli ulcer, which is a disease of poverty. At each stage in the process, the communities involved have to carry out voluntary actions.

The methodological approach adopted by the Blue Hope project includes an analysis of the epidemiological, clinical and environmental situation of Buruli ulcer in conjunction with a health promotion activity. Four studies of the prevalence of Buruli ulcer have been carried out in the villages covered by the study, with the assistance of teams of volunteers from the villages. This paper describes the important role they played in these surveys.

### **Implementation**

The project, which is scheduled to last 4 years (2009-2013), is a case-comparison study for which 4 villages were selected: 2 pilot villages (Houédota and Azonmé in Allada/Zè/Toffo (AZT) health area) and 2 control villages (Adoukandji Centre and Yamontou in Klouékanmè-Toviklin-Lalo (KTL) health area).

In order to determine the epidemiological and clinical situation of the disease, a survey of prevalence of Buruli ulcer was carried out in each village concerned by the study.

In addition to the principal investigator, the teams of investigators comprised volunteers designated by the villages or chosen (in the case of the community intermediaries) by the referral centres for Buruli ulcer treatment (CDTUB) in each health area.

### **Results**

The prevalence surveys took the form of censuses.

Eleven volunteers collected data on Buruli ulcer. All the volunteers were men, four of them community intermediaries chosen by the referral CDTUB in their health area. Seven volunteers were guides chosen by their villages to help the principal investigator collect data.

Their role was to establish relations with the council of elders and administrative and health authorities of their village, to provide guidance and assistance in the villages and to help establish contact with and secure the collaboration of the families concerned by the survey.

### **Challenges**

- Ensuring the volunteers realize that they are capable of taking responsibility for their own and their community's development;
- Achieving ownership of a health promotion approach by the communities concerned;
- Data collection by the volunteers through a community approach under which they detect cases of Buruli ulcer while respecting the concerns of the inhabitants;

### **Solutions proposed**

- Improvement of the health-promotion skills of the volunteers so as to make the pilot communities autonomous;
- Recruitment of new volunteers, and in particular women, to take part in the action of “Blue Hope”;

**Key word:** Buruli ulcer, health promotion, research-action, volunteers, community intermediaries, participation, community empowerment, prevalence survey.

## **The effectiveness of community and health system supports for BU case detection and treatment: a case study of Obom sub-district in Ghana**

**Presenter: Collins S. K. Ahorlu**

**Authors:** Collins S.K. Ahorlu<sup>1</sup>, Dorothy Yeboah-Manu<sup>1</sup>, Eric Koka<sup>1</sup>, Edwin Ampadu<sup>2</sup>, Isaac Lamptey<sup>3</sup>, Susana Hausmann-Muela<sup>4</sup> and Mark Nitcher<sup>5</sup>

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Like elsewhere in Africa, the establishment of Buruli Ulcer control measures in Ghana is hampered by lack of understanding of many aspects of the disease. However, the recent successful introduction of antibiotic therapy has opened up new opportunities and challenges to control programs. The most important of these are how to get people into early treatment, supporting them to complete the 56-days treatment regimen and continue dressing wounds that do not heal within the period of antibiotic treatment. Moreover, a number of social, cultural and economic factors influences where BU-affected individuals and families seek help from, which requires community level interventions to improve case detection and reporting at clinics. The health facilities also need to be strengthened to provide quality case management services. There is the need to use the current available tools for the benefit of the patients. We are implementing community outreach and screening programmes, providing transportation for confirmed cases to go to the clinic for treatment and gave breakfast to school children before taking them to school. The clinic was supported with laboratory services to confirm cases before treatment is initiated. Preliminary findings show an increased number of patients receiving treatment at the clinic with higher completion rate. The number of early reporting (stages one and two) at the health facility has also increased. This is an indication that we could achieve more with the current available tool for BU control if attention is given to community and health facility supports.

## **Buruli ulcer surveillance in Ghana: Sentinel sites, seasonality, and defining endemic areas.**

**Presenter: Lance A. Waller**

**Authors:**

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Ongoing surveillance of incidence and prevalence of Buruli ulcer (BU) in Ghana includes developments in the role of sentinel surveillance communities. These communities provide local detail enhancing reports to national and district-level surveillance efforts, which in turn provide higher quality data for both disease control and research. In collaboration with the Ghana Health Services National Buruli ulcer Control Programme, we examined the surveillance data from one sentinel surveillance site in Ghana. The data identify local spatial, temporal, and seasonal patterns in BU reporting and illustrate the enhanced capabilities over passive surveillance. Next, we place the local, district, and national efforts in a broader context, by considering the elusive task of identifying areas of high endemic risk. We review existing definitions of endemic areas for (relatively) high prevalence diseases such as malaria and cholera and contrast these definitions with guidelines under consideration for neglected tropical diseases in general and Buruli ulcer in particular.

In summary, we examine both local and global elements of surveillance and reporting in order to highlight the multiple facets, identify shared components, and strengthen collaboration across the intersection of the BU surveillance and research networks.

# **Case confirmation of Buruli ulcer within the context of decentralization: the experience of the IME/Kimpese Buruli ulcer project**

**Presenter: Delphin Phanzu**

**Authors:** Phanzu MD, Imposo BBD, Lukanu NP, Minuku MJB, Kayinua M, Muyembe TJJ, Kiabanzawoko NO, Mundabi B, Nkuku L, Vandelannoote K, Eddyani M, de Jong B and Portaels F.

## **Introduction**

Confirmed diagnosis of Buruli ulcer is increasingly important since specific treatment with antibiotics has become available. Its use after reliable confirmation of cases by the laboratory will make it possible to avoid treating erroneously patients without Buruli ulcer.

In November 2009, a national workshop on the organization of Buruli ulcer control in the Democratic Republic of the Congo was held in Kinshasa. The workshop was attended by the Chief Medical Officer, the Directors of the national leprosy, tuberculosis and Buruli ulcer control programmes, the Director of the National Biomedical Research Institute, international experts from the Tropical Medicine Institute in Antwerp and from the World Health Organization, as well as national experts, investigators and actors involved with Buruli ulcer.

One key resolution concerned the establishment of a national network to provide case confirmation of Buruli ulcer cases in RDC; the pilot phase of this is to be carried out in Bas-Congo province as part of the decentralization of treatment (Table 2) and the experience garnered will be used to extend it to other endemic foci of the disease in the country. The network comprises four levels of competence:

- i.** Local: peripheral laboratories in the case-detection and treatment centres (CDT), the laboratory at the Nsona Mpangu General Referral Hospital (HGR), for collection of samples and initial microscope examinations (microscopy).
- ii.** Regional: the regional laboratory at the IME/Kimpese HGR (microscopy, culture and histopathology).
- iii.** National: the National Buruli Ulcer Reference Laboratory (LNRUB), for confirmation by PCR
- iv.** Supranational: the Mycobacteriology Unit at IMT/Antwerp for external quality control.

In addition, a recommendation was made that 3 samples should be taken from each lesion, the first of them for local examination after direct tap (CDT), another for transport in a transport medium to the IME laboratory and the third for transport in an appropriate medium to LNRUB.

The purpose of this study is to evaluate the results obtained in 2010 using the SWOT (Strengths, Opportunities, Weaknesses and Threats) method in order to make a number of recommendations.

## **Results**

A total of 51 out of 123 notified cases were confirmed with Buruli ulcer, i.e. 41.5%.

I. Laboratories belonging to the TB network (with the exception of the IME/Kimpese General Referral Hospital)

Out of 67 samples analysed there using Ziehl-Neelsen stain (ZN), 26 were positive (38.8%):

- Laboratory at the Nsona-Mpangu General Referral Hospital: 10 samples examined, 8 of them positive;
- Laboratory of the Songololo Referral Health Centre: 30 samples examined, 13 of them positive;

- Laboratory of the Songa Referral Health Centre: 14 samples examined, 3 of them positive;
- Laboratory of the Kasi Referral Health Centre: 13 samples examined, 2 of them positive.

II. Regional Buruli ulcer Laboratory (IME/Kimpese Hospital)

Of 103 samples examined, 40 were ZN positive (38.8%).

III. National Buruli ulcer Laboratory (LNRUB/Kinshasa)

Out of 262 samples examined, 96 were positive under PCR (36.6%)

IV. Laboratory of the Mycobacteriology Unit of IMT/Antwerp

Table 1: External quality control by IMT on 82 samples

<b>PCR</b>	<b>ITM</b>		
	-	+	Total
<b>LNRUB</b>			
-	19	12	31
+	7	27	34
NT	10	7	17
Total	36	46	82

<b>ZNS</b>	<b>ITM</b>		
	-	+	Total
<b>IME</b>			
-	28	11	39
+	8	24	32
(blank)	5	6	11
Grand Total	36	41	82

Table 2: Decentralization of Buruli ulcer case management in Bas-Congo province

MANAGEMENT	PRIMARY			SECONDARY	TERTIARY OR REFERRAL CENTRE
	HP	HC	RHC	GRH	UHC/INRB
Category of lesion	I	I & II	I & II	II & III	III and sequelae
Clinical suspicion /diagnosis	+	+	+	+	+
Swab	+	+	+	+	+
FNA	-	+	+	+	+
Biopsy	-	-	+	+	+
ZSN	-	-	+	+	+
PCR	-	-	-	-	+
HIS	-	-	-	+	+
CUL	-	-	-	+	+
Dressings	+	+	+	+	+
Administration of antibiotics	+	+	+	+	+
Surgery	-	-	+	+	+
POD	+	+	+	+	+
Documentation	+	+	+	+	+

**HP:** Health post; **HC:** Health centre; **RHC:** Referral health centre; **GRH:** General referral hospital; **UHC:** University hospital centre; **INRB:** Institut National de Recherche Bio-Médicale ; **FNA:** Fine needle aspiration; **ZNS:** Ziehl-Neelsen stain, **PCR:** Polymerase chain reaction ; **HIS:** Histopathology; **CUL:** Culture; **POD:** Prevention of Disability

### Strengths

1. Diagnostic network exists and is operational
2. Diagnostic tests are available free of charge
3. Consumables are available (cotton swabs, slides, transport media, etc)
4. Information aids are available (UB 01, UB 02 et UB 03)

**Weaknesses**

1. Low case confirmation rate
2. Failure to comply with the established case confirmation circuit
3. Failure to comply with instructions for case confirmation
4. Mismatch between the number of suspect cases and the number of samples analysed in the peripheral laboratories
5. Slow feed-back

**Opportunities**

1. Strengthening of the national partnership (INRB, provincial Leprosy-Tuberculosis coordinating office, Health zones, IME, etc.)
2. Strengthening of the international partnership (ALM, IMT, WHO, Burulivac etc.)
3. Existing laboratory network of the TB programme with trained laboratory technicians and essential items of equipment and material
4. Provincial health development plan

**Threats**

1. Staff working in the field are under-paid (high turnover among trained health-care staff)
2. Dependence on external funding

**Recommendations**

1. Retrain actors working in the field on case detection and sample collection
2. Strengthen supervisor training rounds
3. Improve communication between the different levels by using new technologies (cell phones, internet)
4. Ensure strict observance of the established circuit and of the instructions on case confirmation
5. Undertake advocacy to ensure effective involvement of the provincial and district management teams

## **Partnership and support for the control of Buruli ulcer at the Institut Médical Evangélique (IME) in Kimpese , Democratic Republic of the Congo (DRC): assessment and prospects**

**Presenter: Désiré Imposo**

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This year marks the tenth anniversary of our control programme. The programme's successful history has been the fruit of active cooperation with national and international partners. Since its inception in 2001 with scant resources, the programme has gradually developed thanks to the decisive support of numerous international partners.

The Institute of Tropical Medicine (IMT) in Antwerp has been providing the programme with support since 2002. It has trained and continues to train staff as well as providing the laboratory with equipment for diagnosis of Buruli ulcer. Its support has made it possible for a physician and a laboratory technician to serve internships at the Institute's microbiology unit. In terms of equipment, our hospital has been provided with a laboratory for culture of the mycobacterium. IMT has been instrumental in introducing IME into a research project on *Mycobacterium Ulcerans* which is supported by the European Union. Under the project, which was initially known as BURULICO and is now known as BURULIVAC, we have acquired other items of equipment. A physician from our Institute has been seconded to the project as an investigator. In future, the PCR laboratory set up at the National Biological Research Institute (INRB) under the project will test our samples, while the IMT in Antwerp will remain our reference laboratory for quality control.

The American Leprosy Mission joined our programme in 2004. Its financial support has been of vital importance in providing case management for patients, in community-based control measures and in strengthening our staffs' skills by means of seminars and training sessions. ALM has made it possible for our programme to acquire the equipment and material necessary for supervision, IEC and case management:

- a cross-country vehicle
- 2 motorcycles
- 60 bicycles
- audiovisual equipment
- cameras
- megaphones
- office equipment: a computer and printer
- consumables

WHO has made a valuable contribution towards capacity building by organizing meetings and seminars, providing recommendations and documentation. In addition, it has provided us with the antibiotics needed to provide decentralized treatment.

The other partners, which include the National Buruli Ulcer Control Programme (PNLUB), the National Tuberculosis Control Programme (PNLT), the Fondation Damien, Médecins Sans Vacances (MSV), the Kimpese and Nsona-Mpangu health areas, etc...) have each and in accordance with their abilities, made their contribution.

This valuable support has been reflected in a transformation of the control programme:

- Between 2001 and 2004, our team treated 220 suspected cases of Buruli ulcer exclusively at the hospital
- From 2005 to 2008, the number of cases treated at the hospital gradually increased and at the same time some cases were treated in the community. After the third quarter of 2008, as a result of the decentralization of activities to the community level, many cases (more than 120 each year) were detected at an early stage in the disease and treated in the community. This process of decentralization should be pursued with the support of all our partners to ensure that the control programme is well integrated into the local communities.

This shows that the partnership that has so far provided support for our programme is the cornerstone of Buruli ulcer control in our region. At this stage, it is essential that the partnership should continue, diversify and wherever possible expand its sphere of action in the Democratic Republic of the Congo so as to cover other areas of the country where the disease is endemic and which are not yet included in a control programme.

## **Raising awareness of Buruli ulcer in the community in Côte d'Ivoire action by the NGO AFRISOL -Côte d'Ivoire in partnership with the institut pasteur of Côte d'Ivoire**

**Presenter: Marie Constance Kadio**

Buruli ulcer is a serious public health problem in Côte d'Ivoire, where more than 30 000 cases were registered between 1978 and 2009. These figures are swollen by 2500 new cases each year. Children aged from 0 to 15 years are those most affected and make up more than 75% of patients, 62.8% of whom have ulcerative forms of the disease.

The disease has numerous consequences for the population. Buruli ulcer is responsible for considerable suffering and disability, particularly where children are concerned, and causes children to fall behind at school, entails costly treatment and loss of productivity in the populations affected by it. These harmful consequences aggravate poverty in the affected communities.

In 2007, after a visit to a centre providing treatment for more than 100 Buruli ulcer patients, the NGO AFRISOL-Côte d'Ivoire, realized the suffering and disability caused by the disease and included Buruli ulcer among its priorities with the aim of controlling the disease at an early stage.

This decision is in conformity with the NGO's objectives of combating poverty and improving the living conditions of populations.

Accordingly, in 2010, AFRISOL-CI has prepared a programme of actions based on improving community awareness and early case detection.

With limited means provided by its members, and in partnership with the Institut Pasteur of Côte d'Ivoire, AFRISOL-CI visited 6 villages in four regions of Côte d'Ivoire identified as being in endemic areas.

In each village AFRISOL-CI organized a campaign to raise awareness as follows:

- One week before the arrival of its team, the area for the campaign was prospected by the NGO's field worker who, in conjunction with the public health worker and local volunteers, announced the team's arrival and identified local suspected cases.
- Subsequently, from the first day of its intervention, the NGO organized sessions to raise awareness and provide early case detection together with an evening showing of a film on Buruli ulcer followed by a discussion
- The second day was devoted to a public meeting to raise awareness, held in the village meeting place and involving neighbouring villages. Workers from the Institut Pasteur provide information on the causative agent of Buruli ulcer (*Mycobacterium ulcerans*) and the impact of the environment on the disease. We provide as much information as possible on the disease (definition, diagnosis, treatment and prevention) before holding a question-and-answer session and a distribution of promotional items, leaflets and clothes collected in France if the NGO has been able to ship them. Finally, we carry out active case detection at the local health centre, where we take samples from each suspected case for examination by the Institut Pasteur for case confirmation.
- After its intervention, the NGO donates drugs and equipment to the local nurse who takes part in case detection, to help with follow-up of patients. Cases with complications are referred either to the Treichville-Abidjan hospital or to the Raoul Follereau Institute in Adzopé (leprosy hospital) - at the expense of AFRISOL-CI.

- Two weeks later, patients are visited by the NGO's medical team for a further evaluation.

**The results of our interventions,**

- More than 5000 people have been informed about Buruli ulcer. They have been told what precautions they should take to avoid contracting the disease and how to halt its evolution thanks to early action.
- The mode of transmission, influence of the environment and causative agent of Buruli ulcer have been better explained to populations thanks to the presence of the Institut Pasteur.
- 28 samples have been taken during the case-detection sessions and 26 cases of Buruli ulcer have been confirmed by the Institut Pasteur.
- The NGO has provided case management for the 26 new patients, some of whom have complications and have been referred to the Treichville-Abidjan hospital or to the Raoul Follereau leprosy hospital in Adzopé.
- Patients have been provided with first aid and dressings on the spot.
- 6 nurses have received supplies of drugs, medical equipment and dressings.
- 26 cases with extensive ulcers - different from Buruli ulcer - such as necrotizing post-erysipela fasciitis have been diagnosed and also treated by the NGO like the cases of Buruli ulcer.

**However, it is vital to stress that:**

- The disease is continuing to spread, especially in villages in the lakes region close to Abidjan
- The populations still need information in order better to control Buruli ulcer.
- Initial treatment of Buruli ulcer by traditional healers is increasingly common in villages and often initially proves satisfactory for patients, who believe that the disease is the result of a curse.
- The populations need medicaments and above all consumable items, which are often out of stock.

**AFRISOL-CI has set the following objectives for 2011,**

1. To extend its interventions to other regions.
2. To continue its cooperation with the Institut Pasteur of Côte d'Ivoire.
3. To continue to seek financial partners to support and develop its activity on behalf of Buruli ulcer control.
4. To develop collaboration with traditional healers in order scientifically to verify, authenticate and recognize the efficacy of their treatments and to promote interventions by them with the aim of improving access to care and reducing the cost of treatment.

**The measures that need to be taken in order fully to control Buruli ulcer are quite considerable** and any initiative or effort made to achieve this deserves support and encouragement; much remains to be done to control this terrible affliction which is gaining ground in Côte d'Ivoire, which has already suffered for several years as a result of a political and military crisis.

No-one can deny that we are all entitled to health and to physical, mental and social well-being regardless of where we live.

**This is especially true for children (who are most affected by Buruli ulcer), who represent the world's future and for whose development and protection we are responsible.**

## Using community volunteers for yaws surveillance and control

**Presenter : Nsiire Agana**

Four communities (Kwame Akura, Nwane, Bawa Akura and Chamba Akura) in the Krachi East District of the Volta Region of Ghana were surveyed by Community Volunteers for yaws using a guide produced by the National Yaws Elimination Program. By this guide each suspected case of yaws was to be represented as having P (for papiloma), Sy ('small yaws' in lay terms for papular and macular forms of yaws) or C (crab yaws). Mixed presentations were allowed. The data tool was emailed to the District Director who printed and gave it to the District Disease Control Officer who photocopied and gave copies to the Disease Control Officers stationed in the five health subdistricts of Krachi East. These then chose communities considered high problem areas of yaws in their subdistricts, oriented the community based surveillance volunteers (CBS) in these communities on the forms and tasked them to register all yaws cases. The CBS are already trained and do surveillance on other health problems but not yaws and they were not specifically trained on yaws for this survey, relying on their local knowledge of the disease. The results were cross checked by the National Yaws Program Manager, a Doctor with experience in yaws, and the District and Subdistrict Disease Control Officers who also have a fair knowledge of yaws. The following findings were made.

Community	CBS diagnosis of papiloma (P)	Cross checked for P	CBS diagnosis of Crab yaws (C)	Cross checked for C	CBS diagnosis of maculopapular yaws (Sy)	Cross checked for Sy	Comments
<b>Bawa Akura</b>	6	1	9	2	49	6	2 crab yaws among the Sy
<b>Chamba Akura</b>	7	2	0	0	37	5	1 p among Sy
<b>Kwame Akura</b>	0	1	1	2	19	3	
<b>Nwane</b>	9	7	3	3	38	9	2 Ps absent

Yaws papilomas and crab yaws were diagnosed with 100% accuracy by the Nwane CBS. However only 9 out of the 38 'small yaws' forms (24%) were confirmed. In Chamba Akura however only 2 of the 7 papilomas (29%) and 5/37 of 'small yaws' (14%) were confirmed. No crab yaws were identified. The performance of the CBS in Bawa Akura was similar to Chamba Akura. 17% of 6 papilomas, 22% of suspected crab yaws and 12% of 'small yaws' were confirmed. In Kwame Akura the CBS could not pick up the only papiloma present, picked up only one of 2 crab yaws and only 3 of the 19 (16%) of small yaws forms suspected were confirmed. In general sensitivity for diagnosing yaws by community volunteers is very high but specificity is variable (high in some communities and low in others). Specificity tends to be high for papiloma and crab yaws and low for the macular and popular forms of yaws which are generally lumped with all other forms of skin disease which were scabies and ring worm in most cases. The findings corroborate closely with findings by the same investigator of the use of guinea worm volunteers for diagnosing yaws in the Tamale Municipality of Ghana in 1994 where there was high positive predictive value mainly for the papiloma forms of yaws and low negative predictive value for ulcerated and maculopapular forms of yaws. In short the classical presentations of yaws (papilomas and plantar or palmar yaws) are more easily recognized by the community volunteers than other forms of yaws. These classical forms help give definite names to yaws in many endemic communities. This was demonstrated in one primary school in Central Region where the pupils said they knew yaws and when asked what it was one pupil called it 'yellow

*mpompor'* (yellow boils). And indeed the pupils referred us to a child in the community with yaws and when we traced, the case was confirmed. Some yaws local names are given below.

*Gyator, Due* (pronounced duway), *Dobe, Dee* and *Edo* in various Akan dialects (Twi, Fante, Abrem, Ahanta, Sefwi, etc.)

*Dobi* by the Chokosis (in Chereponi, Northern Ghana)

*Ekli, Jorbu* and *Atsakpa* (pronounced Achakpa) in various Ewe dialects

*Kunkpasa* by the Kabre people of Togo resident in Ghana

*Mgbangba* by the Kokombas

*Jaga* by some languages and dialects in Northern Ghana.

In conclusion yaws is a disease known in the communities but effective surveillance and control requires training of already existing community based health workers and child caretakers and linkage with trained health workers and treatment facilities.



# **Prevention of disability and surgery**



## **Buruli ulcer sequelae in lower and upper limbs: clinical features, prevention and principles of treatment**

**Presenter: Henri Assé**

**Authors: H. Assé, P. Meredith, A Kouakou-Adonis, A, Yao, N. Assie**

Buruli ulcer (BU) disproportionately affects the lower and upper limbs. In its ulcerative form, localization of BU in the locomotor apparatus and prehensile organs is undeniably a *prima facie* sign of morbidity. As the disease progresses, tissue reshaping processes dominated by fibrosis and the resulting contractures give rise to profound changes in limb morphology and function. These infirmities present various clinical forms that all lead to significant disability.

Although treatment of BU is currently inconceivable without prevention of disability, it has to be acknowledged that many BU patients are overlooked because most national BU control programmes make no real provision for this aspect.

Thus demand for treatment of the disabling sequelae of limbs resulting from BU is on the increase and is becoming a real challenge. The techniques for repairing the sequelae of BU, which draw on the principles of reconstructive plastic surgery and hand surgery, basically comprise three stages corresponding to the step-by-step repair of tissues that have sustained primary or secondary damage as a result of the disease.

Knowledge of the formation of these sequelae and the principles of prevention and treatment must be taught to medical and surgical practitioners to guarantee more effective management of BU.

# **Buruli ulcer functional disability trends in the Ejisu-Juaben municipality of Ghana**

**Presenter: Pius Agbenorku**

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## **Background**

Information on the current functional disability trends caused by Buruli Ulcer Disease (BUD) in the middle belt of Ghana is scarce.

## **Aim**

This retrospective study was carried out to describe the trends and category of functional disabilities caused by BUD and body parts mostly involved.

## **Methods**

Information on Buruli Ulcer (BU) patients at the Global Evangelical Mission Hospital, Apromase, was utilized for the study. Data was retrieved from the WHO form BU1 case registry book, surgical theatre register and BU patients' records book of the hospital. Data obtained included: demographic features of patients, category and location of lesion(s). Data obtained was recorded and displayed in tables and graphs by using SPSS version 16.0.

## **Results**

A total of 336 BU cases [more males (53.9%, N=181) than females (46.1%, N=155)] were recorded during the study period. A total of 113 (33.6%) cases of functional disabilities were identified. The age of the BU disabled patients cut across all the age groups (a mean age of 52.5 years), with the bulk of them fallen within 60-74 years. For the trend of functional disabilities, the year 2009 recorded the highest (30.1%, N=34). The lesions were mostly located at the lower limbs (57.5%, N=65), with few (6.2%, N=7) located in the Head and Neck Region of the patients. Lesions with diameter > 15 cm and usually multiple, at critical sites such as the joints and osteomyelitis, were the majority (59.3%) category of lesions.

## **Conclusion**

The trend of functional disability reveals a proportional increase with increasing years. Impaired range of motion due to contraction at the knee and ankle joints was the highest functional disability recorded. Management difficulties and disabilities caused by BUD could be avoided by early detection of the disease, promoted by intensive health education campaigns.

## **Multicentre study of Buruli ulcer disabilities in the head and neck region**

**Presenter: Pius Agbernorku**

Reconstructive Plastic Surgery & Burns Unit, Komfo Anokye Teaching Hospital, School of Medical Sciences, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana

### **Objective**

To identify the various disabilities caused by Buruli Ulcer Disease (BUD) in the Head and Neck Region (HNR) and suggest possible ways to overcome the complications involved.

### **Methods**

The study involved six different hospitals in the central part of Ghana from 2004 – 2009. The sources of information were the operation registers and the case notes of the patients.

### **Results**

The age of the 38 patients ranged from 0 –56 years. Most (76.3%, N=29) patients had severe and mild ulcers located around the HNR. For deformities, most (55.3%, N=21) of the patients had deep scars around on the eye, neck, cheek and forehead. Few (13.2%, N=5) of them had lost one eyeball.

### **Conclusion**

Visual impairment and psychological effects due to facial scars were the common forms of disabilities. Management difficulties and BUD disabilities could be avoided by early detection of the disease and training of health professionals at district levels.

# **Implementing prevention of disability at the Allada Centre for the Detection and Treatment of Buruli Ulcer (CDTUB): organization and results, 2005 - 2009**

**Presenter: Jean Gabin Houezo**

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## **I. Introduction**

Buruli ulcer is a skin condition caused by *Mycobacterium ulcerans*, a microorganism related to the causative agents of tuberculosis and leprosy. Very little information is currently available about prevention of disability (POD), despite the fact that POD is an increasingly important element in management of the disease. Patients should not only have good scar formation but also maintain good limb function after completion of treatment. Physiotherapy, "the art of healing using all the techniques of movement", is one of a range of therapies to prevent or reduce the functional problems patients are exposed to. All these techniques considered together comprise POD.

In this paper we shall focus on the Allada CDTUB's experience of implementing this essential component of treatment.

## **II. Framework and method**

POD is conducted in a number of areas of intervention of the CDTUB: physiotherapy, inpatient treatment, wound dressing and surgery.

**Upon admission:** an initial evaluation is made of the patient's condition. This evaluation involves a set of assessments, for example of pain, joint and muscle contracture, cutaneotrophic involvement and functional impairment. An assessment form is filled out, after which a course of treatment is prescribed for each patient. At this stage in the process it is very important to organize an interview between the patient and the person supervising their treatment for the purpose of securing the patient's adherence to treatment. This is done by explaining to the patient in simple terms the findings of the various assessments and the various stages of management of the disease. The need to complete the course of treatment in order to avoid functional sequelae at a later stage should be stressed.

**During management of the disease:** actual treatment options will vary according to the individual health post (for example physiotherapy, wound dressing, surgery or inpatient admission)..

The minimum range of activities proposed includes joint movement, joint postures involving progressive muscle and tendon stretching exercises aided by custom-made splints, pressure wraps to decrease oedema, muscle strengthening, proprioception to reposition deformed joints, and gentle massage of scars with shea butter to nourish and soften the skin and thus protect it.

**On discharge:** A final evaluation is carried out involving a comparison with the different assessments carried out before the start of the treatment in order to gauge the difference. At the conclusion of this discharge evaluation, another form is filled out.

**Follow-up:** Following discharge, a follow-up programme is proposed involving periodic assessments to evaluate the patient's functional capabilities. The duration of this follow-up depends on the nature of the patient's lesions.

### III. Results

Table 1: Comparison of functional impairment rates on admission, 2005-2009

Year	2005 (%)	2006 (%)	2007 (%)	2008 (%)	2009 (%)	TOTAL (%)
<b>Admitted with functional impairment</b>	102 (90.3)	112 (81.8)	44 (72.1)	11 (61.1)	44 (66.67)	313 (79.2)
<b>Admitted without functional impairment</b>	11 (09.7)	25 (18.2)	17 (27.9)	07 (38.9)	22 (33.33)	82 (20.8)

Table 2: Comparison of discharge rates without sequelae, 2005-2009

Year	2005 (%)	2006 (%)	2007 (%)	2008 (%)	2009 (%)
Discharged without functional sequelae	27 (62.8)	51 (85)	23 (88.5)	13 (76.5)	46 (90.2)
Discharged with functional sequelae	16 (37.2)	09 (15)	03 (11.5)	04 (23.5)	05 (09.8)

Between 1 January 2005 and 31 December 2009 the physiotherapy unit treated 313 new BU patients. A large proportion of these patients were male (56%) and children under 15 (45%). The proportion of patients admitted for treatment with functional impairments (tightness, contractures, deformity, etc.) is approximately 79% of total admissions during this period. The proportion of patients discharged with no functional sequelae was highest in 2009 (90.2%).

### IV. Discussion

Since being instituted in 2004, POD has progressively improved. Being the final component of disease management implemented by CDTUBs, the effective participation of all units is essential if POD is to be successful. But it has proved difficult to integrate POD into health workers' routines. The various health posts (operating theatre, dressings stations, inpatient wards) have gradually started to work in tandem with the physiotherapy unit.

A comparison of results from one year to the next shows an improvement in the indicators for admissions and discharges, which points to a progressive improvement in the quality of overall care. The first component of POD is early detection in the sense that it contributes to a reduction of disability on admission. Patients are being screened much earlier at the CDTUB and hence there are fewer disabilities on admission.

A number of factors have contributed to this situation. Among the most significant are training of health workers, the availability of premises and equipment that meet proper standards and the progressive improvement of the treatment system.

### Conclusion

Buruli ulcer is a disabling rather than a fatal disease. Over time, POD has become an important component of the treatment offered to patients at Allada CDTUB. Better infrastructure, trained health workers and - most of all - a better organized treatment system and a team-based approach have over time reduced rates of functional impairment on admission and discharge.

## **Problems posed by case management of former Buruli ulcer patients with disabilities living in the community**

**Presenter: Désiré Imposo**

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Case management of Buruli ulcer patients with sequelae has not received much attention from our control programme, and this may also be the case in other countries where the disease is endemic. However, historically, approximately 25% of patients who are cured are left with some form of disabling sequelae. What becomes of them when they return into the community?

From the social angle, those with minor disabilities are more or less well integrated into the community; however, those with more severe disabilities are unable to do so, and become a burden on their family. They are often considered to be the victims of mystical attacks by sorcerers. They themselves are occasionally regarded as sorcerers, which explains why they are stigmatized. Children are unable to continue their education.

In medical terms, these patients expect us to provide a solution to their problem. We meet them during our supervisory rounds and awareness-raising campaigns, but as time goes by there is still no solution to their problems. However, some sequelae can be remedied by plastic and reconstructive surgery. This involves a number of conditions which it is not always possible to satisfy (a surgical team, funding, logistics, etc.).

Financially, these persons are poor and are unable to pay for their treatment. Our control programme has no funds to cover the cost of surgery for them or of social and economic rehabilitation and even less to pay for the education of children.

### **Sequelae:**

These can be avoided or limited by early detection and case management, and by proper POD. However, disabilities do not always result from inadequate case management. Some sequelae are almost unavoidable. This is the case of lesions that are extensive from the outset, of lesions on multiple sites and lesions affecting bones and joints. During the last five years we have registered more than 44 cases of sequelae of different levels of severity from Buruli ulcer which have been treated in our hospital. There are other cases which have been treated in the community. We have focused in particular on a few severe cases which would call for proper case management if possible:

### **Face:**

- one case involving loss of tissue from the left hemisphere
- loss of an eye, incomplete occlusion of the eyelids of the remaining eye

### **Upper limb:**

- cases of elbow contracture

### **Lower limb:**

- cases of contracture of the knee
- leg amputations

We should like to take this opportunity to request from WHO, NGOs and all persons of good will a commitment, a resolution or practical recommendations to ensure that physical, social and economic rehabilitation is actually available and that these oft-forgotten victims of Buruli ulcer receive comprehensive case management.

## **From preventing disabilities caused by Buruli ulcer to community-based rehabilitation (CBR)**

**Presenter: Valérie Simonet**

### **Responses to the issue of the institutional approach in the context of Buruli ulcer**

In almost all countries, prevention of disabilities caused by Buruli ulcer has progressed from an institutional approach to one which is increasingly community-based.

In Cameroon too, it was quickly appreciated that institutional case management in a referral hospital posed a problem of access, cost and sustainability. Consequently, the rehabilitation component of prevention developed towards decentralization, family-based case management, involvement of community intermediaries and the introduction of a circuit to provide information in place of the outreach strategy.

This strategy has borne fruit in a number of ways. Rehabilitation measures have been brought closer to the target population, home-based rehabilitation is often sustainable over time, monitoring activities are generally integrated within routine health system activities from the intermediaries to the district.

### **Further steps: beyond the top-down approach**

We might well content ourselves with what already exists; nevertheless, FAIRMED wanted to benefit from hindsight and to consider the coherence of these measures by examining both Buruli ulcer and leprosy not simply within the narrow context of a top-down programme, but from a broader perspective that includes the multiple physical disabilities.

This bird's-eye view, which puts measures to prevent disability into their global context, allows us to highlight several shortcomings of the current strategy:

- Despite decentralization and the involvement of families and intermediaries, the approach has remained broadly institutional and as a consequence poses once again questions of cost, access, sustainability as well as of equity.
- Decentralization does not guarantee sufficient proximity for professionals to be able to provide rehabilitation themselves with the appropriate frequency and for the time necessary after hospitalization.
- If they support a top-down programme vertical, the financial, organizational and training efforts focus on a single disease. This is costly and therefore difficult for the Government to sustain and jeopardizes sustainability. It also poses the question of equity between those who are supported by the programme and those without the « luck » of having a Buruli ulcer but who have another physical disability for which no form of support exists.
- Interventions focus on a biomedical model in which the priority is physically to « repair » as well as possible the persons concerned before returning them to their community. Scant attention is given to the psycho-social and rehabilitation approach.
- Although intermediaries are involved, community participation is still weak in so far as the community itself does not take responsibility for all the issues involved in the re-education and rehabilitation of people affected by Buruli ulcer. It is certainly very difficult to mobilize a community around a problem which is marginal and whose case-management is moreover already organized by a programme. However, community investment is one of the cornerstones of sustainable action.

### **Beyond Buruli ulcer: a project for integrated disability prevention and rehabilitation**

In order to broaden the approach to preventing disabilities caused by Buruli ulcer, at a workshop organized in September 2010 a start was made on the development of a tripartite project involving FAIRMED, the Ministry of Welfare and the Ministry of Health and which favours the participative approach. The project addressed the following question: why is it so difficult for the population to access a reliable disability prevention system of satisfactory quality?

Examination of this question resulted in an operational plan whose purpose is, inter alia, to develop a national disability prevention and rehabilitation based on two pilot experiments:

1. Development of a network of facilities providing disability prevention in each province
2. Introduction of community-based rehabilitation in a pilot district.

As a whole, the above approaches would provide an appropriate response to concerns about the availability at the local level and more broadly about access to interventions, their equity, cost and community involvement. An institutional, biomedical and top-down approach would shift towards a community-based one taking into account psychological, social and rehabilitation issues. This change could be an opportunity to develop disability-prevention and rehabilitation measures that are sustainable over time.

## **Reliability of assessing limitation of movement (LOM)**

**Presenter:** Linda Lehman

**Authors:** Linda Lehman, Paul Saunderson

### **Brief introduction(background)**

Standard reporting of new cases of Buruli ulcer, using the WHO-BU1 form, includes the question “Limitation of movement at any joint – yes/no?” The aim is to understand in the simplest possible way, how much disability is already present in new cases, which is related to the delay in case-finding. LOM should also be measured again at the end of treatment to give a clear indication if disability management has been successful during antibiotic treatment and if further care is indicated. Because of the important use of the LOM results, it is vital that the measurements themselves are reliable. Although the question appears simple, the reliability of this data has never been studied.

### **What was done (implementation)**

At the start of a POD training sessions and during supervision, we asked health workers to assess limitation of movement in BU patients. Results were compared to a gold standard.

### **What was achieved (results)**

In many training and supervision situations 22 – 25% of persons with LOM may not be identified. In addition, workers have difficulty knowing how to register movement limitations of the eye and mouth as it appeared to not be “Joint Limitation” but Movement Limitation.

### **What were the challenges during the implementation**

LOM is not assessed very reliably in the field; in particular, it may be missed in 20-25% of new cases. Identifying cases with mild LOM are important, as they may be easily corrected by simple interventions; also if not corrected early on, they may lead to chronic disability. In order to make the assessment more reliable and the data reported to WHO more useful, the challenge is to develop some simple guidelines to standardize these procedures.

### **What are your proposed solutions to these challenges**

Rules for examining LOM in BU cases can be established. Health workers can be asked to observe limb and other body part movements of both the affected and non-affected sides together and to compare whether the movements are the same or different. LOM was identified when the BU affected side demonstrated less movement than the non-affected side, whether it is at a joint or other site such as the eye or mouth.

### **–Conclusions**

BU Control programs may find that LOM may not be identified in 20-25% of new cases in the field. In order to improve the accuracy of these assessments, we propose that some simple guidelines be used, and practiced during training and supervision activities. This presentation will give participants the opportunity to see examples of LOM that were not identified.



# Research



# **Environment and transmission**



## Highlights and Review of Transmission and Related Research on Buruli Ulcer Disease for 2010

**Presenter: Rich W. Merritt**

**Authors:** Richard W. Merritt<sup>1</sup>, Janet A. M. Fyfe<sup>2</sup>, Pam L. C. Small<sup>3</sup>, John R. Wallace<sup>4</sup>, Mark E. Benbow<sup>5</sup>, and other members of the WHO Transmission sub-working group

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This presentation will briefly present an overview and significant highlights of published and ongoing research on Buruli ulcer transmission during the year 2010. Several research areas will be discussed including: a) reported risk factors associated *M. ulcerans* infection world wide; b) laboratory studies on interactions of *Mycobacterium ulcerans* with mosquito species and the implications for transmission and trophic relationships; 3) the role of mammals (mainly arboreal folivores) and mosquitoes in the ecology and potential transmission of *M. ulcerans* disease in Australia; 4) seasonal and regional dynamics of *M. ulcerans* transmission and deciphering the role of water bugs as hosts or vectors in Cameroon; 5) specific modeling techniques to assess landscape features, climate, and human occupational activities to understand BU disease dynamics in southeast Australia; 6) evaluation of demographic, environmental and socio-cultural components involved in transmission of *M. ulcerans* from the environment to humans in Benin; 7) an intellectual framework for indicting the roles of living agents as biologically significant reservoirs and/or vectors of pathogens in disease transmission; and 8) recommended transmission research directions on Buruli Ulcer disease;

## Laboratory studies on the ability of *Mycobacterium ulcerans* to infect through open wounds

**Presenter: Heather R. Williamson**

**Authors:** Heather R. Williamson<sup>1</sup>, Maha Aqqad<sup>1</sup>, Lydia Mosi<sup>1,2</sup>, and Pamela Small<sup>1</sup>

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One hypothesis associated with *Mycobacterium ulcerans* transmission is that the bacterium can enter the body through a preexisting wound following environmental exposure. Although several case-control studies have found an increased risk for Buruli ulcer in those who regularly swim or wade through water, experimental studies have not been conducted to support this hypothesis. In an effort to address this question, an experimental model was developed using hairless Harley guinea pigs. Buruli ulcer was induced experimentally in guinea pigs by intradermal inoculation, a method that reproduces similar clinical manifestation and pathology to that produced in human skin. In this experiment, abrasions were made on the backs of hairless Hartley guinea pigs and the animals were exposed topically to *M. ulcerans*. In addition, *M. ulcerans* were injected intradermally as a positive control. Lesions were apparent at the injection site in 5 of 7 guinea pigs within two weeks post infection, and within three months for all 7 guinea pigs. Histopathology of the lesions revealed a large area of necrosis filled with extracellular acid-fast bacilli. Injection sites were positive when assayed via qPCR with high copy numbers of genome units, and *M. ulcerans* were recovered upon culture.

In contrast, all infected abrasions healed within 7 days with no clinical signs of infection during the three-month study. No gross pathology was observed at the abrasion sites, and microscopic histopathology of the abrasion sites was identical to uninfected skin. All abrasions were culture negative, and qPCR analysis is underway. Results from these studies suggest that injection of *M. ulcerans* facilitates the production of Buruli ulcer, and raises questions regarding route of transmission in the environment.

## "Micro-geography of *Mycobacterium ulcerans* and epidemiology of Buruli ulcer in Tandji village Benin.

**Presenter: Pamela Small**

**Authors:** Pamela L. C. Small<sup>1</sup>, Heather Williamson<sup>1</sup>, Ghislain Sopoh<sup>2</sup>, Yves Barogui<sup>2</sup>, Charlie Darr<sup>2</sup>, Rich Merritt<sup>3</sup>, Eric Benbow<sup>4</sup>, John Wallace<sup>5</sup>, Julie Clennan<sup>6</sup>, Lance Waller<sup>6</sup> and Christian Johnson<sup>2</sup>.

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In the past three years we have studied the demography of *M. ulcerans* and Buruli Ulcer in the village of Tandji, Lalo Commune, Benin using active surveillance data, case-to-house mapping, and standard environmental sampling in the village and peripheral work spaces. In this study we have used spatial GIS based analysis of the distribution of *M. ulcerans* and BU patients to identify high risk environments. Our primary study site, the village of Tandji, is comprised of 1288 residents distributed between 5 hamlets. A house-to-house mapping of BU cases validated data provided by the Lalo Health center and showed that there was a 5-fold greater prevalence between the most and least prevalent hamlet. Spatial analysis of sites within each hamlet did not reveal positive clusters of BU cases; however, in one of the largest hamlets, Ganlohoue, a negative cluster was identified. In this cluster the expected number of BU cases was 16, whereas the observed number was 3 (P value=.019). Age-specific data was investigated in the villages of Tchi-Ahomadegbe and Tandji. In both villages over 50% of the cases occurred in children between the ages of 4-12, with a 50% decrease in the 13-15 age group. However, these data were not normalized against the population structure of the villages studied.

The distribution of *M. ulcerans* was examined by analyzing standard samples from water sources, agricultural soil, and invertebrates using qPCR. Data from this analysis identified two "hot spots" in activity spaces surrounding the villages where *M. ulcerans* was identified in water, invertebrate, soil and macrophyte samples. These "hot spots" included an established paddy field, and a woman's bathing area. Some of the samples from these sites contained greater than 100,000 genome equivalents of *M. ulcerans*. Longitudinal data from an established paddy field documented the presence of *M. ulcerans* over a three year period in samples taken during 3 seasons. In contrast, sites taken from within the village were negative for *M. ulcerans* DNA with the exception of 1 invertebrate sample which contained less than 1000 genome equivalents of *M. ulcerans*. Over 40 soil samples collected in a grid from two maize fields were also *M. ulcerans* negative.

In summary, the distribution of *M. ulcerans* and Buruli Ulcer are not randomly distributed even within a small geographic area of less than 20 square kilometers. This information can be useful for helping Buruli Control focus educational and surveillance efforts. Identification of a high risk age group, children between the ages of 4-12, suggests that studies to identify the activity spaces shared by children and *M. ulcerans* could be important for identifying transmission pathways and framing interventions.

## Survey of aquatic bugs' species in Bankim, a new endemic area in Cameroon

**Presenter: Solange Meyin**

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Bankim district located in the Northern part of Cameroon (Adamaoua region: N 06°04'05" E 10°27'37"), has been recently described as a new endemic site in Cameroon. This region benefited from the construction of a dam which considerably modified the environment. Previous collections of some aquatic bugs in this region were shown positive for *M. ulcerans*. But aquatic bugs' biodiversity and biology still poorly documented. In the afore-mentioned context the present study was carried out to identify the commonly occurring medium and large size aquatic bugs fauna and workout their relative abundance, diversity according to type of water bodies and comparing with those trap in the night by light trap.

Insects were collected daily from June 1st to June 30 2010 in ponds formed around dam flooded area, in streams and a river. Light traps made up of a 250 W bulb connected to an electrical generator put in front of a white sheet, were installed from 6 PM to 11PM during one lunar cycle, in 3 sites (near the dam, near habitations and in the forest) in the same month.

We collected 338 aquatic bugs in different water bodies belonging to 6 families. Belostomatidae was numerically the most abundant group constituting of 33.13 % of the total aquatic insects followed by Naucoridae, Ranatridae (27.81%, 18.63%). The other families identified were Nepidae, Notonectidae, and of Gerridae representing respectively 9.46%, 5.91% and 5.02%. All families identified were present in streams and ponds but only two families (Ranatridae and Nepidae) were collected in the river; Among these 338 aquatic bugs, 59.17% (200) were collected in the streams, 38.16% (129) in the ponds and only 9 (2.66%) in the river.

Through the light trap only 2 families were identified among a total of 390 aquatic bugs caught. Belostomatidae, predominant with 80.51% and Notonectidae 19.49%. Notonectidae were caught all along the month and during the full moon, but Belostomatidae were absent during full moon. According to the site of collection, we obtained 25.64% (100) of Belostomatidae and 11.94% (46) of Notonectidae near the dam; near habitations 21.53% (84) of Belostomatidae and 2.56% (10) of Notonectidae and in the forest, 33.33% (130) of Belostomatidae and 5.12% (20) of Notonectidae.

This preliminary entomological survey shows the variation of aquatic bugs' diversity according to the types of water bodies in the same endemic region and according to light attraction and the moon phases.

## Entomological investigations carried out from 2002 to 2010 into the involvement of water bugs (*Heteroptera - Hemiptera*) in transmission of *Mycobacterium ulcerans* to humans in Côte d'Ivoire (West Africa)

**Presenter: Julien Doannio**

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ulcer is a disease caused by a mycobacterium present in the environment: *Mycobacterium ulcerans*. This communicable disease occurs essentially in wet tropical regions, and in particular in west Africa where it is endemic. It is the third most common mycobacterial disease affecting humans after leprosy and tuberculosis, although it is more prevalent than either leprosy or tuberculosis in some rural areas of several countries (Benin, Côte d'Ivoire and Ghana). This has led WHO to act, and in 1998 to declare Buruli ulcer an « emerging disease » and to recognize it as a neglected tropical disease. Its development is a source of concern in Côte d'Ivoire, the country most affected in the world, with an aggregate number of 30 000 cases and more than 2000 cases detected each year. It particularly affects children living in isolated rural areas around bodies of stagnant or slowly flowing water. In order to control the disease, it is essential fully to understand its epidemiology. In this connection, there are several hypotheses on the mode of transmission of *M. ulcerans* to humans. Since 1999, the involvement of water bugs belonging to the order of the hemiptera has been invoked by Portaels. In 2002, this hypothesis was confirmed by Marsollier *et al.* for water bugs of the genus *Naucoris* taken from the region of Daloa in Côte d'Ivoire, where the disease is endemic. In 2008, Portaels also found *M. ulcerans* in samples taken from the environment (Gerridae) in Ghana. In 2007, studies began in Côte d'Ivoire into the specific diversity, biology, ecology, ethology and role of aquatic heteroptera in the transmission of *M. ulcerans* to humans. Samples of aquatic heteroptera were collected each month from different aquatic environments in endemic areas of Côte d'Ivoire. The insects were identified by family, genus and occasionally species. Their distribution, population dynamics and ecological distribution in the water points investigated were correlated with human activities. Monospecific batches of water bugs were regularly composed in order to identify the molecular signatures of *M. ulcerans* using PCR at the bacteriology laboratory of the Institut Pasteur in Côte d'Ivoire and at the bacteriology laboratory of the Groupe d'Etudes des Interactions Hôtes-Pathogènes (Host-Pathogen Study Group) at the University Teaching hospital in Angers, France. Eighteen (18) species belonging to 8 families were identified. After the aquatic insects collected had been identified, 283 monospecific batches were composed and sent to the Institut Pasteur in Côte d'Ivoire (IPCI) for PCR. Twenty four (24) of the 283 batches i.e. 8,5% containing the following, 14 *Diplonychus sp*, 2 *Naucoris sp*, 3 *Micronecta sp*, 2 *Ranatra fusca*, 2 *Anisops sp* and 1 *Laccotrephes ater*, respectively belonging to the

families Belostomatidae, Naucoridae, Corixidae, Ranatridae and Nepidae tested positive under PCR. Thirty five (35) samples of saliva were collected from specimens of the genus *Diplonychus*. Six of the samples (i.e. 17%) tested positive under PCR. Out of 109 other monospecific batches sent to the laboratory in Angers, France, 33 (i.e. 30%) tested positive under PCR. They comprised 11 batches of *Diplonychus sp* (Belostomatidae), 8 batches of *Micronecta sp* (Corixidae), 2 batches of *Laccocoris sp* (Naucoridae), 4 batches of *Ranatra fusca* (Ranatridae), 3 batches of *Anisops sp*, 1 lot de *Anisops sardea* et 1 lot de *Enithares sp* (Notonectidae), 2 batches of *Plea pullula* (Pleidae) and 1 batch of de *Laccotrephes sp* (Nepidae). Clearly, not only is *Diplonychus sp* the genus most commonly found, it is also that most affected by *M. ulcerans*. This justifies the decision to breed this genus in the laboratory since 2008, in order to improve our understanding of its biology and ethology and to standardize physical and chemical parameters so as to determine the best conditions for breeding the insect which would provide an animal model for experimental infections. We have now bred six successive generations in the laboratory. To conclude, although some aquatic heteroptera that host *M. ulcerans* are strictly phytophagous, (e.g. the Corixidae), the great majority of water bugs are carnivorous predators that are hosts and vectors of *M. ulcerans*. The absence of a reliable key for determining the family, genus and species in central and west Africa has led us to draw up an iconographic catalogue to determine the taxonomy of these insects.

## Complex Systems Approach for Studying Buruli Ulcer in Central Ghana

**Presenter: Heidi Hausermann**

In central Ghana, areas of high Buruli ulcer incidence largely correspond with landscapes altered by gold mining activities and deforestation. We believe such land disturbances combine with flooding events to create sites of stagnant water suitable to the presence and growth of *Mycobacterium ulcerans*. We also hypothesize that the degree to which individuals are exposed to *Mycobacterium ulcerans* varies according to individual participation in everyday activities like swimming, collecting water, and farming. We employ interdisciplinary collaboration and a complex systems science framework to understand the dynamic relationships between the environmental characteristics of these endemic areas (e.g. land-cover change and water quality) as well as individuals' everyday activity patterns and spaces. This paper presents an overview of this five-year project, highlighting how we have linked together different methods—from soil sampling to surveys—in an attempt to better understand socio-ecological relationships as they relate to Buruli ulcer outbreaks. At the regional scale, for instance, remote sensing of satellite imagery allows us to track land-cover change and flooding events over space and time. Community-level participatory mapping was used to query land-use change, as well as the seasonal spatial distribution of stagnant water bodies and perceptions of contamination. These maps help us identify possible *Mycobacterium ulcerans* habitat and Buruli ulcer risk areas. They also serve as the basis from which we develop sampling plans to test water and soil quality. At household and individual scales, we conduct surveys with Buruli and match cases to understand how different behaviors and activity patterns might influence infection. This paper also presents preliminary results from survey data, which resonate with existing scholarship to suggest increased risk of infection is associated with unprotected exposure to stagnant water. Finally, we also discuss the educational and outreach components of this project.

Key words: Buruli ulcer, central Ghana, complex systems science, mixed methods

## **Identify Associations of Behavioral Patterns with BU in Hopes of Better Elucidating Transmission Modes.**

**Presenter: Gyasi Samuel**

### **Background**

*Mycobacterium ulcerans* disease, or Buruli ulcer (BU), is an indolent, necrotizing infection of skin, subcutaneous tissue and, occasionally, bones. It is the third most common human mycobacteriosis worldwide, after tuberculosis and leprosy and is characterized by subcutaneous tissue necrosis giving rise to chronic, progressive ulcers. The primary risk factor associated with Buruli ulcer is proximity to slow moving water and exposure to wetlands.

### **Methodology/Principal findings**

This work is prelude to a main research that seeks to investigate environmental distribution of *M. ulcerans* in some selected communities in the Amansie west district (Ghana), relate to some heavy metals as well as study behavioral patterns in a hope of better elucidating transmission modes. In this paper, a total of 400 respondents were randomly selected from 6 systematically chosen communities based on their Buruli ulcer case endemicity. With the help of questionnaires, data was collected followed by focus group discussions. Statistical analysis using respondent's demographic data revealed that when the studied population were stratified based on age and endemicity, the proportion of subjects who fall within the age group 16-20 were significantly higher ( $p = 0.0017$ ) among the endemic area (15%) as compared to the non-endemic area (5.5%). However, the adult population (i.e. age > 50 years) was more common among the non-endemic area compared to the endemic. Majority of the subjects interviewed for the study in the endemic communities believe water to be a possible mode of transmission (29.0%) which was significantly higher (13.5%) than those in the non endemic communities ( $p=0.0002$ ) and this was twice as much more than their counterpart in the non endemic population. Socioeconomic status when assessed revealed that greater proportion of study participants in endemic areas (43.5%) trekked to farm barefooted. A greater proportion of children in the study population in endemic areas (43.5%) also trek to farm barefooted compared to their non endemic counterpart (8.5%) and this was significant. ( $p=0.0001$ ). When domestic hygiene was compared based on what children in the study population wore to fetch water, it was revealed that about two times more children in the endemic areas (45.5%) trekked to fetch water barefooted compared to children in non-endemic areas (24.5%) (OR = 2.6;  $p = 0.0001$ ) with more women from the endemic area (9.0%) occasionally walking barefooted doing domestic chores in house as compared to those in non-endemic areas (6.5%).

### **Conclusions**

Results from this work suggests that people living in endemic communities with low socioeconomic status could be at risk of having infection. These findings support earlier reports that BU is a disease of the poor and rural dwellers and the fact that focal demography, along with patterns of human water contact, may play a major role in transmission of Buruli ulcer.

# **Diagnosis and Pathogenesis**



# Use of VNTR to detect *M. ulcerans*: its value in laboratory diagnosis of Buruli ulcer

**Presenter: David Coulibaly**

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## Introduction

Buruli ulcer is an endemic skin infection in Africa. Confirmation of cases of the disease and of the presence of the mycobacterium is of vital importance for early case management and control measures thanks to identification of the disease reservoir. This is especially true in low-income countries which are those most affected by the disease and are beginning to set up molecular biology laboratories. One or several targets are used for molecular detection of the disease pathogen. These include insertion sequences, minisatellites, and genes coding for the toxin produced by *M. ulcerans*. A study was conducted to assess the value of using minisatellites as a target for laboratory confirmation of Buruli ulcer cases.

## Material and Methodology

This preliminary study was carried out using a total of 29 samples made up of 9 strains isolated at the Tuberculosis and Atypical Mycobacteria Unit at the Institut Pasteur in Côte d'Ivoire and 20 clinical samples. The samples were subjected to conventional PCR.

The targets used to detect the mycobacterium were the IS2404 and IS2606 insertion sequences, widely used for clinical and environmental diagnosis of the mycobacterium, and three genetic markers: VNTR1, ST1 and VNTR19 used in molecular typing of the bacterium. On account of their high sensitivity and specificity, these markers have previously been used to detect *M. ulcerans* in environmental samples.

Samples that tested positive for both the IS2404 and IS2606 targets were compared with the results obtained using minisatellites markers.

## Results

Of the 29 samples analysed, 24 (9 strains and 15 clinical samples) i.e. 83% were positive for both IS2404 and IS2606. As regards the minisatellites markers, all the strains contained both the ST1 and VNTR19 markers, while just one strain carried the VNTR1 marker. It was not possible to amplify this VNTR1 marker in the clinical samples, 5 of which (25%) contained both the ST1 and VNTR19 markers.

## Conclusions

This preliminary study shows that the IS2404 and IS2606 insertion sequences are targets of choice for molecular diagnosis of *M. ulcerans*. The ST1 and VNTR19 minisatellites could be used in additional complementary tests for laboratory confirmation of the presence of *M. ulcerans* in a sample, regardless of its nature.

**Key words:** Buruli ulcer- *Mycobacterium ulcerans*- molecular diagnosis- minisatellites- Côte d'Ivoire.

# Report on second quality assessment program for molecular detection of *M. ulcerans* in environmental samples

**Presenter:** Caroline Lavender

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## Background

Quality Assessment Programs (QAPs) are performed to assist laboratories maintain high standards. They enable participants to check that samples are being processed correctly, that results are being appropriately recorded, and that assays are performing and being performed in an accurate and reproducible manner. In 2008, the WHO Collaborating Centre for *Mycobacterium ulcerans* in Melbourne, Australia, coordinated the first QAP for the detection of *M. ulcerans* in environmental samples. Here we present the results of the second QAP conducted in 2010.

## Objectives

The objectives of the QAP were to enable each participating laboratory to:

- Assess its performance in detecting *M. ulcerans* in environmental samples;
- Identify problems with DNA extraction protocols and PCR assays;
- Develop and maintain strategies to improve and maintain the quality of testing; and
- Be part of a network for exchanging information, troubleshooting etc.

## Program overview

Eight laboratories in Belgium, Cameroon, Côte d'Ivoire, France, French Guiana, Ghana, Switzerland and the USA took part. Five of these laboratories participated in the first QAP in 2008, while three laboratories were participating for the first time. Participants were sent 10 heat-sterilised environmental samples (including soil, pond water, pond algae and animal faeces) and six DNA extracts. Participants tested the samples using the DNA extraction and/or PCR methods they routinely use for environmental samples in their own laboratories.

## Results

At the time of writing, seven laboratories had returned results. There was moderate agreement between the expected results and participants' results for the 10 environmental samples (Table 1). Of those laboratories that obtained incorrect results, more reported false negatives (suggestive of poor DNA extraction efficiency, low PCR sensitivity and/or PCR inhibition) than false positives (suggestive of cross-contamination). There did not appear to be any correlation between participants' results and sample type, DNA extraction method, PCR method/target, laboratory type, or number of environmental samples tested per year.

With regard to performance over time by laboratories that also participated in the 2008 program, three laboratories had similar or improved scores than previously, while one laboratory reported a higher number of false positives in 2010. One laboratory did not test any samples in 2010 so no comparison could be made.

The results of the DNA extracts were omitted from the analysis as the DNA appears to have been degraded during transport.

### Conclusion

The results of this QAP indicate that continued work is needed to ensure the sensitivity and specificity of molecular testing of environmental samples for *M. ulcerans*.

**Table 1. 2010 environmental QAP results by laboratory**

Lab ID	No. correct results/ no. samples analysed	No. false positives	No. false negatives	Laboratory type	No. environmental samples tested per year
A	10/10 (100%)	0 (0%)	0 (0%)	Reference	Less than 100
B	No samples analysed	NA	NA	Other	None
C	5/10 (50%)	1 (10%)	4 (40%)	Reference	Less than 100
D	10/10 (100%)	0 (0%)	0 (0%)	Research	100 to 500
E	10/10 (100%)	0 (0%)	0 (0%)	Research	Less than 100
F	8/10 (80%)	2 (20%)	0 (0%)	Research	Less than 100
G	7/10 (70%)	0 (0%)	3 (30%)	Reference	Less than 100
H	Testing in progress	NA	NA	Hospital	100 to 500

# Laboratory Examination of Buruli Ulcer in Japan

**Presenter: Kazue Nakanaga**

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## Background

The first case of Buruli ulcer in Japan was reported in 1980 of a 19-year-old Japanese woman who had never been abroad. The causative agent is not fully equal with *Mycobacterium ulcerans* but closely related to “*Mycobacterium ulcerans* subsp. *shinshuense*” Since then, the reported number of cases in Japan were gradually increased, and 19 cases in total by Dec. 2010. Unfortunately, the Buruli ulcer in Japan have a risk of being late for diagnosis because usual Japanese dermatologists and surgeons are unaware Buruli ulcer or recognize Buruli ulcer as tropical disease. For making an earlier diagnosis in the future, we conducted a validation study on these all 19 cases and clinically comparable cases about laboratory examinations for differential diagnosis.

## Materials and Methods

All the subjects are received at Leprosy Research Center from 2006 to Dec. 2010. 1. Clinical samples of the patient suspected as Buruli ulcer: There were 15 frozen or chilled skin biopsy tissue (from 13 cases), and 10 thin sections of formalin fixed paraffin embedded skin biopsy tissue (from 10 cases) included. 2. Isolates: Ten isolates, which were successfully isolated from the cases of Buruli ulcer in Japan, and additional 5 strains of mycobacteria, which were isolated from the suspected patients.

Laboratory diagnostic tests were as follows: 1. Bacterial isolation test (with frozen or chilled skin biopsy sample) , 2. PCR test targeting IS2404 (154bp), 3. 16S rRNA gene sequencing, 4. Urease test, and 5. DDH test (Commercially available identification test of 19 mycobacteria by DNA-DNA hybridization)

## Results and Discussion

The bacterial isolation was successful in 11 cases (11/19, 58%). The isolation period was variable (the shortest took 4 weeks and the longest 11 months, median 6 weeks). Although it is important for the drug susceptibility test and for further research, bacterial isolation test was not fit for early diagnosis because of its low rate of success and taking long period. PCR test targeting IS2404 was positive for the DNA of frozen or chilled skin biopsy tissue of 11 cases (11/15, including 10 Buruli ulcer cases, positivity 100%). This PCR test was considered to be most important for the early diagnosis. The equal PCR test was also positive for 8 samples of formalin fixed paraffin embedded skin biopsy tissue (8/10, including 8 Buruli ulcer cases, positivity 100%). This PCR test with paraffin embedded skin sample is proven to be very useful for retrospective analysis. All 10 isolates have the identical 16S rRNA gene sequences which were virtually similar with those of *M. ulcerans*. Only different sites between them were the 492, 1288, 1449-1451 (*E. coli* positions), the nucleotide of those positions were A, C, TTT in *M. ulcerans* (African strain), and were G, G, --- in “*M. ulcerans* subsp. *shinshuense*”. All 10 isolates have apparent urease activities though they are not detected with *M. ulcerans*. Of the additional 5 mycobacterium strains , one is identified as *M. peregrinum*, and the rest of 4 are identified as *M. marinum*. The results of the DDH tests were *M. marinum* with all 9 isolates tested. Both “*M. ulcerans* subsp. *shinshuense*” and *M. ulcerans* were not included in defined 19 species of DDH test, misdiagnosis have been possibly occurred in clinical assay.

## Conclusion

PCR test targeting IS2404 was considered to be the most important test for the early diagnosis, and also for the retrospective analysis. However, if we cannot solve the problem that usual dermatologists in Japan are lacking awareness of Buruli ulcer, we cannot achieve early diagnosis.

# Inaugural Report of the WHO BU Laboratory Network Working Group

**Presenter: Françoise Portaels**

**Authors:** Miriam Eddyani<sup>1</sup>, Caroline Lavender<sup>2</sup>, Françoise Portaels<sup>1</sup>

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## Background

In March 2010, the WHO Buruli ulcer Initiative established six working groups to advance work on key Buruli ulcer (BU) priority areas. The Laboratory Network Working Group was formed to strengthen work on laboratory confirmation of cases, including capacity building, quality control and reporting of activities.

## Aim

To serve as a platform for coordination and communication among all laboratories involved in the diagnosis of BU to advance the work on laboratory confirmation of cases and related activities.

## Terms of reference

1. Provide support to national control programmes to confirm clinical cases and to studies involving laboratory detection of *M. ulcerans*;
2. Provide global policy guidance on appropriate laboratory techniques and best practice;
3. Assist with laboratory capacity development, including training;
4. Coordinate annual laboratory quality control programmes for clinical specimens;
5. Coordinate standardized reporting of activities relating to the confirmation of BU cases to the WHO;
6. Monitor drug resistance; and
7. Liaise with the other Working Groups to ensure coherence in activities.

## Membership

<b>Country</b>	<b>Laboratory</b>	<b>Representative(s)</b>
Australia	Victorian Infectious Diseases Reference Laboratory (VIDRL), Melbourne	Janet Fyfe Caroline Lavender
Australia	Queensland Mycobacterium Reference Laboratory (QMRL), Brisbane	Jim Psaltis
Belgium	Institute of Tropical Medicine (ITM), Antwerp	Miriam Eddyani Françoise Portaels Bouke de Jong
Benin	Mycobacterial Reference Laboratory, Cotonou	Dissou Affolabi
Cameroon	Institut Pasteur, Yaounde	Sara Eyangoh
Central African Republic	Institut Pasteur, Bangui	Fanny Minime-Lingoupou
<b>DRC</b>	Institut National de Recherche Biomédicale	Anatole Kibadi Kapay

<i>Country</i>	<i>Laboratory</i>	<i>Representative(s)</i>
	(INRB)	Léontine Nkuku
Côte d'Ivoire	Institut Pasteur, Abidjan	Solange Kakou Ngazoa
France	Universitaire d'Angers, Angers	Laurent Marsollier
French Guiana	Institut Pasteur de la Guyane, Cayenne	Anne-Sophie Drogoul
Germany	University Hospital, Ludwig-Maximilians University, Munich	Marcus Beissner Gisela Bretzel
Ghana	Noguchi Memorial Institute for Medical Research (NMIMR), Accra	Anthony Ablordey Diana Ackon Amissah
Ghana	Noguchi Memorial Institute for Medical Research (NMIMR), Accra	Dorothy Yeboah-Manu
Ghana	Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi	Nana Yaa Awua-Boateng
Ghana	Komfo Anokye Teaching Hospital (KATH), Kumasi	Richard Phillips
Japan	Leprosy Research Centre, National Institute of Infectious Diseases, Tokyo	Kazue Nakanaga

### Activities

In its first year, the Group focussed on the development of a standardised report form to capture information on the types of laboratory methods used (e.g. PCR, direct smear examination [DSE], culture, histology) and the number and types of diagnostic specimens submitted for testing (e.g. fine needle aspirate [FNA], swab, tissue).

At the time of writing, members had completed the form for samples tested in their laboratories during the first half of 2010 (January to June) and were in the process of completing the form for samples tested in the second half of the year (July to December). A summary of the results compiled from all laboratories for the first half of 2010 is below, however it should be noted that the positivity rates for all methods varied greatly between laboratories.

- 1010/2241 (**45%**) samples tested were **PCR positive**
- 499/1916 (**26%**) samples tested were **DSE positive**
- 142/900 (**16%**) samples tested were **culture positive**
- 794/1609 (**49%**) patients\* tested were **PCR positive**
- 416/1454 (**29%**) patients\* tested were **DSE positive**

\* A patient was defined as any person from whom a specimen had been collected for laboratory investigation of BU, regardless of the degree of clinical suspicion.

The full report for 2010, with an analysis and discussion of the results, will be available for the meeting in March and copies may be obtained from the WHO website.

The group will meet in March to discuss and plan activities for 2011, which may include training sessions, plans to decentralise microscopy and a second round of external quality assurance (EQA) for PCR.

## Report of activities carried out in 2010 by the National Buruli ulcer Reference Laboratory in the Democratic Republic of the Congo

**Presenter : Anatole Kibadi Kapay**

**Authors:** KIBADI Kapay ; MUNDABI Pemba PEMBA Bibiche, NKUKU Léontine, KOEN, NKWEMBE Edith, MBALA Placide, LUNGUYA Octavie, PHANZU Delphin, IMPOSO Desiré, KINZOZOLO Mungindu, SINGA Jackie, TIENDREBEOGO Alexandre, PORTAELS Françoise, MUYEMBE Tamfum

*The Buruli ulcer Unit or National Buruli Ulcer Reference Laboratory (LNRUB) was established at the National Institute for Biomedical Research (INRB) in 2005 on the recommendation of the First National Congress on a Policy to Control Buruli Ulcer (BU) in the Democratic Republic of the Congo, which was organized between 27 and 29 September 2004 by the Ministry of Health and WHO. In 2010 LNRUB received 347 specimens of which 109 (31.4 %) were confirmed by PCR assay as *Mycobacterium ulcerans* infections. The following table summarizes the test results:*

Description	Province of origin of specimens	Number of specimens	%
Specimens received by province: Total: 347	1.- Bas Congo	260	74.92 %
	2.- Maniema	63	18.15 %
	3.- Kinshasa	23	6.62 %
	4.- Equateur	1	0.28 %
<b>PCR positive</b> results by province Total: 109 ( <b>31.4 %</b> )	1.- Bas Congo	100	91.74 %
	2.- Maniema	8	7.33 %
	3.- Kinshasa	1	0.91 %
	4.- Equateur	-	-
Distribution of PCR positive results by sex		Female (58)	53.31 %
		Male (51)	46.78 %
Distribution of PCR positive results by age	< 6 years	12	11 %
	6-15 years	34	31.19 %
	> 15 years	62	56.88 %
	Undetermined case	1	0.91 %

### Research and training activities

- 1) A BU seminar was organized at the IME-Kimpese Hospital in collaboration with INRB
- 2) Two seminars (training sessions) on PCR were organized at INRB by the Antwerp Institute of Tropical Medicine in collaboration with INRB
- 3) A BU case confirmation mission was carried out in Maniema Province in collaboration with the National Buruli Ulcer Control Programme, WHO and INRB
- 4) Training sessions.

## Case confirmation by molecular diagnosis at the Institut Pasteur in Côte d'Ivoire (IPCI) in 2010

**Presenter : Solange Kakou-Ngazon**

**Authors:** Kakou-Ngazon E. Solange, Aka N'Guetta, N'Golo Coulibaly David, Sangare Flany, Mambe Perpetue, Aouassi S., Dosso Mireille

Infection of the skin by *Mycobacterium ulcerans*, which is responsible for Buruli ulcer, was discovered in Australia by MacCallum in 1948 (1). The first observations in Côte d'Ivoire date from 1978, when Perraudin reported the first case in the country in a young Frenchman who had spent some time in Côte d'Ivoire. After 1989, the disease has developed into an epidemic, to such an extent that in 1995 the political and health authorities set up the national mycobacterial ulcer control programme (PNUM). Buruli ulcer is still a health problem in Côte d'Ivoire despite the development of a national control programme and the efforts made to eradicate the disease by the World Health Organization. In 2009 more than 2000 cases were diagnosed and confirmed by PCR at IPCI, in conformity with the recommendation by WHO that 50% of cases should be confirmed by this method.

In order to meet this target for confirmation of 50% of cases of Buruli ulcer, IPCI, which is the national reference laboratory, received thanks to the collaboration of its partners (NGO Afrisol, Guinea), numerous samples from ulcers on suspected Buruli ulcer patients.

### Material and methods

PCR makes it possible to detect the presence of *M. ulcerans* DNA in clinical samples using the IS2404, IS2606 et ketoreductase (KR) targets. 74 swabs from patients (28 from Côte d'Ivoire and 46 from Guinea) sampled from ulcers during the campaign period were sent to the laboratory at the molecular biology unit. After extraction of nucleic acids using the Nuclisens Kit (Biomérieux), 5 µl of DNA were tested in the different PCR panels. These involved four PCR tests: classical nested IS2404 PCR, IS2606, and real time PCR IS2404 and KR (2-4).

### Results and Conclusion

A sample is considered positive if at least 2 of the 4 PCR tests are positive. In the case of Côte d'Ivoire, 78.5% (22/28) of the samples from the Afrisol NGO campaign to raise awareness and collect samples were positive. In the case of Guinea, 30.4 % (14/46) were positive and 70% suspected cases.

To summarize, in 2010, 48.6% of the samples sent to IPCI were found to be positive by PCR case confirmation. Thanks to the involvement of NGO volunteers it was possible to confirm cases of Buruli ulcer in Côte d'Ivoire and in Guinea. In contrast to previous years, in 2010 samples for case confirmation by the National Buruli Ulcer Control Programme have not yet been sent to IPCI despite the introduction of laboratory diagnosis by PCR and the availability of the necessary skills. Transport of samples needs to be improved to provide case confirmation in Guinea and to offer reliable laboratory diagnosis. Additional efforts are also needed via awareness raising campaigns in order to control and eradicate *M. ulcerans* infection in Africa.

## **Direct Chemical Detection of Mycolactone Detection in human Patients as a Diagnostic test**

**Presenter: Pamela Small**

**Authors:** Pamela Small, Thomas Spangenberg, Kate Jackson, Yoshito Kishi, Dorothy Yeboah-Manu, Lydia Mosi and Fred Sarfo.

**Abstract:** A workshop was held at Noguchi Institute of Medical Sciences in order to evaluate a new diagnostic method for Buruli Ulcer based on chemical detection of mycolactone in patient samples. Nine participants from Benin, Côte d'Ivoire, and Ghana brought patient samples to the workshop including 49 swabs, 39 swabs in transport media, 14 samples in ethanol, 15 needle biopsies and 29 tissue samples. Many of the samples however were from treated patients. Bacterial pellets of *M. ulcerans*, uninfected human tissue spiked with mycolactone and uninfected human biopsy material were included as controls. Results from this workshop show that the technique can be readily taught as all 9 participants successfully isolated mycolactone from spiked human tissue.

A wide variety of patient samples were submitted for analysis: 37 were analyzed during the workshop. Mycolactone was not detected in 26/37 samples tested. Of these negative samples 13 were from treated patients, and the remaining negative samples were from untreated patients. Mycolactone was tentatively identified in 11/37 samples. Nine of the mycolactone positive samples were from untreated patients, 1 was from a patient treated for 12 days, and the treatment status of one patient was unclear. Six of the positive samples were submitted as swabs. Out of 13 swabs submitted from untreated patients, 6 were positive. Extraction of mycolactone from dry swabs was particularly effective. Mycolactone was also detected in 2 fine needle aspirates submitted in very small volumes and 2 biopsy samples. Control samples were also successfully analyzed: mycolactone was not detected in normal skin, whereas detection of mycolactone in spiked human skin was highly successful.

With regards to human samples, mycolactone was detected most often in samples which also contained numerous other lipids, whereas "clean" extracts were generally not only devoid of mycolactone, but lacked other lipids as well.

Dry swabs as well as biopsy material in ethanol were the best type of samples for use with this method. The use of transport media introduced technical problems and this type of sample is not ideal for mycolactone extraction.

This workshop demonstrated the feasibility of direct detection of mycolactone in human tissue as a diagnostic for *M. ulcerans*. Further work must be done now to map the distribution of mycolactone in tissue in order to develop optimal methods for obtaining samples for mycolactone detection. In addition, further development of biochemical methods could be used to improve the sensitivity of the procedure as well as to streamline the technique. Workshop attendees included: Ange Dissou, Buruli Ulcer Center, Allada, Benin; Henri Assi, Ministry of Health, Cote d'Ivoire, Emmanuel Kacou, Centre de Santé St Michel de Zoukougbeu, Cote d'Ivoire, Pierre Marie Akochy, Institute Pasteur, Cote d'Ivoire; Enoch Aninagyei, Amasaman District Hospital, Ghana, Henry Awusu, Agogo Presbyterian Hospital, Ghana, Fred Steven Sarfo, KCCR, Kumasi, Ghana, Nana Yaa Awua-Boteng, KCCR, Kumasi, Ghana; Michael Erimpong, KRRC, Kumasi, Ghana; Edwin Ampadu, Ministry of Health; Tony Ablordey, Diana Amisah Ackon, Phyllis Addo, Alfred, Dodoo, Charles Quaye, Amelia Danso, Daniel Boakye, Zand Zulichatu Nokobu, Noguchi Memorial Institute of Medical Science, Accra Ghana

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## **Sero-reactivity to the 18KDa small heat shock protein of *M. ulcerans*: is it an indication of BU endemicity?**

**Presenter: Dorothy Yeboah-Manu**

**Authors:** D. Yeboah-Manu<sup>1</sup>, K. Roeltgen<sup>2</sup>, W. Opare<sup>3</sup>, K. Asan-Ampah<sup>1</sup>, A. Asante-Poku<sup>1</sup>, Z. Nakubo<sup>1</sup>, E. Ampadu<sup>3</sup>, K. Koram<sup>1</sup>, C. Ahorlu<sup>1</sup> and G. Pluschke<sup>2</sup>.

<sup>1</sup>Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, <sup>2</sup>Swiss Tropical and Public Health Institute and University of Basel, <sup>3</sup>National Buruli ulcer Control Programme, Disease Control Unit - GHS, Accra, Ghana

Previous analysis with sera of a limited number of Buruli ulcer (BU) patients, household contacts and individuals living in BU non-endemic regions have indicated that antibody responses to the 18KDa small heat shock protein (shsp) reflect exposure to *M. ulcerans* (Diaz et al., 2006). To investigate this further, we have tested 482 human sera collected from people aged between 5 and 90 years living in BU endemic and non endemic communities along the Densu river of Ghana. Sera were analyzed both by Western blot and ELISA for antibody responses against the 18 kDa shsp. Additionally, we analysed 99 sera from people living in the BU non-endemic Volta region of Ghana and 20 sera from European controls.

0% (0/20) of the sera from the European controls and only 12% (12/99) of the sera from people living in the non-endemic Volta region were positive for anti-shsp IgG. The positivity rate for people living in the Densu river basin was significantly higher, but did not differ for non-endemic and endemic villages (30% versus 33%, respectively). Generally, the positivity rate in children was higher than in adults.

It remains to be investigated, whether immune responses found in individuals from the non-endemic Volta region are caused by exposure to environmental mycobacteria other than *M. ulcerans*. The observation that positivity rates in endemic and non-endemic communities along the Densu river valley were comparably high, may speak for exposure to immunologically cross-reactive strains with different virulence or for co-factors enhancing susceptibility to disease in endemic villages.

*Diaz D; Döbeli H; Yeboah-Manu D; Mensah-Quainoo E; Friedlein A; Soder N; Rondini S; Bodmer T; Pluschke G. Use of the immunodominant 18-kiloDalton small heat shock protein as a serological marker for exposure to Mycobacterium ulcerans. Clinical and Vaccine Immunology: 2006;13: 1314-21.*

## Comparative genomics of *Mycobacterium ulcerans*: small changes, big impacts

**Presenter: Tim Stinear**

**Authors:** Sacha J. Pidot<sup>1</sup>, Ken Doig<sup>1</sup>, Torsten Seemann<sup>2</sup> and Tim Stinear<sup>1</sup>

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To gain deeper insights into the evolution of pathogenesis in *M. ulcerans* and to address the key research priorities of understanding transmission and developing new diagnostics for Buruli ulcer, we have now sequenced 24 *M. ulcerans* (including recently described mycobacteria that produce mycolactones) and five *M. marinum* strains from around the world. By focusing our analysis on subsets of strains from local BU endemic regions we have been able to identify single nucleotide polymorphisms (SNPs) for high-resolution molecular typing that link *M. ulcerans* in humans with a potential terrestrial animal reservoir. We have also developed a novel sequence comparison method that has allowed us to objectively identify DNA sequences that are found in all *M. ulcerans* strains but not closely related *M. marinum* strains. We have used these data to find genes encoding potential specific antigens and to demonstrate their application in BU seroepidemiology. One of the most interesting outcomes of our multi-strain comparative approach has been to infer a most recent common ancestor of all *M. ulcerans* strains, which has highlighted a relatively limited pool of chromosomal changes that distinguish *M. ulcerans* from *M. marinum*. We reasoned that these changes are likely to be important for the specific pathogenicity of *M. ulcerans*. Indeed, experiments to test hypotheses generated from these comparisons have shown that both gene acquisition and point mutations have led to the distinctive pathogenicity of *M. ulcerans*. For example, point mutations within the promoter region of a gene encoding the 18kDa heat shock protein antigen cause constitutive expression of this protein among all *M. ulcerans* strains and confer a significantly enhanced biofilm-forming phenotype on these strains. Multi-strain, comparative genomics is providing deep insights into how *M. ulcerans* is evolving and causing disease.

## Mycolactone targets epithelial cell control of adhesion and motility

**Presenter: Caroline Demangel**

**Authors:** Laure Guenin-Macé<sup>1</sup>, Romain Veyron-Churlet<sup>1</sup>, Fabien Le Chevalier<sup>1</sup>, Nicolas Blanchard<sup>2</sup>, Anne-Caroline Chany<sup>2</sup>, Virginie Casarotto<sup>2</sup>, Hui Hong<sup>3</sup>, Peter L. Leadlay<sup>3</sup>, Timothy P. Stinear<sup>4</sup>, Marie-France Carlier<sup>5</sup> and Caroline Demangel<sup>1</sup>

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Here we explored the impact and molecular basis of mycolactone on the epithelial cell cytoskeleton. In HeLa cells, mycolactone induced the formation of microspikes and impaired the cell capacity to adhere and migrate, indicating a possible interference with the Cdc42/Rac GTPase signalling pathway. Proteins of the Wiskott Aldrich Syndrome protein (WASp) family were considered, as they mediate Cdc42 signals to the actin cytoskeleton via their Cdc42/Rac interactive binding (CRIB) domains. Notably, mycolactone was able to bind significantly and high affinity the CRIB domain of N-WASp, to modulate its actin-polymerization activity. Structural variants of mycolactone and recombinant domains of the target protein allowed us to delineate the interacting domains in each partner. Although ubiquitously expressed by mammalian cells, N-WASp plays key functions in cutaneous tissues, where its deficiency causes spontaneous ulcerations. Our data strongly suggest that mycolactone targets N-WASp *in vivo*, with consequences on skin tissue cohesion and repair.

## 'Is *M. ulcerans* able to colonize neuronal cells?'

**Presenter:** Estelle Marion

**Authors:** E. Marion, C. Deshayes, C. Dantec, E. Garcion, T. Stinear L. Letournel, J. Eyer, L. Marsollier.

Buruli ulcer, or *Mycobacterium ulcerans* infection, is an emerging disease, principally diagnosed in humid tropical countries and inducing large skin ulcers. These lesions are painless, a distinct feature that suggests that the mycolactone toxin and/or *M. ulcerans* impedes the signal transmission by the nervous system. In this context, the aim of this work was to study the interaction between *M. ulcerans* and neuronal cells by using *in vitro* and *in vivo* models. We showed that a virulent strain of *M. ulcerans* is able to enter into neurons cultivated from neonatal rat hippocampus. On the contrary, this phenomenon was less observed with a mycolactone-deficient strain. To support these data, we analysed nerve fibres from mouse-infected tissues and few bacilli were found in close contact with nerve fibres. The invasion process established by *M. ulcerans* to colonize the nervous system remains uncharacterised, but we hypothesise that this ability could be involved in the painless of the *M. ulcerans* infection.

## **Mycolactone circulates in the peripheral blood of Buruli ulcer patients - Consequences for diagnosis and disease monitoring**

**Presenter: Laure Guenin-Macé**

**Authors:** Fred S. Sarfo<sup>1</sup>, Fabien Le Chevalier<sup>2</sup>, N'Guetta Aka<sup>3</sup>, Richard Phillips<sup>1</sup>, Mireille Dosso<sup>3</sup>, Ivo Boneca<sup>4</sup>, Pascal Lenormand<sup>5</sup>, Abdelkader Namane<sup>5</sup>, Romain Veyron-Churlet<sup>2</sup>, Laure Guenin-Macé<sup>2</sup>, and Caroline Demangel<sup>2</sup>

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Simple and field-compatible diagnostic tests for Buruli ulcer (BU) are needed for this disease to be treated locally and inexpensively. Moreover, the optimization of antibiotic treatments against BU would be greatly facilitated if biomarkers of the infection were identified. Here we asked whether mycolactone could be detected in the peripheral blood and ulcer exudates of patients, and thereby constitute an interesting basis for the development of novel diagnostic approaches and for monitoring disease progression. Samples were collected at the beginning, middle or end of antibiotic treatment in patients from two endemic countries, namely Ghana and Ivory Coast. Lipids were solvent extracted from serum, white cell pellets, red cell pellets or ulcer exudates then analyzed in parallel by two quantitative approaches. The first one was derived from a recently published method using TLC migration followed by coupling to 2-naphthylboronic acid and fluorescence detection. The second one was adapted from our previous studies in animal models, and based on HPLC-MS/MS. We identified structurally intact mycolactone in both ulcer exudates and peripheral blood, thus providing the proof of concept that assays based on mycolactone detection in circulating blood can be used to diagnose BU. Importantly, mycolactone was found to persist at the local and systematic levels after the end of antibiotic treatment, suggesting that the molecule is produced under antibiotic pressure and/or badly eliminated by infected hosts.

## **Inhibition of inflammatory protein synthesis by mycolactone**

**Presenter: Rachel Simmonds**

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Both *in vitro* and *in vivo* studies have demonstrated that one mechanism important to mycolactone-dependent immunosuppression is the inhibition of inflammatory mediator production. This is a tightly regulated process involving several steps including; 1) rapid and strong induction of gene transcription; 2) stabilisation of these mRNAs that would otherwise degrade rapidly; 3) de-repression of translational blocks that would otherwise prevent protein synthesis and (in some cases); 4) further processing of polypeptides to facilitate export/activity. Several lines of evidence agree that mycolactone acts post-transcriptionally and my previous work in primary human monocytes has shown that it selectively targets the translation step. Mycolactone's selectivity means that not all protein translation is affected; only that of a class of induced inflammatory mediators including TNF, IL-6 and COX-2.

These studies have now been expanded and show that mycolactone is a selective inhibitor of translation in many different types of cell. This has facilitated the development of a model *in vitro* system to study this function. Similarly to professional immune cells, mycolactone also selectively inhibits the translation of IL-6, IL-8 and COX-2 in the HeLa and HEK293 fibroblastic cell lines at immunosuppressive doses and independently of cytotoxic effects. Detailed molecular approaches are now being employed to understand the mechanism underlying this activity and in particular to identify what confers mycolactone-sensitivity to certain mRNA transcripts. In addition, global translational profiling is being used to explore the full range of mycolactone-sensitive transcripts. These studies will not only further our understanding of the pathogenesis of BU, but also the mechanism of translational de-repression in inflammation.

# The kinetics of gamma interferon secretion during antibiotic therapy in *Mycobacterium ulcerans* disease

**Presenter: Stephen Sarfo**

**Authors,** Sarfo FS<sup>1</sup>, Phillips RO<sup>1, 2</sup>, Amoako YA<sup>1</sup>, Frimpong M<sup>3</sup>, Adentwe E<sup>4</sup>, Fleischer B<sup>5</sup>, Wansbrough-Jones M<sup>6</sup>

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## Introduction

We have recently shown that secretion of gamma interferon (IFN- $\gamma$ ) in response to Mu sonicate increases during antibiotic therapy for Buruli ulcer disease<sup>1</sup>. We have examined the secretion IFN- $\gamma$  of further in a sub-study of a randomised controlled trial comparing the efficacy of SR2CR6 vs SR8. The aim of this study was to investigate the kinetics of IFN- $\gamma$  secretion during normal and paradoxical clinical responses to antibiotic treatment.

## Methods

83 patients with Mu disease were recruited from villages near Tepa, Agogo and Nkawie in Ghana. The diagnosis of Mu infection was confirmed by PCR for IS2404 on a 4mm punch biopsy. Patients were randomised to be treated with either rifampicin 10mg/kg by mouth and streptomycin 15mg/kg IM daily for 8 weeks (RS8) or rifampicin 10mg/kg and streptomycin 15mg/kg for 2 weeks followed by clarithromycin 7.5mg/kg daily for 6 weeks (RS2CR6). Whole blood was collected from patients at 0, 6, 12 and 32 weeks and stimulated with Mu sonicate and PHA as previously described<sup>1</sup>. Clinical response to antibiotic treatment was assessed at fortnightly visits and time to complete healing noted. Patients were judged to have a paradoxical response using standard criteria. Paradoxical response was defined as new inflammatory disease within or close to a Buruli ulcer leading to extension of the existing ulcer or new ulceration, usually with pus formation. The Mann-Whitney's U-test was used to compare the medians of serum IFN- $\gamma$  at each time point.

## Results

Median IFN- $\gamma$  secretion at baseline was significantly higher in oedemas 1770 (1238 – 2669 pg/ml) and ulcers 753 (88 – 2377 pg/ml) compared with early nodules 355 (56-1736 pg/ml) and plaques (390 (136 – 2384 pg/ml). Taking all lesion types together, there was a significant increase in IFN- $\gamma$  secretion from a median of 573 (56 – 2669 pg/ml) at baseline to 1071 (50 – 3114 pg/ml) at 6 weeks, 1986 (247 – 2881 pg/ml) at 12 weeks and a fall to 1411 (103 – 3716 pg/ml) at 32 weeks. There were no significant differences between the two arms of the study. Lesions that had not healed at 6 and 32 weeks had significantly higher IFN- $\gamma$  secretion than those that had completely healed. Six lesions underwent paradoxical enlargement of which four had at least 2 IFN- $\gamma$  measurements performed and there was a trend towards an increase in IFN- $\gamma$  concentration during the paradoxical reaction (Figure 1).

## Discussion

As observed previously, clinical healing of BU lesions during antibiotic therapy was accompanied by recovery of IFN- $\gamma$  secretion in response to Mu sonicate with no significant difference between the two treatment arms. The response was maintained longer in late healing lesions which may relate to persistent presence of Mu antigens in the skin. Healing of BU is characterised by the appearance of granulomas in lesions which is consistent with a Th-1 type immune response. Paradoxical responses may represent an exuberant Th-1 response to Mu antigens as observed in other mycobacterial infections<sup>2</sup>. Further studies are required to explore the potential to predict paradoxical reactions and the reasons for delayed healing in some BU patients on antibiotic treatment.

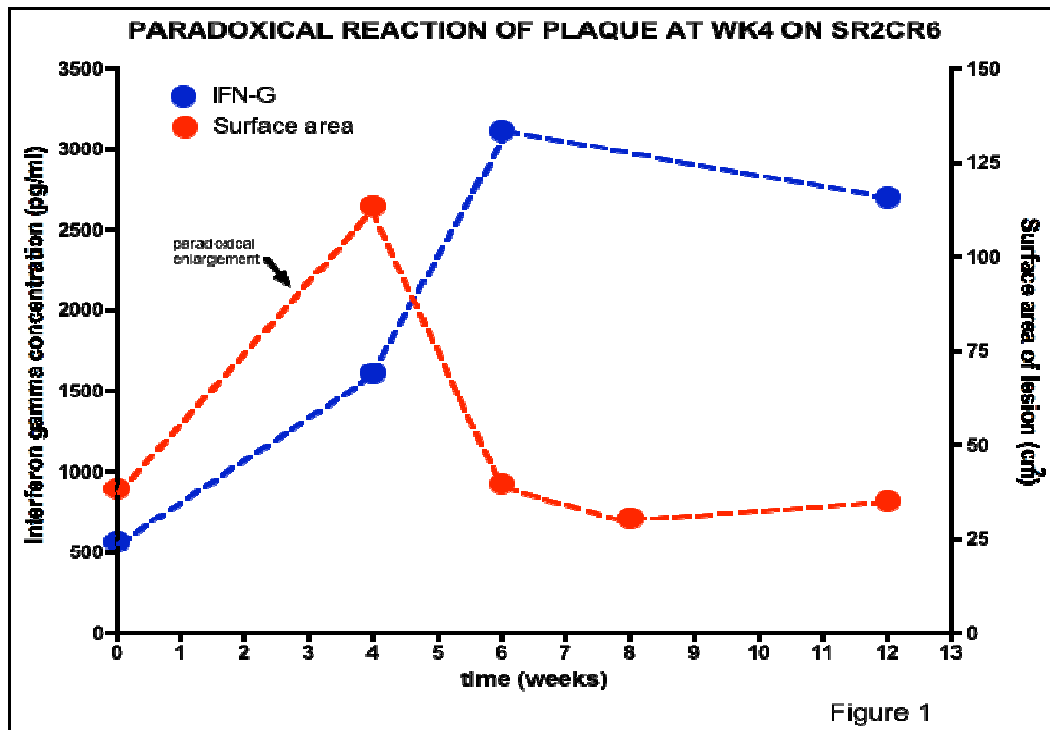


Figure 1

## Reference

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## **Mouse model of Buruli Ulcer: protection conferred by *Mycobacterium bovis* BCG or with a mycolactone negative strain of *M. ulcerans***

**Presenter: Teresa Martins**

**Authors:** Alexandra G. Fraga,<sup>1</sup> Teresa G. Martins,<sup>1</sup> Egidio Torrado,<sup>1</sup> António G. Castro,<sup>1</sup> and Jorge Pedrosa<sup>1</sup>

<sup>1</sup>Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal

Buruli ulcer (BU) is an emerging necrotizing skin disease caused by *Mycobacterium ulcerans*. Although progresses have been made in early detection and treatment, efficient vaccination would be the best approach for the effective control of this neglected disease. Several reports show some degree of protection conferred by immunization with *M. bovis* Bacille Calmette-Guerin (BCG) against both experimental *M. ulcerans* infections and BU. Alternatively to BCG would be the development of live-attenuated vaccines based on mycolactone-negative strains of *M. ulcerans*.

In this study we evaluated the immunological mechanisms underlying the protection conferred by vaccination with BCG or with a mycolactone-negative strain of *M. ulcerans* in the mouse footpad model of experimental BU and the associated. We observed that BCG vaccination conferred transient protection against infection with virulent *M. ulcerans*, which was associated with an earlier and heightened Th1 type of immune response, not only at the site of infection, but also in the draining lymph node (DLN). Nonetheless, mycobacteria persisted in the footpad leading to the eventual increase in bacterial loads and progression to ulceration, followed by DLN necrosis. Immunization with the mycolactone-negative *M. ulcerans* strain also significantly delayed the progression of infection by virulent *M. ulcerans* but, eventually, the pathogenic mechanisms in the DLN and in the footpad prevailed with the consequent emergence of ulceration.

These findings further support the importance of Th1-mediated immunity in the protective response triggered by vaccination. However, it will be important to determine when and what protective mechanisms are compromised by *M. ulcerans* infection and consequently lead to the loss of protection induced by vaccination.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement N° 241500.

## **Buruli Ulcer: apoptosis and antibiotic therapy.**

**Presenter: Elisa Zavattaro**

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Forty-five skin biopsies taken from Beninese patients affected by Buruli ulcer (BU) in different clinical and therapeutical phases were histologically and immunohistochemically studied. Oedema, extensive necrosis and granulocytic infiltration were the main changes in the first phase of the disease, and significantly decreased in the course of therapy. By contrast, dermal and subcutaneous sclerosis, lymphocytic and plasmacytic infiltration and non-necrotizing granulomas were mainly represented in the advanced stage of disease and at the end of therapy. Moreover, regressive changes and inflammation of deep nervous bundles were observed after therapy. To clarify a hypothetic participation of apoptotic mechanism in the pathogenesis and maintenance of the process, we investigated the presence and number of apoptotic bodies by using Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling (TUNEL): the number of positive bodies significantly increased from beginning of the disease to advanced stages, in parallel with decrease of necrosis, and were also detected inside or around the nerves. We also investigated, by an immunohistochemical tool, the expression of caspases 3 and 8 and Bax, three molecules directly involved in the apoptotic process, and found that they were consistently down-regulated after therapy.

Our results may confirm that apoptosis, obviously triggered by mycolactone activity, plays a non secondary role in the development and the course of BU. Furthermore, the detection of apoptotic bodies inside of nervous bundles may account for the well-known anaesthesia, clinically demonstrated in some phases of BU.

## **Histopathological characteristics of suspected Buruli ulcer injuries on admission**

**Presenter: Luc Brun**

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Diagnosis of Buruli ulcer (BU) depends on confirmation by one or more laboratory tests including PCR and a histopathology exam. The diagnostic value of the histopathology exam is significant when acid fast bacilli (AFB) are observed on a histological slide associated with coagulation necrosis detected by Ziehl-Neelsen stain. The question is whether histopathology has value in diagnosis of BU on admission when no AFB are detected on the histological slide.

### **Objective**

This study aims to identify histopathological lesions that could be considered diagnostic for BU in tissue specimens taken on admission to the Allada Centre for Buruli Ulcer Detection and Treatment (Allada CDTUB) in Benin.

### **Methodology**

An analytical retrospective study was carried out of 37 patients admitted to Allada CDTUB in the period from 23 January 2008 to 10 May 2010. 37 tissue specimens were taken on admission. PCR and a histology exam using hematoxylin and eosin stain and Ziehl-Neelsen stain techniques were performed on each tissue specimen.

### **Results**

Of the 37 patients evaluated, 28 were under 15 (75.7%). The M/F sex ratio was 0.95. Ulcerative lesions accounted for 40.5% of the total. 24 patients were PCR positive (64.8%) .

**Analysis of the lesions according to the PCR results on admission** reveals that psoriasiform hyperplasia ( $p=0.01$ ) and necrosis of the subcutaneous tissue ( $p=0.01$ ) and vascular walls ( $p=0.00$ ) were significantly higher when the PCR was positive.

On the other hand there was no significant difference with regard to the inflammation of the vascular walls and vascular thrombosis of the dermis and subcutaneous fatty layer.

**The presence of AFB was observed in the dermis** in 1/23 PCR positive specimens and 1/10 PCR negative specimens. In subcutaneous tissue, AFB were found in 1/24 PCR positive specimens and 2/11 PCR negative specimens

**In conclusion**, this study has enabled us to focus on the following elements:

1. Two problems of BU diagnosis, namely:
  - (i) Differential diagnosis of BU (are other mycobacteria the causal agent of PCR negative lesions in cases where AFB are detected by histopathology?)
  - (ii) The need for clear histopathological criteria in diagnosis of BU (we have demonstrated that the absence of AFB does not exclude the possibility of BU on histopathological examination)
2. The importance of histopathology in BU diagnosis which, in combination with other bacteriological tests, facilitates a more accurate diagnosis of PCR negative lesions.

**Key words**: Buruli ulcer, AFB, histopathology, PCR.

## **A multiplex kindred with severe Buruli Ulcer displaying Mendelian inheritance**

**Presenter: Quentin Vincent**

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Buruli Ulcer (BU), caused by *Mycobacterium ulcerans*, is the third most common mycobacteriosis worldwide after tuberculosis and leprosy, and has been flagged in 1998 by the World Health Organization as an emerging neglected infectious disease. The physiopathology of *Mycobacterium ulcerans* infection primarily involves the lipidic toxin mycolactone, a unique feature among mycobacteria. The resulting extensive skin ulcers and/or osteomyelitis cause pathologic scarring responsible for severe life-lasting functional disabilities in the affected population, mainly composed of children of less than 15 year of age.

Buruli ulcer mainly strikes in Western Sub-Saharan Africa but cases have been reported in more than 30 countries worldwide. A common characteristic of the endemic countries consists in the extreme clustering of BU cases in families living in the vicinity of slow-flowing or stagnant waters in rural areas. However, only a fraction of these heavily exposed individuals develop Buruli ulcer, which leads us to hypothesize a genetic etiology accounting for this variability.

To tackle this issue, we adopted an extreme-phenotype strategy, which consisted in recruiting the most severe of the >1,500 BU cases diagnosed and treated during the last 7 years at the Centre de Détection et de Traitement de l'Ulcère de Buruli in Pobè, Benin. We report here the analysis of a single highly-informative consanguineous family in which two siblings were affected with exceptionally severe PCR-confirmed BU. The index case suffered from a multifocal edematous form of BU, which disseminated under treatment and involved the four limbs, eventually requiring amputation to heal. Her sister suffered from an edematous form which affected the right arm from shoulder to fingers.

Blood was obtained from the 2 parents, 2 affected and 3 unaffected children. DNA was processed for the genotyping of >900,000 Single Nucleotide Polymorphisms by the Affymetrix Genome-Wide 6.0 array. After quality control procedures, 120,156 independent SNPs were used for linkage analysis by homozygosity mapping. Three regions, on chromosome 5 and 8, cosegregated with the affected status following a Mendelian recessive inheritance mode, i.e. were shared homozygous by descent by the 2 affected individuals but not the 3 unaffected siblings (yielding the maximum possible LOD score given the pedigree. Sequencing of genes in these regions is currently ongoing and show promising results. This first description of a genetic etiology for extremely severe BU will have far reaching biological and medical implications.

## **A histopathological analysis of skin lesions emerging several months after completion of antibiotic treatment of Buruli ulcer**

**Presenter: Marie-Therese Ruf**

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Clusters of extracellular acid fast bacilli (AFB) and poor inflammatory responses at the site of infection are typical histopathological features of an untreated Buruli ulcer (BU). After the recommended eight weeks standard treatment with rifampicin and streptomycin, a reversal of the local immunosuppression caused by the macrolide toxin mycolactone of *M. ulcerans* is observed. In some patients secondary lesions develop during or after completion of antibiotic treatment.

We have conducted a detailed histopathological and immunohistochemical analysis of tissue specimens from two patients who developed multiple new skin lesions 46 to 409 days after completion of antibiotic treatment and complete healing of the initial ulcerative lesions. We found typical histopathological hallmarks of BU and AFB with degenerated appearance. However, other than in active disease, lesions contained massive leukocyte infiltrates including large B-cell clusters, as typically found in cured lesions.

Our histopathological findings demonstrate that the secondary skin lesions which emerged several months after completion of antibiotic treatment were associated with *M. ulcerans* infection. It appears that these lesions were resolved by immune responses primed by the successful treatment of the primary lesion.

## **Cytotoxic effect of mycolactone produced by *M. ulcerans***

**Presenter: Masamichi Goto**

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Buruli ulcer is characterized by its painless nature of the lesion. We have studied the pathological mechanism of this phenomenon, and revealed that local nerves are invaded and damaged by *M. ulcerans* and similar nerve damage is evoked by the injection of mycolactone in mouse models. In both models, Schwann cells showed vacuolar degeneration.

In order to further elucidate the mechanism of the nerve damage in Buruli ulcer, we tested the cytotoxic effect of mycolactone on the cultured Schwann cells. As mycolactone is known to evoke cell death and apoptosis to fibroblasts, macrophages, adipocytes and keratinocytes, it is necessary to compare the cytopathic pattern of Schwann cells produced by mycolactone to other cells. Also, for the evaluation of function of mycolactone alone, we used synthesized mycolactone in this study.

In the first study, L929 mouse fibroblast cells (ATCC CCL1) and J774A.1 mouse macrophage cells (ATCC TIB 67) were cultured for 24 hrs. Synthesized mycolactone A/B (supplied from Prof. Yoshito Kishi, Harvard University, U.S.A.) was first diluted by ethanol and further diluted by PBS. Mycolactone with final concentration of 30 ug/ml, 3 ug/ml, 300 ng/ml, 30 ng/ml, 3 ng/ml, 300 pg/ml was added to the culture wells and incubated for 24 hrs and 60 hrs. For the negative control, 100% ethanol similarly diluted by PBS was used. Both fibroblasts and macrophages showed cell death (detachment of most of adhered cells) 24 hrs after the addition of mycolactone 30 ug/ml, 3 ug/ml and 300 ng/ml. 30 ng/ml of mycolactone showed partial detachment, and 3 ng/ml and 300 pg/ml showed no floating cells as well as negative control. At 60 hrs, floating cells increased, but 3 ng/ml and 300 pg/ml showed no floating cells. These results are similar to the study by George KM et al. (2000), but they observed massive fibroblast cell death by 3 ng/ml at day 3, that is about 1/10 of mycolactone concentration compared to our present study.

In the second study, mouse SW10 Schwann cell (ATCC CRL-2766), L929 cells and J774A.1 cells were cultured. Synthesized mycolactone A/B with final concentration of 300 ng/ml, 30 ng/ml, 3 ng/ml, 300 pg/ml was added to the culture wells and incubated. Both fibroblasts and macrophages showed cell death (shrinkage and detachment) 24 hrs after the addition of mycolactone 300 ng/ml and 30 ng/ml, but 3 ng/ml and 300 pg/ml did not show morphological changes. Schwann cell also showed cell death 24 hrs after the addition of mycolactone 300 ng/ml, and 30 ng/ml of mycolactone showed partial detachment, but 3 ng/ml and 300 pg/ml did not show morphological changes. At 48 hrs, 30 ng/ml showed floating cells. Cytotoxic concentration of mycolactone for the Schwann cells was approximately 10 times higher than that for fibroblasts and macrophages.

In this study, we could demonstrate the cytotoxic property of synthesized mycolactone A/B to mouse fibroblasts, macrophages and Schwann cells. Schwann cells were damaged by mycolactone, but contrary to our expectation, Schwann cells were relatively resistant to the mycolactone in the present study. Quantitative analysis of dead cells and apoptotic cells is in progress.

## ***Mycobacterium ulcerans* infections induce progressive muscle atrophy and dysfunction and mycolactone impairs satellite cell proliferation.**

**Presenter: Jérôme Frenette**

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### **Introduction**

Clinical observations from Buruli ulcer (BU) patients in West Africa suggest that severe *Mycobacterium ulcerans* (*M. ulcerans*) infections can cause skeletal muscle contracture and atrophy leading to significant impairment in function.

### **Material and Methods**

In the present study, male mice C57BL/6 were subcutaneously injected with *M. ulcerans* in proximity to the right biceps muscle, avoiding direct physical contact between the infectious agent and the skeletal muscle. The histological, morphological, and functional properties of the muscles were assessed at different times after the injection.

### **Results**

On day 42 post-injection, the isometric tetanic force and the cross-sectional area of the myofibers were reduced by 31% and 29%, respectively, in the proximate-infected muscles relative to the control muscles. The necrotic areas of the proximate-infected muscles had spread to 7% of the total area by day 42 post-injection. However, the number of central nucleated fibers and myogenic regulatory factors (MyoD and myogenin) remained stable and low. Furthermore, Pax-7 expression did not increase significantly in mycolactone-injected muscles, indicating that the satellite cell proliferation is abrogated by the toxin. In addition, the fibrotic area increased progressively during the infection. Lastly, muscle-specific RING finger protein 1 (MuRF-1) and atrogin-1/muscle atrophy F-box protein (atrogin-1/MAFbx), two muscle-specific E3 ubiquitin ligases, were upregulated in the presence of *M. ulcerans*.

### **Conclusion**

These findings confirmed that skeletal muscle is affected in our model of subcutaneous infection with *M. ulcerans*, and that a better understanding of muscle contractures and weakness is essential to develop a therapy to minimize loss of function and promote the autonomy of BU patients.