WHO Meeting on Buruli ulcer Control and Research

20–22 March 2017

WHO Headquarters
Geneva, Switzerland

ABSTRACTS
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Plenary sessions

Updates on Buruli ulcer
The role of surgery in the treatment of Buruli ulcer – a randomized clinical trial

Presented by Anita Wadagni

A Wadagni, YT Barogui, RC Johnson, G Sopoh, TS van der Werf, F Hédiblè, F Ahoou, J de Zeeuw, J Kleinnijenhuis, Y Stienstra

Background

Surgical intervention used to be the mainstay of the treatment for Buruli ulcer disease, a neglected tropical disease caused by Mycobacterium ulcerans. Since the introduction of streptomycin and rifampicin for eight weeks as standard care, the role of surgery has been unclear. Small lesions have shown to be able to heal without surgery. This study investigates the effect of delaying the decision to perform surgery.

Methods

A single centre, controlled trial with equal randomization was performed in Benin (ClinicalTrials.gov, NCT01432925) between July 2011 and December 2015. Patients aged three years or older with confirmed disease were randomized to evaluate the need of surgery eight (standard care) or 14 weeks after start of antimicrobial treatment. The primary endpoint was healing without the need for surgery. A doctor masked to treatment assignment checked the indications for surgery with predefined criteria.

Findings

119 patients were enrolled with two patients per arm being lost for follow-up. Fifty-five (96·5 %) participants in the delayed decision group and 52 (89·7%) participants in the standard care group were healed one year after start of the treatment (RR= 1·08 CI 95% [0·97 – 1·19]). Among the healed participants in the delayed decision group, 37 (67.3%) patients healed without the need of surgical intervention while in the standard care group, out of the 52 who were healed, 25 (48·1%) patients healed without surgery (RR= 1·40 CI 95% [1·00 – 1·96]). The time to heal and functional limitations did not differ between the two arms. Hospital admissions and duration of wound care were reduced by postponing the decision.

Interpretation

Delaying the decision to perform surgery means even large ulcers can heal with antibiotics only without negative effects on healing rate and functional limitations.
Update: Buruli ulcer control activities Ghana in 2016

Poster: Edwin Ampadu

Introduction

Ghana’s annual reports over the past three years have shown a steady decline in the number of cases reported. However, it is of the view that there are more cases than reported. Interestingly it has also been observed that any time case detection activities step up, more cases are identified with high number of category 3.

In order to bring the disease to low level of public health importance national programme steps up assistance for continuous case detection activities in the districts.

Over the last three years, the programme has had some support from ALM/MAP to strengthen national programme control office. This help tremendously and we wish to continue.

Major activities carried out

- National Programme with the support from ALM/MAP and ANESVAD carried out support visits and organized training to 5 endemic regions Ashanti, Brong Ahafo, Eastern, Central and Greater Accra Regions
- Activities included:
  - Ensuring standard disease control activities and support to treatment. They include surveillance, documentations, use of various BU forms and wound care activities. In all 18 endemic districts were visited
  - Capacity development for the districts and some major treatment centres were also carried out. In all six such training activities were carried out: -in all, 100 health workers of various ranks who have interest in the control and management of Buruli ulcers, wound care, and also 29 community health volunteers participated.
  - Post training follow ups visits [4] such visits took place to both the regions and the districts. The objective was to appreciate the new skills gained and how effective they are applying after the training. There were remarkable interests in the control activities.

- The Methodist relief services, Ghana has since 2014 been working in some endemic communities in Ghana where the disease is prevalent providing portable drinking water to the communities and caring for some cases of Buruli ulcer seen.
  - In 2016, the Methodist Relief Service decided to support the national programme with assorted surgical dressings as their contribution to the disease control activities. These were distributed to the endemic treatment districts and centres. Twelve major centres in Ashanti, Brong Ahafo and Greater Accra and Eastern regions have benefited. The items include Crepe bandages, Cotton wool Cotton gauze, Vaseline gauze and plasters

- International collaboration: A Visit by the WHO drug trial/wound care team in November 2015 and also 2016 took place. The Objective of the visit: To follow up and assess progress of the drug trial and the wound management of the cases enrolled in the trial. This is the last year in the proposed three year trial. Glad to mention that the outcome looks assuring.

- International Meeting on [Case management] CM- NTDs held in Benin September, 2016. Ghana participated.
- Visit by WHO surveillance and Data expert. The visit was to compliment Ghana DHIMS 2 as data collection tool for disease control and how NTDs could be captured on it. The Global coordinator for Buruli ulcer was present.
- Programme collaboration: Buruli ulcer, Yaws, and Leprosy Programmes have come together to develop a proposal to integrate control the diseases using common resources. In 2016 these three
programmes, Buruli ulcer, Yaws and Leprosy met to produce an integrated plan of action to be supported for action. Hopefully ANESVAD will support this action.

**Research works**

Case confirmation using fTLC [fine thin layer chromatography] technique as a novelty in diagnosis of Buruli ulcer. With Department of Chemistry, University Ghana Legon in the lead. This is in addition to the exciting PCR.

At the end of the day the national programme is keen in relying on a point of care diagnosis which is robust and will not make clinicians miss opportunities

**Challenges to the National Control Programme**

- Poor cross country vehicles to support disease control activities.
- High attrition of trained staff in the endemic centres making the disease control challenging
- Irrational Logistics support to the programme
- Laboratory confirmation is not assessable – point of care
- Awareness of the disease within the public still low

**SURVEILLANCE AND DATA**

![Annual total cases reported- Three year trend](image)
The laboratory confirmation coverage has also shown some decline. Collaborators in laboratory case confirmation to the national programme have increased to 5 tests include [PCR, fTLC, LAMP etc. ]

**Collaborators /Acknowledgement**
ALM/MAP International, ANESVAD, WHO

**Local**
Country WHO, WATER, BUVA, GHS, FHI 360, METHODIST CHURCH – Department for rural development

**Research centres**
Noguchi Memorial Institute of Medical Research; Department of chemistry, Legon; Komfo Anokye Teaching Hospital; Plastic Surgery Department, KCCR
In Guyana in the years 2015 and 2016, we diagnosed 8 new cases of *M. ulcerans* infection: sex ratio: 3; mean age: 52 years [20-75]; geographical origin of patients: Europe: 3; Guiana: 3; West Indies: 1; Haiti: 1. Place of residence: Cayenne region: 7; Kourou region: 1. Lesions were ulcerative in all cases with localization in the lower limbs in 6 patients, and in the upper limbs in 2 patients. Categories were: I in 4 patients and II in 4 patients. Diagnosis was laboratory confirmed: Ziehl +: 5/8; anatomo-pathology +: 6/6. Treatment included rifampicin - clarithromycin for all patients except 1 who was prescribed moxifloxacin - clarithromycin because he was on antiretroviral therapy with a protease inhibitor for HIV infection. Good treatment efficacy in all the patients treated.

An incidence analysis by age group taking account of census data of the Guianese population since the 1960s shows a change in the epidemiology of *M. ulcerans* infection in Guiana from 1969 to 2013, from African epidemiology (i.e. a prevalence of infantile cases) to Australian-type epidemiology (a prevalence of cases in adults). The likely explanation is the change in lifestyles and the rise in the standard of living in French Guiana over this period.

We reviewed observations of patients treated with a combination of antibiotics containing clofazimine: 44 patients were treated with clofazimine (during the period 1970-2001). For the most part, surgery was also involved, which rules out any conclusions regarding the benefit of using clofazimine in humans, based on our retrospective data.
Epidemiological surveillance of Buruli ulcer in Gabon in 2015 and 2016

Poster: Dr Annick Mondjo
National Communicable Diseases Control Programme (Gabon)

Introduction

In Gabon, Lambaréné and the surrounding area have remained the main focus of Mycobacterium ulcerans infection since 1961. Nevertheless, during the last five years, patients living outside the Centre health region have had diagnoses confirmed by PCR.

Organization of epidemiological surveillance of Buruli ulcer

The characteristics of Buruli cases are reported to the central level, on a monthly basis in the case of referral facilities in the Centre health region, which keep a BU 02 register, and on a case-by-case basis for other health facilities. Specimens for diagnosis are transmitted centrally through the Communicable Disease Control Programme (PLMI). PCR-confirmed diagnosis is performed free of charge by the bacteriology unit of the Franceville International Medical Research Center (CIRMF). The data recorded at the central level in the anonymous Excel BU 02 database were used to assess the performance of Buruli control during the evaluation of the National Health Development Plan (PNDS) 2011-2015, in July 2014 and January 2016.

Characteristics of new cases

Thirty-nine (39) new cases of Buruli ulcer were reported in 2016, compared with forty-three (43) in 2015 (updated data). Most of the patients are treated in the region where they live. The majority of new Buruli cases recorded in 2015 and 2016 were from the Center health region (71%). Some were from Libreville (12%), North Region (6%) or Maritime Region (7%). In both years, the proportion of women decreased from 53% to 43%, while the proportion of children under 15 showed no significant change (43.5%). In the same period, the proportion of ulcerative forms remained above 90%. By contrast, the proportion of category III forms declined sharply from 42.9% to 27%. In addition, the frequency of functional limitation decreased threefold (from 16.3% to 5.3%).

Confirmation of diagnosis by PCR

The proportion of PCR-confirmed Buruli ulcer diagnosis performed by CIRMF increased from 25.6% in 2015 to 38.5% in 2016 (compared with 10% in 2014). This increase can be explained, principally, by the fact that the proportion of patients from whom specimens were collected increased from 67% to 76% (compared with 13% in 2014). The improvement in the MU1 / MU2 positivity rate of the specimens was more modest, increasing from 45% (= 11/24) to 54% (= 15/28), without it being possible to say more precisely what role was played by clinicians and what role was played by the laboratory. However, the performance of health facilities in the Centre Region appears to have been decisive, considering that the confirmation rate of Buruli ulcer diagnoses was close to 50% in 2016, compared to less than 25% the previous year. Since the identification of the new focus at Port Gentil, most of the cases reported by the Maritime Region have been confirmed by PCR. Health facilities in other regions were less successful in this regard in 2016.
Comments

The integration of the case management component of activities to control Neglected Tropical Diseases has helped to build capacity among the stakeholders involved in Integrated Surveillance of Disease and Response (IDSR) and has partially offset the reduction of human resources in the Centre Region. The current improvement in some indicators is perceptible, yet remains fragile. The new global target for **PCR-confirmed Buruli diagnosis of over 70%**, introduced in the new Health Development Plan 2017, was validated in July 2016 together with a goal of achieving a specific treatment completion rate of at least 80%.

Outlook

Additional objectives should be discussed during the finalization of the specific multiannual plans and the case management component of integrated control of neglected tropical diseases. In the meantime, the quality of referral services in the region forming the historic focus of the disease should be sustained, and the vigilance of health workers and the population must be strengthened also. In addition to the **operational guidelines** institutionalizing the data and specimen-collection loop, the PLMI plans to set up an **Epi-Info database** to extract data from the UB 01 data sheets (disease history, treatment outcome, etc.).

**Table 1 : Principal characteristics of new Buruli ulcer cases, Gabon, 2011-2016**

<table>
<thead>
<tr>
<th>Year</th>
<th>New Buruli ulcer cases</th>
<th>% ulcerative forms</th>
<th>% category 3 forms</th>
<th>% forms with functional limitation</th>
<th>% PCR positive examinations</th>
<th>% IS2404-based confirmation MU1/MU2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Former targets</td>
<td>n/a</td>
<td>&lt;85%</td>
<td>&lt;33%</td>
<td>&lt;25%</td>
<td>n/a</td>
<td>&gt;50%</td>
</tr>
<tr>
<td>2011</td>
<td>59</td>
<td>44%</td>
<td>38%</td>
<td>5%</td>
<td>33%</td>
<td>27%</td>
</tr>
<tr>
<td>2012</td>
<td>45</td>
<td>70%</td>
<td>10%</td>
<td>7%</td>
<td>44%</td>
<td>33.3%</td>
</tr>
<tr>
<td>2013</td>
<td>59</td>
<td>83.1% (n=49)</td>
<td>42.4% (n=25)</td>
<td>13.6% (n=8)</td>
<td>74.2% (=23/31)</td>
<td>39%(*) (=23/59)</td>
</tr>
<tr>
<td>2014</td>
<td>47</td>
<td>72.3% (n=34)</td>
<td>37.0% (n=17)</td>
<td>42.6% (n=20)</td>
<td>83%(*) (=5/6)</td>
<td>10%(*) (=5/47)</td>
</tr>
<tr>
<td>2015</td>
<td>43</td>
<td>90.7% (n=39)</td>
<td>42.9% (n=18)</td>
<td>16.3% (n=7)</td>
<td>45.8% (=11/24)</td>
<td>25.6% (=11/43)</td>
</tr>
<tr>
<td>2016</td>
<td>39</td>
<td>92.3% (n=36)</td>
<td>27.0% (n=10)</td>
<td>5.3% (n=2)</td>
<td>53.6% (=15/28)</td>
<td>38.5% (=15/39)</td>
</tr>
</tbody>
</table>

**Buruli targets in PNDS 2017-2021.**

| n/a       | <25%             |                |                    |                                  |                             | >70%                             |

*Sources:* Communicable Disease Control Programme / BU 02 reports from health regions – results of PCR tests carried out in the laboratory of the Antwerp Institute for Tropical Medicine (2011-2012) and the Franceville International Medical Research Centre (2013-2016).
Table 2: Geographical origin of new Buruli ulcer cases (where known), Gabon, 2011-2016

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<tbody>
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<td>Libreville-Owendo</td>
<td>Libreville</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>14</td>
<td>3</td>
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<td>West</td>
<td>Ntoum</td>
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<td>South-East</td>
<td>Franceville</td>
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<td>Centre</td>
<td>Lambaréné</td>
<td>2</td>
<td>59</td>
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Sources: national PLMI database / BU 02 registers of the Centre health region
(*) data correct as at 31 January 2017

Map A – Mapping of Buruli ulcer in Gabon, 2017

[key: black = endemic health regions; grey = health regions with suspected Buruli ulcer; white = health regions free from Buruli ulcer]
Buruli ulcer in Japan: update 2015-2016

Poster: Rie Roselyne Yotsu

Rie Roselyne Yotsu1,2, Chiaki Murase3, Mariko Sugawara4, Koichi Suzuki5, Yuji Miyamoto 6, Mitsunori Yoshida 6, Norihisa Ishii 6

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Epidemiology of BU in Japan, up to 2016

Japan is one of the few non-tropical countries reporting cases of Buruli ulcers (BU). Starting with the first case report in 1982 by Mikoshiba et al. there have been sporadic reports in a wide geographic area, including four and two in 2015 and 2016, respectively. To date, a total of 60 cases have been reported in 17 out of the country’s 47 prefectures (36%). Forty-one cases (68%) have been confirmed as caused by \textit{M. ulerans} subsp. \textit{shinshuense}, a subspecies exclusively isolated in Japan, using PCR and 16S rRNA gene sequencing.

Characteristics of the new BU cases: 2015 and 2016

Most of the new cases (4 of 6) were among elderly subjects (>80 years). One case was in a 6 year-old and another was in a 33 year-old. Sex ratio was 1:1. The geographic distribution was again wide, with no obvious clustering: 3 cases were from the Nagano prefecture (with 3 previously reported cases), 2 from the Shiga prefecture (previously 5 cases), and 1 from the Tottori prefecture (previously 5 cases). Also, there was again no clear epidemiologic association: two cases had known contact with some water source. Five patients noticed onset of their lesions during wintertime, a trend observed also for many of our previous cases. All six cases were ulcerated. Five cases were BU category I, and one case was category II.

Treatment

Leprosy Research Center is currently performing a study on the effect of rifampicin, clarithromycin, and levofloxacin (replaced by tosufloxacin for children) combination oral therapy for BU. So far, we have been observing positive treatment outcomes. All six new cases were treated or under treatment with this regimen, with clinical improvement. Two of them received skin grafting.

Skin-NTDs in Japan

We will briefly report on case numbers for other skin-NTDs in Japan.

Challenges and way forward

We identify the following as our challenges and way forward:

- Need for increased awareness among physicians and health-related workers (given their unfamiliarity with BU, it’s possible that many cases remain unreported).
- Involvement of more physicians, health-related workers, and other actors in BU
- Search for mode of transmission in Japan
- Further research on diagnosis and treatment
- Collaboration between other countries (West Africa, Southeast Asia, etc.)

The overall goal of these activities is to contribute to improve the lives of those affected with BU worldwide.
Plenary sessions

Other skin NTDs
Mycetoma a neglected dilemma

Presented by Ahmed Fahal

The Mycetoma Research Centre, University of Khartoum, Khartoum, Sudan

Mycetoma is one of the most neglected tropical diseases; it is a debilitating medical and health condition. It is characterised by massive deformities and disabilities, progresses relatively inaudible, producing little pain, but resulting in high morbidity and can be fatal. Mycetoma is caused either by certain bacteria (actinomyctoma) or by fungi (eumycetoma). The nonexistence of pain, and health education and the low socio-economic status commonly contribute to the late presentation of affected patients. Mycetoma has numerous adverse bearings on patients, families, communities and health authorities in endemic areas.

Currently, the actual incidence and prevalence of mycetoma are not well defined. Furthermore, the route of infection, resistance and susceptibility to the infection are not well understood. The mycetoma distribution is unique as it frequently seen in the mycetoma belt. However, it is reported globally.

Clinically mycetoma presents with the triad of painless subcutaneous mass, multiple sinuses and discharge that frequently contains grains. The infection then progresses to affects the skin, the deep structures and the bones. It is commonly seen in the foot and hand. However, every body part can be affected. Mycetoma is more frequently reported among the young adults and children. It is more frequent among farmers, workers but in endemic areas, no one is immune from developing the disease.

The mycetoma diagnostic tools and tests are invasive, expensive of low sensitivity and specificity and not available in mycetoma endemic areas.

The present treatments are neither safe, effective nor affordable. For eumycetoma, the treatment requires extensive and destructive surgery and prolonged antifungal treatment. The currently available antifungals, ketoconazole and itraconazole, are proving to be ineffective and have serious side effects; what is more, the FDA and EMA have restricted the use of ketoconazole due to its toxicity. Treatment with Itraconazole lasts twelve months, at the cost of approximately 900 USD per month, making it too expensive for patients and health authorities in endemic areas, resulting in high drop-out rate from treatment. Treatment outcomes are disappointing, characterised by low cure rate and high amputation rates. The current treatments have a 25-35% efficacy rate.

Certainly, more research is needed on how this disease occurs and on new diagnostics and treatments that are safe, efficient and affordable.
Clinical features and management of cutaneous Leishmaniosis: a typical skin NTDs example

Presented by Mourad Mokni
La Rabta Hospital - Tunis – Tunisia

Cutaneous leishmaniasis (CL) is an infection with parasitic flagellates of the genus Leishmania. It is usually a widespread zoonotic disease transmitted between wild and peridomestic animals, especially rodents and canines, mainly by phlebotomus sand flies, and from these reservoirs to man. CL is found in all countries of the tropical and subtropical regions of the world except New Zealand, Australia, and the islands nations of the pacific. Epidemiology of CL may be influenced by environmental Factors, demographic aspects, population migration, seasonal and climatic conditions. Leishmania species identification is mainly based on biochemical characters (isoenzymes) which have been exploited to establish numerical classifications. Molecular studies have given much new information on structure of the Leishmania genome, the study of the function of which is considered as a priority for understanding and solving several acute clinical problems, such as pathogenicity, tissue tropism, and drug resistance. Although each of the leishmania species may have its peculiar manifestations and areas of endemicity, yet none of the clinical manifestation is unique to a particular species because of considerable clinical diversity and overlap. Ulcero-crusted nodule and plaque are the main clinical presentations. All other clinical forms will be reviewed. The clinical picture is dependent on determinants related to the infecting species of Leishmania and the host. These include infectivity, virulence of the parasite, extent of lymphatic, siege of the lesions, immune response, and genetic susceptibility of the host. Clinical and histologic manifestations depend upon the strain of the organism, the size of inoculum, and the immunologic status of the individuals in the endemic or nonendemic areas. Clinical classifications already described are complex and not practical because of the polymorphism of the disease. The natural history of leishmaniasis must be considered in therapeutic strategy. Lesions of CL heal spontaneously over 1 month to 6 years. Multiple therapeutic options had been considered over the years. Unfortunately, few have proven effective and withstood the test of time. To date, antimonials, both intralesionally and parenterally are still the standard agents. Their toxicity, however, precludes indiscriminate use ans necessitates close medical observation. The enhanced interest in leishmaniasis over the past few years soon may yield less toxic, effective, orally administered agents and a protective vaccine.
Leprosy, the disease – diagnosis, treatment, prevention and elimination strategy

Presented by Venkata Ranganadha Rao Pemmaraju

Leprosy

Leprosy, a chronic infectious disease, caused by *Mycobacterium leprae* primarily affects skin, peripheral nerves, mucosal surfaces of upper respiratory tract and the eyes. The mode of transmission is believed to be through droplets, during close contact with untreated cases. Incubation period is long with an average of two to seven years. Left untreated, leprosy leads to deformities mainly seen in hands, feet and eyes. Physical deformities are considered to be the main cause for stigma and discrimination against persons affected by leprosy.

Diagnosis and treatment

Hypo-pigmented skin lesion with definite loss of sensation is the commonest symptom of leprosy. In most of the programmes leprosy is diagnosed on clinical grounds. Slit skin smear examination, DNA based polymerase chain reaction (PCR) assay and histopathological examination of skin biopsies are carried out in some programmes to confirm the diagnosis. Multidrug therapy (MDT) comprising of Rifampicin, Dapsone and Clofazimine constitutes treatment for leprosy. The duration of treatment ranges from 6 months for pauci-bacillary (PB) cases and 12 months for multi-bacillary (MB) cases.

Global leprosy strategy 2016 – 2020

Though elimination of leprosy as a public health problem was achieved globally in 2000 and by many countries by 2005, the new case detection particularly those with grade 2 disabilities (G2D) showed a very slight decrease for the past ten years. This remains a concern for programme managers. ‘Global Leprosy Strategy 2016–2020; Accelerating towards a leprosy-free world’ was prepared with a goal to further reduce the disease burden due to leprosy. It is structured on three pillars to strengthen government ownership, coordination and partnerships, stop leprosy and its complications and stop discrimination and promote inclusion. The Global Leprosy Strategy 2016–2020 calls for achievement of the following targets:

- Zero G2D among new child cases
- Less than one new case with G2D per million population
- Zero countries with legislations that allow discrimination against persons affected by leprosy

Integrated programming

Leprosy is listed as one of the NTDs and Buruli Ulcer, Cutaneous Leishmaniasis, Mycetoma, leprosy, lymphatic filariasis, Onchocerciasis and yaws are some of the NTDs which have skin manifestations. Grouping Skin NTDs is an opportunity to synergize the programme efforts and make them more efficient. Wider consultations on these grounds under ‘Skin NTDs’ is recommended to achieve enhanced coverage of the NTD programmes and achievement of targeted goals as per NTD Road Map.
Efficacy of single dose azithromycin for treatment of latent yaws: a longitudinal comparative cohort study

Oriol Mitjà

Authors
Oriol Mitjà,1,2 Camila González-Beiras,1,3 Charmie Godornes,4 Wendy Houinei,5 Haina Abel,6 August Kapa,6 Raymond Paru,6 Eric Mooring,7 Sivauk Bieb,5 James Wangi,8 Sergi Sanz,1 Quique Bassat,1,9,10 Sheila Lukehart,4,11

Introduction
As in venereal syphilis, latent infection also occurs in yaws. Treatment of latent cases is a crucial component of the WHO yaws eradication strategy. The objective of treating persons in latent stage of disease is twofold: to prevent relapsing episodes and resurgence of transmission to uninfected children and to avoid progression to the destructive late manifestations of the tertiary stage. We aimed to assess the efficacy of single-dose azithromycin to treat patients with latent yaws infection compared to patients with active yaws.

Methods
This population-based cohort study included children in Lihir Island (Papua New Guinea) with high titer (a titer of at least 1:8 on RPR) latent yaws or active yaws between May 2013 and October 2016. Latent yaws was defined either on the basis of no suspicious skin lesions or ulcers negative by PCR for Treponema pallidum subsp. pertenue (TP), and active yaws by the presence of a TP-PCR positive ulcer. These two groups were treated with a single 30mg/kg oral dose of azithromycin. The primary endpoint was treatment efficacy, with cumulative cure rate defined serologically as a decrease in RPR of at least two dilutions by 24 months after treatment or earlier.

Findings
277 participants (176 with latent yaws and 101 with active yaws) qualified for inclusion and completed follow up. Cure rates were 92.1% (95%CI 86.9-95.3) in the latent yaws group, and 95.7% (89.5-98.3) in the active yaws group (p-value for the difference 0.264). Cure rates also did not differ significantly between the latent yaws group and the group of children with active yaws at 6 and 12 months and in all subgroups. Cure rates at 6 months were 81.1% (70.7-88.4) in the latent yaws group and 89.2% (79.4-94.7) in the active yaws group and at 12 months were 88.8% (82.4-93.1) and 92.0% (83.6-96.3) respectively. In subgroup analysis participants with higher RPR at treatment were more likely to present a ≥2 dilution drop of the titer compared to participants with lower RPR.

Interpretation
Based upon declines in serological titres, a single oral dose of azithromycin is efficacious for the treatment of latent yaws. This finding supports the WHO strategy for the eradication of yaws based on treatment of all members of a yaws endemic community irrespective of their clinical status.
An unusual case of leprosy – just an exception of the rule?

Poster: Gisela Bretzel

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Department of Infectious Diseases and Tropical Medicine (DITM), University Hospital, Ludwig-Maximilians-University, Leopoldstrasse 5, 80802 Munich, Germany

We report on a leprosy patient of Pakistani origin who has been living in Germany for more than 20 years. The initial diagnosis of multibacillary leprosy (MB) was made in March 2009 based on clinical criteria and laboratory confirmed by means of positive Ziehl-Neelsen microscopy of skin smears [bacteriological index, BI 1+] and PCR, and a high titre of anti-PGL-I antibodies (>100 antibody units). The patient was treated with 12 months MDT (rifampicin, dapsone, clofazimine), furthermore received prednisolone for 12 weeks due to a type I reaction.

The clinical symptoms had completely receded in May 2010 and a significant decrease of anti PGL-antibodies (<30 antibody units) was noted. From then on follow up of the patient including PGL-I serology was done in yearly intervals.

In October 2015 the patient presented in our outpatient clinic with painful swelling of the middle finger joints. A significant rise of the anti-PGL-I antibody level (>100 antibody units) was noted, unambiguous clinical signs of a relapse were however absent. Due the external diagnosis of rheumatoid arthritis the patient received methotrexate therapy over six weeks.

In February 2016 the patient presented again in our outpatient clinic with multiple reddish lesions indicative for a relapse of the MB leprosy, this time confirmed by histopathology, Ziehl-Neelsen microscopy and RLEP qPCR of a skin biopsy and nasal swabs, as well as an unaltered high anti-PGL-I antibody titre. The bacillary load of the patient as determined by microscopy (BI 4+) and quantification by RLEP qPCR (skin biopsy: ~300.000 bacteria, nasal swab: ~110.000 bacteria) was remarkably high. Molecular drug resistance testing conducted at the Global Health Institute of the Ecole Polytechnique Federal de Lausanne, Switzerland revealed no resistance to rifampicin, dapsone or ofloxacin. Furthermore, corresponding to the morphological index (MI) showing a significant proportion of solid stained bacilli (> 50% per nasal swab), a molecular viability assay (16S rRNA RT qPCR) proved the presence of viable M. leprae in nasal swabs. Subsequently the patient was put on a second course of MDT.

Treatment monitoring was conducted by means of microscopy (MI, BI), quantification of bacillary load by RLEP qPCR, 16S rRNA RT qPCR of nasal swabs and PGL-I-serology. A tenfold decrease of bacillary load was noted three days after start of rifampicin treatment. However, surprisingly – and in contrast to the common opinion – the RNA assay detected viable bacilli until day 110 after start of treatment, and solid stained bacilli were seen until day 67 (BI 2+; MI 10%). Over a period of six months anti-PGL-I antibodies decreased again to low levels.

The infectious dose of M. leprae for humans is not known. As far as our patient is concerned, in view of the initially high BI and the prolonged excretion of viable M. leprae through the upper respiratory tract, the possibility of infectiousness for contact persons cannot be ruled out. We furthermore assume a chronic focus of infection in our patient, therefore, to prevent another episode of relapse, 24 months of MDT (possibly in combination with other bactericidal agents), alternatively treatment until M. leprae is not detectable anymore in diagnostic samples is considered. To identify similar cases at greater risk of transmitting M. leprae or experiencing a relapse we strongly recommend treatment monitoring by means of diagnostic tools such as microscopy (BI and MI), RNA assay and PGL-I-serology in monthly or at least quarterly intervals.
Plenary sessions

Diagnosis of Buruli ulcer
Diagnostic indicators of Buruli ulcer in patients with skin lesions in a Buruli ulcer endemic region

Presented by Miriam Eddyani

Miriam Eddyani 1, Ghislain Sopoh 2,9, Gilbert Ayelo 2, Luc Brun 3, Jean-Jacques Roux 4, Yves Barogui 5, René Fiodessihoue 2, Ramanou Logbo 2, Dissou Affolabi 6, William R. Faber 7, Annelies Van Rie 8, Françoise Portaels 1, Bouke de Jong 1

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3Université de Parakou, Parakou, Benin
4Centre Hospitalier de Chambéry, Chambéry, France
5Centre de Dépistage et de Traitement de l’Ulcère de Buruli, Lalo, Benin
6Laboratoire de Référence des Mycobactéries, Cotonou, Benin
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9Institut Régional de Santé Publique, Ouidah, Benin

In most Buruli ulcer (BU) endemic settings, the diagnosis of BU is made on clinical and epidemiological grounds, after which treatment with BU-specific antibiotics is initiated empirically pending microbiological confirmation where available. Following the current decline in BU incidence in several endemic countries, there has been a proportional decrease in BU lesions and increase in non-BU lesions treated in BU facilities. Clinical expertise in the recognition of BU is thus likely to wane, potentially resulting in diagnostic misclassification. In this context, laboratory confirmation of BU becomes essential.

The development of a diagnostic algorithm that is accurate, cost effective, rapid and feasible in remote and resource-limited settings has great potential to improve the management of patients presenting with BU-like skin lesions. We therefore aimed to determine the diagnostic accuracy of clinical, epidemiological and microbiological signs of BU and to establish the optimal diagnostic approach for patients presenting with lesions clinically compatible with BU in a low income setting.

A total of 226 consecutive patients with skin lesions compatible with BU were recruited between March 2012 and March 2015 at the Centres de Dépistage et de Traitement de l’Ulcère de Buruli of Allada and Lalo and in 10 decentralized health posts in southern Benin. The laboratory tests included direct smear examination (DSE) after Ziehl-Neelsen and auramine staining, IS2404-PCR, culture and histopathology. The clinical and epidemiological indicators recorded were the WHO diagnostic criteria plus additional signs associated with BU. In the absence of a gold standard, the accuracy of each test was estimated using a composite reference standard (CRS) which included clinical information, laboratory results and an evaluation by an expert panel.

Among the 205 patients with complete data, clinicians recognized BU with a sensitivity of 88% (95%CI: 81%-93%) which was higher than the sensitivity of any of the microbiological tests. The sensitivity of PCR was 62% (95%CI: 53%-70%), lower than has been reported in studies using only laboratory tests as reference standards. In our study setting, 22% (95%CI: 13%-33%) of patients clinically not suspected to have BU were classified as BU by the CRS. In clinical practice, these patients would not be assessed by microbiological or histological assays, nor be treated for BU.

An accurate diagnosis of BU will allow improved patient management by initiating appropriate antibiotic therapy for skin infections in non-BU patients and limiting treatment with a prolonged course of more toxic antibiotics to those who truly have BU.
Laboratory confirmation of Buruli ulcer disease at the Noguchi Memorial Institute for Medical Research, Ghana, 2008-2016

Presented by Dorothy Yeboah-Manu

Yeboah-Manu D, A. Asante-Poku, Aboagye S, Ampah K and E Danso

Affiliation: Noguchi Memorial Institute for Medical Research (NMIMR), Bacteriology Department

Introduction

Buruli ulcer (BU), caused by Mycobacterium ulcerans, is a neglected tropical disease. With the introduction of antibiotic treatment in 2006, laboratory confirmation became very necessary to avert unnecessary drug treatment due to misdiagnosis. Thus the Ghana Health Service requested the Noguchi Memorial Institute for Medical Research to assist in laboratory confirmation.

Method

Clinical samples collected from suspected cases outside the Greater-Accra-Region (GAR) were transported through courier services, while most from the GAR were collected during weekly site visits.

Results

Between 2008 and 2016, we analyzed by PCR 2,525 samples originating from 2,206 cases from various health facilities within Ghana except the Western, Upper East and Upper West Regions. The average annual positivity rate of 46.2%; ranged between 14.6% and 76.2%. The positivity rates of the annual confirmed cases over the eight year period are; 2008 (52.3%), 2009 (76.2%), 2010 (56.7%), 2011 (53.8), 2012 (41.2%), 2013 (41.5%), 2014 (22.9), 2015 (28.5) and 2016 (14.6%). Of the overall 1,020 confirmed BU cases, the ratio of female to male was 518 and 502 respectively. Patients ≤15 years accounted for 52% (median age 14 years) of all cases. The confirmed clinical forms of BU presented were; ulcer (69.2%), nodule (9.6%), plaque (2.9%), edema (2.5%), osteomyelitis (1.1%), ulcer/edema (9.5%) and ulcer/plaque (5.2%). Lesions frequently occurred on the lower limbs (57%) followed by the upper limbs (38%), the neck and head (3%) and the least found on the abdomen (2%). The highest number of confirmed cases were from Greater Accra (71.4%) followed by Eastern (13.3%) regions of Ghana and the least from Volta region (<1%).

Conclusion

Our findings show a decline in microbiological confirmed rates over the years and therefore call for intensive education on case recognition to prevent over-diagnosis as BU cases decline.
Update on the development of a rapid diagnostic test for Buruli ulcer

*Presented by Katharina Röltgen*

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The major burden of Buruli ulcer (BU) lies on populations in remote, rural areas of West and Central Africa with limited access to treatment centres and laboratory services. Consequently, diagnosis and subsequent antibiotic treatment are often based only on clinical findings. However, due to the broad differential diagnosis of the various disease stages, laboratory reconfirmation of clinical diagnosis is crucial to ensure appropriate treatment. Therefore, a simple point-of-care diagnostic test that can easily be implemented in rural health facilities is of urgent need.

We have been developing low-tech antigen detection systems based on monoclonal antibodies (mAbs) raised against the *Mycobacterium ulcerans* protein MUL_3720, which was shown to be highly expressed by the pathogen. Within the framework of this project we have developed a highly specific sandwich ELISA aimed for usage at the district hospital level. Extensive optimization of all assay parameters, including sample processing, identification of ideal mAb combinations and concentrations, and incorporation of a signal amplification step, was performed to improve the sensitivity of this test system. Moreover, a lateral-flow rapid diagnostic test is currently being developed in a partnership between Swiss TPH, the Foundation for Innovative New Diagnostics (FIND) and Alere/Standard Diagnostics (SD), which is intended for direct application at the point of care. Sensitivity and specificity of prototypes of these two assay formats are currently being analyzed with BU lesion specimens that have first been characterized by IS2404 real-time PCR testing.

This project is funded by the UBS Optimus Foundation and the Swiss Agency for Development and Cooperation (SDC) through FIND.

Poster: Solange Kakou-Ngazoa

Kakou Ngazoa S¹, Aka Nguetta¹, Coulibaly Ngolo D¹, Sylla A¹, Kouakou H¹, Aoussi S¹, Faye-Kette H¹, Assé H², Dosso M¹.

Poster:
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²National Program of BU control and surveillance, Côte d’Ivoire

Introduction

(BU) is a neglected skin disease caused by Mycobacterium ulcerans and occurs in rural regions in central and in West Africa. The biological confirmation of BU required molecular diagnosis test confirmation. Since 2009, the number of BU cases was increased in Côte d’Ivoire. The surveillance was applied in the country with the implication of World Health Organization (WHO) to control and to eradicate BU. The clinical evolution of the disease has two steps by early nodula and ulcers forms. To improve the diagnosis and the surveillance of BU infection, WHO has recommended the laboratory confirmation of at least 70% of suspected BU cases. - The objective of this study is to present the biological molecular diagnosis of MU in Côte d’Ivoire in 2015-2016.

Materials and methods

The samples were collected in 2015-2016 from suspected cases of BU in all sanitary districts coordinated by the national program of Buruli Ulcer. The patients were from the national program, from NGOs and other clinical structures. Briefly, 2 ml water were added to the clinical samples and well mixed. After centrifugation the pellet was resuspended in 200 µl sterile water. The alkaline lysis was applied and the extracted DNA was used in PCR methods. The real time PCR was done with 5 µl DNA and 20 µl PCR Master-mix.

Results and conclusion

980 clinical suspected patients were tested in molecular tests. The age distribution was 52.6% (531/1009) for male and 47.3% (478/1009) for female. The national distribution of the patients has shown that the Buruli ulcer is located in 10 districts sites: Bas-Sassandra, Comoé, Goh Djiboua, Lacs, Lagunes, Montagnes, Sassandra-Marahoué, Vallée du Bandama, Woroba, and Zanzan. The positivity rate was high in 4 districts: Goh-Djiboua (65%), Lacs (61%), Montagnes (59 %) and Vallée du Bandama (57%). The positivity rate of PCR was 46.82% and 50.52% for 2015 and 2016. Our results reveal BU endemic districts sites in the country.

The molecular diagnosis of MU has recently become a routine test for all BU cases and has improved the biological confirmation of BU in Côte d’Ivoire.

Mots clés: Buruli ulcer, M. ulcerans, PCR, Côte d’Ivoire, West Africa.
Exploration of differential diagnosis and therapeutic outcomes in suspected cases of Buruli ulcer at two Buruli Ulcer Screening and Treatment Centres in Benin

**Poster: Ghislain Sopoh**

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**Introduction**

Buruli ulcer (UB) remains a public health problem, especially in regions of West Africa. Even if the means to manage the disease exist, completely reliable diagnosis is still a problem. The aim of this study is to identify the frequency of various lesions that can result in a differential diagnosis for Buruli ulcer and determine the aetiological diagnosis of clinically suspected UB lesions that are not confirmed by microbiology.

**Method**

We carried out a prospective analytical study on patients living in areas endemic for Buruli ulcer treated at the Allada and Lalo Buruli ulcer screening and treatment centres in southern Benin between March 2012 and March 2015. The studies included all patients with skin lesions suggestive of Buruli ulcer. Patients admitted for traumatic ulcers of less than two weeks’ onset and relapsed Buruli ulcer cases were excluded. The final consensus diagnosis was based on all epidemiological, clinical, microbiological and histopathological findings and after discussion by an international and multidisciplinary panel of experts.

**Results**

205 patients of median age 19 (Q1 = 10, Q3 = 45), of whom 116 were men (56.6%) and 89 women (43.3%), were included in the study. The lesions were generally localized in the lower limbs (144 or 70.24%), with the remainder in the upper limbs and other locations (61 or 29.67%). The lesions were respectively category 1 (28 or 13.7%), 2 (103 or 50.2%) and 3 (72 or 35.1%). A clinical diagnosis of Buruli ulcer was suspected on admission in 129 (62.9%) patients but ultimately confirmed in 127 (62.0%) by EP-CRS. It remained questionable in 11 cases (5.4%). For confirmed non-Buruli lesions (67/205), the aetiologies were established as being infectious (17, 25.4%), traumatic (7, 10.4%), vascular (2, 3.0%) or tumoral (3, 3.5%). The aetiology could not be established in the case of 38 lesions (no image on EP-25 evaluation; old lesions with non-conclusive images - 13). The therapeutic outcome was favorable in 121 patients, including 80 Buruli ulcer cases (39.0%), 9 probable Buruli ulcer cases (4.4%) and 32 non-Buruli ulcer cases (15.6%). It was unfavorable in 39 patients, 16 of them Buruli ulcer cases (16.8%), 2 probable Buruli ulcer cases (1.0%) and 21 non-Buruli ulcer cases (10.2%). The aetiologies of cases with an unfavorable outcome were: necrotic fasciitis, pyodermitis, ulcers of vascular origin, and tropical ulcers. This outcome could not be documented in 45 patients.
Conclusion

This study confirms that, under equal epidemiological and socio-demographic conditions, a number of lesions are easily confused with Buruli ulcer. The final diagnosis is not always easy despite the availability of laboratory tests for investigation. The therapeutic outcome of non-Buruli ulcer cases is not always favorable due to the absence of adequate aetiological treatment. It is important to use effective diagnostic methods for suspected cases and ensure availability of aetiological treatment for non-Buruli cases.

Keywords: Buruli ulcer, differential diagnosis, composite reference standard
Evolution of mycolactone detection in Buruli ulcer patients treated with antimicrobial

*Poster: Anita Wadagni*

Authors: GBEDEVI Mirabelle, GOMIDO Inès, WADAGNI Anita Carolle, AMEWU Richard, AKOLGO Gideon, DOSSOU Ange, SOPOH Ghislain Emmanuel, HOUEZO Jean Gabin, BAROGUI Yves Thierry, JOHNSON Roch Christian, PHILLIPS Richard, ASIEDU Kingsley

**Background**

Buruli ulcer (BU) is a chronic cutaneous disease caused by *Mycobacterium ulcerans*, the only human pathogen known to be associated with mycolactone secretion responsible for the necrosis of the dermis, subcutaneous fat and fascia, resulting in large ulcers. Standard routine laboratory techniques for the confirmation of BUD are *M. ulcerans* isolation by culture, histopathology, smear microscopy for acid-fast bacilli (AFB) and polymerase chain reaction (PCR) for detection of the *M. ulcerans* specific insertion sequence IS\(2404\). Recent research efforts have focused on direct detection of mycolactone in tissues by f-TLC and studies are showing promising results. This paper is a sub-study of a larger research aimed at evaluating the performance of f-TLC against standard IS\(2404\) PCR as a diagnostic tool and determine its usefulness as a treatment monitoring tool. Patients admitted at the CDTUB, Allada are treated with a combination of rifampcin (10 mg/kg) and streptomycin (15 mg/kg) once daily for 8 weeks.

**Objectives**

This study aims to monitor the levels of mycolactone in tissue at different times during antibiotic treatment.

**Method**

Prospective, observational study including patients admitted to the hospital in 2016, with positive TLC test at admission. Apart from samples collected at W0 to confirm the diagnosis, further samples were collected for follow up at W2, W4, W8 and W12 after start of antibiotics. Healed lesions were not sampled.

**Results**

40 patients have been recruited as suspected BU patients. Of these, 18 patients tested positive for mycolactone at W0 and included in this analysis. The other 22 patients were tested negative for mycolactone and 06 out of the 22 were positive for PCR but 13 PCR results were still pending. Most of the patients were female (61.1%). The median age of patients was 11.5 years (IQR: 4.75-26.25). Among the 18 patients positive for mycolactone test, PCR result was available for 5 patients (27.8%) of whom 4 had returned positive.

We observed that at W2, 6 patients out of 18 remained positive. At W4, there were only 4 patients out of 18 that still positive for the mycolactone detection. From W8 to W12 no patient was positive for the mycolactone test.

**Conclusion**

Subject to confirmation of these results on a larger sample, we can conclude that the detection of mycolactone decreases during antibiotic treatment and disappeared around the eighth week. In addition to its usefulness for diagnosis, the detection of mycolactone by thin layer chromatography could be an indicator of control and monitoring of the course of treatment in patients with Buruli ulcer.
Control sessions

Integration

—

Experience and tools
Integrated Strategy for Case Management of five Neglected Tropical Diseases (Buruli ulcer, Human African Trypanosomiasis, Leishmaniasis, Leprosy and Yaws) in the WHO African Region.

Presented by Dr Alexandre Tiendrebeogo

Further to the adoption of resolutions on Neglected Tropical Diseases (NTDs) by the World Health Assembly and the Regional Committee for the WHO African Region, an integrated strategy for addressing case management NTDs (Buruli ulcer, Human African Trypanosomiasis, Leishmaniasis, Leprosy and Yaws) is being promoted by the WHO Regional Office for Africa (AFRO). The objective of this integrated strategy is to contribute to the achievement of the Global NTD roadmap targets and goals by 2020, which include elimination of leprosy and human African trypanosomiasis, eradication of yaws and control of Buruli ulcer and leishmaniasis. The rationale behind this strategy is the high burden of case management NTDs in the African Region and reducing available resources and support for supporting the control and elimination of these five NTDs.

For the promotion of the integrated strategy for five case-management neglected tropical diseases (CM-NTDs) a strategy document was developed in 2014 by a group of CM-NTD National Programme Managers, Experts and WHO Staff members. This strategic document was accompanied by the revised terms of reference and *modus operandi* of the NTD Regional Programme Review Group for Preventive Chemotherapy to include some items for a sub-group on Case Management. Later in 2015, three guidance documents on CM-NTDs were further developed by the same group of Programme Managers, Experts and WHO Staff. These three guidance documents are as follows:

- A manual on five case management NTDs for use by peripheral health workers
- A guide for supervision to be used by health district managerial teams
- A guide for monitoring and evaluation of case management NTD Programmes

Five documents were developed and finalised in French and then translated into English and Portuguese for dissemination and use in Member countries of the African Region. This dissemination was initiated during the first regional meeting of CM-NTD National Programme Coordinators and Managers and first meeting of the CM-NTD Sub-group of the Regional Programme Review Group. Some countries (Liberia and Togo) were proposed to receive support and pilot the use of these documents for integrating CM-NTD case finding and treatment in health districts. With the support of AFRO and AIM, Liberia developed an integrated case-management strategy for Leprosy, Buruli ulcer, yaws and complications lymphatic filariasis (hydroceles and lymphedema), which was included in the NTD Master Plan for 2016-2020. Training of health workers is ongoing in five counties with materials adapted from AFRO guidance documents. Togo has used the same guidance documents to carry out two workshops for district level staff covering the entire country.

With the adoption of the integrated strategy for case management neglected tropical diseases and dissemination of guidance documents, the WHO Regional Office for Africa aims to implement more effective and efficient approaches for addressing these five diseases and achieve the 2020 NTD goals in the Region.
Buruli ulcer / Neglected Tropical Diseases’ project in the Upper Denkyira East District, Ghana

Presented by Godfred Sarpong

Godfred Kwabena Sarpong, Dunkwa Offin (Ghana), George Amofa, Dunkwa Offin (Ghana)

Background
The Upper Denkyira East District implemented a year’s project to improve on access to quality health services for persons affected by Buruli ulcer/Neglected Tropical Diseases through the primary health care approach in 30 endemic communities.

Method
Between 15th August, 2015 and 15th August, 2016, we conducted an integrated Buruli Ulcer / Neglected Tropical Diseases training, and resourced over 130 peripheral health workers and community volunteers using standard and national modules and guidelines.

Results
Over the 12-month period, 24 Buruli ulcer, 63 yaws and 8 leprosy cases were recorded, confirmed and managed according to national guidelines. 1,635 other skin lesions (e.g. Scabies, fungal skin infections) were also identified, referred and treated. Community self-referrals improved and accounted for over 30% of all cases identified.

Conclusion
The integration of Buruli ulcer control activities with other neglected tropical diseases, as well as community engagement on neglected tropical diseases surveillance systems is feasible and improves on access to quality health care.

Acknowledgement
This work was supported by Anesvad (NGO), Spain
Integrated approach to control neglected tropical skin diseases in Lalo, Benin

Presented by Yves Barogui

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\textsuperscript{c}Raoul Follereau Foundation, France
\textsuperscript{d}Anesvad Foundation, Spain
\textsuperscript{e}National Leprosy and Buruli Ulcer Control Programme, Ministry of Health, Benin
\textsuperscript{f}Department of Control of Neglected Tropical Diseases, World Health Organization, Geneva, Switzerland

Introduction

Of the five neglected tropical diseases (NTD) with case management, 4 have a cutaneous manifestation. Only Buruli ulcer and leprosy have been definitely proven to be endemic in Benin. The presence of yaws in Benin has not yet been proved. In recent years, the number of cases of Buruli ulcer and leprosy has decreased considerably in Benin as in most African countries. Given the reduction in the number of cases and the scarcity of resources, the World Health Organization has recommended an integrated approach to the control of NTDs. Here we share our experience of integrated control of NTDs with cutaneous manifestations in Lalo, Benin.

Method

Between 1 May 2016 and 31 December 2016, the following initiatives were organized:

- Integrated training of health workers, community intermediaries, teachers and former Buruli ulcer patients in recognizing the signs of leprosy, Buruli ulcer and yaws;
- Integrated awareness campaigns and screening for these three diseases in villages and schools;
- Routine rapid diagnostic testing for yaws in all patients with suspicious skin lesions.

The data were analyzed using IBM SPSS Version 20 software.

Result

In all, 1106 people (37.05% women) were examined in 27 schools and 32 villages in areas endemic for Buruli ulcer. The median age of the subjects was 11.

- 34 (3.1%) suspected cases of Buruli ulcer, 15 of which were confirmed
- 8 (0.7%) suspected cases of leprosy, 3 of which were confirmed
- 185 (16.5%) suspected cases of yaws, all negative on rapid testing
- 879 (79%) cases of other skin diseases including fungal infections, cellulitis, eczema and chronic ulcers.

Conclusion

Integrated NTD screening provides optimal screening and management for NTDs with cutaneous manifestations. But the sustainability of this approach will depend on the training of health workers not only in NTDs with cutaneous manifestations but also in basic dermatology.
Yaws resurgence in Bankim Cameroon: The relative effectiveness of different means of detection in rural communities

*Presented by Alphonse Um Boock*

Authors: Alphonse Um Boock, Paschal Kum, Ferdinand Mou, Mark Nichter

**Background**

Yaws is an infectious, debilitating and disfiguring disease of poverty that mainly affects children in rural communities in tropical areas. In Cameroon, mass-treatment campaigns carried out in the 1950s reduced yaws to such low levels that it was presumed the disease was eradicated except among groups of pygmies inhabiting the dense forest. In 2010 an epidemiological study of leprosy, yaws, and Buruli ulcer conducted in Bankim Health District made it evident that cases of yaws existed and that the afflicted either were not coming to clinics or that health staff were failing to recognize the disease. Five different means of detecting yaws in clinical and community settings were initiated in the district over the following four years from 2012 to 2015.

Figure one: Map of Cameroon’s 10 provinces and location of Bankim District

**Aim**

To identify the most effective strategies for eradicating yaws in Cameroon

**Methodology**

This observational study reviews data on the number of cases of yaws identified by each of five yaws detection approaches. The five detection approaches include:

1) Passive yaws detection stepped up at local clinics after staff attended NTD awareness workshops,
2) Community-based case detection carried in remote villages by hospital staff who relied on community health workers to identify cases,
3) Yaws screening following an innovative mass Buruli Ulcer (BU) outreach program being piloted in the district,
4) School-based screening programs conducted as stand-alone and follow-up activities to mass outreach programs, and
5) House to house active surveillance activities conducted in forty-eight villages.

Implementation of each of the four community-based approaches was observed by a team of health social scientists who were tasked with assessing what was required for each approach to be effective, and to identify its strengths and limitations.

Two tests were used to confirm suspected cases: RPR and TPHA. Patients confirmed of having yaws were treated on the spot with free injections of benzathine Penicillin.

Findings
In 2010 and 2011 35 cases of yaws has been notified. During an intensive Buruli ulcer outreach program carried out between 2012 to 2015, 815 cases of yaws were identified and treated.

Five yaws detection approaches were ongoing during this time period yielding with the follow percentages of cases:
Screening according to the type of intervention is as follows:

- 60 (7%) for passive detection at clinics
- 64 (8%) for NTD community outreach programs
- 328 (40%) for mass BU program followed by school screening
- 253 (31%) for school-based program alone
- 110 (14%) for House-to-house search (1889 households visited)

Discussion
The five interventions for detecting yaws had a synergistic effect and proved to be valuable components of a yaws eradication program. Well planned, integrated, and interactive mass NTD educational programs supported by local chiefs and traditional healers, facilitated by health volunteers, and accompanied by school-based programs proved to be particularly important in Bankim.

Conclusion
Including yaws detection in a Buruli ulcer outreach program constituted a win-win situation, as the demonstration effect of yaws treatment (rapid cure) increased confidence in early Buruli ulcer treatment. Outreach programs functioned as magnets for both BU, yaws and other kinds of chronic wounds that future integrated wound care programs need to address.
Integrating the entire management and early detection approach at the community level

Presented by Franz Wiedemann

The presentation shows the need for and usefulness of integrating the entire management and early detection approach at the community level.

To initiate a national Buruli ulcer control programme, it was necessary to focus on a centralized system: all actions were implemented through a national programme.

Over time, through awareness-raising activities and active case-finding by the team of leprosy and tuberculosis control workers, patients were detected earlier and case management was simplified.

The next step was the integration of community health workers into early research, post awareness-raising campaign activities, establishment of diagnostic systems and direct confirmation between stakeholders. Local peripheral care units were given responsibility for case management (training).

In 2015 and 2016, the German Leprosy and Tuberculosis Relief Association (DAHW) helped to launch the decentralized detection and management approach by strengthening training and raising awareness among community teams.

Following the first phase of the project to integrate neglected tropical skin diseases including Buruli ulcer into the community health system, we found that:

- Case management costs have gone down
- Patients attended earlier
- Knowledge of Buruli ulcer has progressed at all levels
- Thousands of other cases of skin disease have been treated under the Buruli ulcer programme
- The general community health situation has improved.

The national programme is increasingly geared to policy formation, development of guidelines and supervision of stakeholders.

Given the impact of decentralization and integration across the community, integration into the general health system is working.

Conclusions

Decentralization and integration of Buruli ulcer at the community level is the logical way forward.

Integration into the general health system is beneficial for patients and the country.

National programmes must therefore review their role and become the guarantors of a high level of quality of care and epidemiological surveillance.
Results of integrated awareness-raising and screening activities for leprosy, Buruli ulcer and other skin diseases in three health districts in Côte d’Ivoire

Presented by Koffi Aboa Paul

Koffi Aboa Paul, Henri Assé, Abbet Abbat R, Christian Jonhson, Kouakou Yao ange Théodore

Introduction

Tropical diseases are a global concern. Côte d’ivoire is endemic for leprosy, Buruli ulcer and yaws. In addition to these diseases, other diseases with cutaneous manifestations constitute differential diagnoses for Buruli ulcer, leprosy, and yaws. In order to provide a comprehensive response to these health problems in communities in co-endemic localities, the two programmes that coordinate Buruli ulcer and leprosy control activities have opted for an integrated control strategy. For example, in 2016, integrated awareness-raising activities and screening for Buruli ulcer, leprosy, yaws and other skin diseases were carried out in the form of mobile consultations in the health districts of Zouan-hounien, Divo and Oumé.

Objective

The main objective of this article is to present the results of integrated awareness-raising and screening activities in localities co-endemic for Buruli ulcer and leprosy in three health districts in Côte d'Ivoire.

Methodology

Each campaign to raise awareness of and screen for skin diseases lasted five days in each district. The implementation began with a preparatory phase consisting of (i) identification of the localities to be targeted, (ii) social mobilization, and (iii) strengthening nursing capacity for disease management and training community health workers how to recognize common skin diseases.

Following this phase, dermatological consultations in conjunction with community awareness sessions on leprosy, yaws and Buruli ulcer were conducted by five teams: an awareness-raising team, four patient consultation teams and a team of supervisors. Each team was made up of stakeholders from health districts and the two relevant health programmes. Awareness of Buruli ulcer, leprosy and yaws was raised through radio broadcasts (rural radio), films and posters/images with commentaries. In the various localities these sessions took place the evening before and on the same day as the consultations. The consultations took place in rooms with plenty of natural light, but in a way that respected the patients’ privacy. The patients seen by the teams were pre-registered, having gone through a triage process. Patients with dermatological lesions were interviewed and received free on-site treatment. Only complicated cases were referred to the specialist hospital. The team dispensed antibiotics specifically for Buruli ulcer and leprosy, as well as common medicines for skin diseases such as antifungal agents, ascariicides, and Marseille soap.
Results

The following tables show the results of these activities:

**Table 1: Awareness-raising activities**

<table>
<thead>
<tr>
<th>Health districts</th>
<th>Number of localities</th>
<th>Targeted co-endemic districts</th>
<th>Number of people targeted by awareness-raising campaigns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ivo</td>
<td>10</td>
<td></td>
<td>3 700</td>
</tr>
<tr>
<td>Zouan-hounien</td>
<td>34</td>
<td></td>
<td>8 378</td>
</tr>
<tr>
<td>Oumé</td>
<td>20</td>
<td></td>
<td>8 540</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>64</strong></td>
<td></td>
<td><strong>20 618</strong></td>
</tr>
</tbody>
</table>

**Table 2: Screening**

<table>
<thead>
<tr>
<th>Health districts</th>
<th>Number of people consulted (presenting with skin lesions)</th>
<th>Type of lesions detected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BU  Leprosy  Yaw</td>
<td>Scabies  Ringworm</td>
</tr>
<tr>
<td></td>
<td>P  M  B</td>
<td></td>
</tr>
<tr>
<td>Divo</td>
<td>1 167 02 03 00 08 87 232 314</td>
<td>138</td>
</tr>
<tr>
<td>Zouan-hounien</td>
<td>8 805 01 14 06 00 00 - -</td>
<td>187</td>
</tr>
<tr>
<td>Oumé</td>
<td>1 062 03 04 03 00 54 243 492 72</td>
<td>72</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>11 034</strong> 06 21 09 08 141 473 806 397</td>
<td>397</td>
</tr>
</tbody>
</table>

Conclusion

The integrated awareness-raising and screening for diseases with cutaneous manifestations implemented in the three health districts constitutes a feasible strategy in Côte d'Ivoire. It has facilitated pooling of human resources, equipment and funding, and has made it possible to screen for a number of skin diseases in the same place at the same time. Thus, by providing a solution to the problems of screening for leprosy and Buruli ulcer in a context of reduced incidence of both diseases, the initiative has made it possible to note the coexistence of other common skin diseases such as scabies, ringworm and especially chronic ulcers with aetiologies that can be managed easily at the peripheral level.
Skin disease prevalence survey among primary schoolchildren in Côte d’Ivoire: focus on Buruli ulcer, leprosy, and yaws (skin NTDs): project implementation and preliminary results

Presented by Rie Roselyne Yotsu

Rie Roselyne Yotsu1), Amari Akpa2), Konan N’Guessan2), Aubin Yao2), Aka N’Guetta3), Emma Yeboue4), Norihisa Ishii5), Kouamé Kouadio3), Rigobert Abbet Abbet4), Bamba Vagamon6)

1) National Center for Global Health and Medicine, Japan; National Suruga Sanatorium, Japan; 2) MAP International, Côte d’Ivoire; 3) Pasteur Institute, Côte d’Ivoire; 4) National Leprosy Control Program, Ministry of Health, Côte d’Ivoire; 5) Leprosy Research Center, National Institute of Infectious Diseases, Japan; 6) Raoul Follereau Institute, Côte d’Ivoire

Background

Many neglected tropical diseases – including Buruli ulcer, leprosy, and yaws – present with skin symptom(s) (skin NTDs). This characteristic feature may facilitate early detection by village nurses / fieldworkers in endemic areas. We aim to leverage village nurses / fieldworkers to implement a school-based skin survey in early detection of skin NTDs in Côte d’Ivoire; a country with the highest reported number of Buruli ulcer cases globally. We are presenting our program implementation and preliminary results in the Adzopé health district.

Objectives

To establish school-based skin survey for early detection and treatment of skin NTDs and to describe the distribution and the disease burden of these diseases in Côte d’Ivoire.

Methods

This program took place in Adzopé from 16 Nov 2015 to 13 Jan 2016, and consisted of two phases: 1) screening by a team of village nurses of all primary school children aged 5 to 15 in a total of 50 schools (38% of all schools in the district), and selection of those presenting with any skin lesion(s): eczema, ulcers, warts; 2) in-school examination and management of screened children by two dermatologists.

Results

Among 13,019 children screened, 3,504 (27%) presented with skin lesion(s). Due to financial constrains and unavailability of dermatologists, only 986 were able to be consulted, and they presented a total of 1,633 skin diseases. The majority of diagnoses were fungal infection (n=1,153, 70%), followed by eczema (n=238, 15%), and scabies (n=36, 2.2%). One early case of multi-bacillary leprosy case was detected in a 12-year-old girl, before disability development. Treatment was provided for all diseases other than tinea capitis (omitted due to large number cases, benignity and potential for spontaneous healing after adolescence). The program had a high rate of acceptability.

Conclusions

This was the first attempt at an integrated, multi-skin NTD screening and diagnosis in the country. This strategy has the potential of improving early detection of NTDs, especially in areas of co-endemicity. We plan to expand the project to a wider region in Côte d’Ivoire and it could also be relevant in neighboring countries.

Further progress

We are currently in preparation to implement the project in the Gagnoa health district, Côte d’Ivoire. For this round, we are targeting a total of 9,930 schoolchildren from 38 villages, which accounts for 9.2% of the schoolchildren in the whole district. The 1st phase is scheduled during 12-16 Dec 2016,
and the 2nd phase is scheduled during 23 Jan-10 Feb 2017. We plan to share the results during the meeting.

**Extension of Buruli ulcer control activities in Kongo Central province, 2015-2016**

*Presented by Delphin Phanzu*

Phanzu DM\(^1\), Luzolo EK\(^1\), Diengidi BM\(^1\), Imposo DHBB\(^1\), van der Grinten E\(^2\), Saunderson P\(^2\)

\(^1\)Kimpese Evangelical Medical Institute, Buruli Ulcer Project, Kongo Central, DR Congo
\(^2\)American Leprosy Missions, Greenville, South Carolina, United States of America.

The Central Kongo Province is the location of one of the major foci of Buruli ulcer in the Democratic Republic of Congo, namely the territory of Songololo in the Cataractes district, where the bulk of control activities have been concentrated for more than a decade. The efforts deployed by the different disease control stakeholders, with support from our various partners, have facilitated a gradual extension of programme coverage. Since 2014, two new health areas known to be endemic have become incorporated into the programme each year. The results achieved over the past two years and the outlook for the future form the subject of this presentation.

Of 11 health areas known to be endemic for Buruli ulcer in the Province, 8 were covered by control activities (73%). A total of 312 patients were screened, 175 in 2015 and 137 in 2016. The proportion of ulcerative forms has remained stable at 85% over the past two years. We have noted no difference in functional limitations on diagnosis \((p = 0.515)\), lesion categories \((p = 0.246)\), or the proportion of patients treated on a decentralized basis, even though this increased from 88% in 2015 to 91% in 2016 \((p = 0.480)\). Patients diagnosed in categories I and II represented 80% of the total in 2015 and 76% in 2016. Last year, 74% of reported cases were confirmed by at least one positive laboratory test compared to 58% the previous year \((p = 0.002)\), and all the patients benefited from specific antibiotic therapy, compared to 79% the previous year.

Cases of Buruli ulcer have been detected and treated in all the health areas involved in our programme, confirming the endemicity of these areas. The decentralization of care in front-line health services has been reinforced through early detection of cases. Maintaining the current momentum, continuing coverage of all endemic areas and strengthening general-purpose health services remain major challenges.
Integrated approach for skin NTDs – Pilot initiatives to collect evidences

*Presented by Bernardo García Izquierdo*

Anesvad has been combating NTD since 1968. Intervention focus was originally addressed to leprosy elimination. In the late 90’s, Buruli ulcer was added to the operation portfolio. Both diseases have in common to be worldwide neglected as well as to have cutaneous manifestations.

Our recently updated mission states that Anesvad is committed to “**Combat the Neglected Tropical Diseases (NTD) in Sub-Saharan Africa from a human rights approach and in coherence with the Primary Healthcare (PH) strategy**”. This concentration effort looks for having more options to have a greater impact with the limited resources we manage. This approach is in correlation with the recent WHO NTD Strategy for achieving 2020 goals. One of the key components to be successful in controlling, eliminating or eradicating NTDs is to follow an integrated approach. There are scientific, organisational, resource mobilisation and political reasons to support this approach as the most logic and reasonable nowadays. Integration to combat NTDs or other infectious diseases is not new. The new thing about it is the historical possibility to take effective steps in reducing radically the burden of these conditions.

The main challenge linked to follow an integrated approach is the absence of evidence track that would allow implementers as well as decision makers to be sure about the way to proceed in each particular context. Another important obstacle is the necessary shift to move from vertical approaches to more horizontal strategies where combating certain neglected conditions is part of a larger programme to strengthen public health systems in areas and communities of extreme vulnerability and poverty. Last but not least is the reorganisation process that will be needed in the structure and organization chart of the health system.

In order to contribute to move forward the 2020 agenda, Anesvad is promoting and accelerating different pilot initiatives of integrated programmes in highly endemic countries of skin NTDs. Lessons learned from the goals, process and definition of these integrated programmes are of high value added both for further scaling up and dissemination to other endemic countries.
Community-based surveillance for case detection in GA West Municipality

Poster: Collins SK Ahorlu

Collins SK Ahorlu¹, Edwin Ampadu²,

¹Noguchi Memorial Institute for medical Research, College of Health Sciences, University of Ghana, Legon, Ghana; ²National BU Control Program Manager, Ghana Health Service Korle-Bu, Accra.

Background
Buruli Ulcer (BU) is one of the most neglected debilitating tropical diseases caused by Mycobacterium ulcerans, which causes considerable morbidity and disability. Building on earlier findings that community-based intervention could enhance case detection and reduce treatment dropout and defaulter rates, this study was initiated to train district and sub-district disease control and selected clinical staff to establish and validate active surveillance and response system for early buruli ulcer case detection, diagnosis and treatment in collaboration with trained community-based volunteers in an endemic sub-district in the Ga West municipality in Ghana.

Methods
Community census was conducted in the 10 selected communities; baseline data collected using a questionnaire survey with a sample size of 600. Training was then organized for selected clinical staff, disease control officers and community volunteers on early buruli ulcer case detection, diagnosis and treatment and how to collect community-based surveillance-response system data.

Result
The census results show that there were 3070 persons living in 837 households with a mean size of 3.7 (±0.52) in the 10 study communities. At baseline, 52.5% of those interviewed were females. Majority of respondents were self-employed. Majority (63.4%) said they could recognize the early signs of BU using Nodule/boil. Four surveillance rounds have been concluded (August – November 2016). In August 2016, three out of 2628 persons examined (one confirmed). In September 2016, 2736 persons examined (none confirmed). In October 2016, three out 2724 persons examined were suspected cases (all confirmed). In November 2016, one out of 2633 persons examined was a suspected case, and was confirmed. Out of the seven suspected cases, five were females. The suspected cases were made up of category 1 (2), category 2 (3) and category 3 (2) and aged between 38 and 85 years.

Conclusion
Preliminary data suggests that it is feasible to establish a community-based surveillance-response system for BU to enhance case detection, diagnosis and treatment to reduce BU-related disabilities, especially as BU cases are beginning to come down in some endemic communities.
Integrated morbidity management & disability prevention for tropical lymphoedema: issues for health systems

Poster: Gail Davey

Tsige Amberbir¹, Abebe Kelemwerk¹, Asrat Mengiste², Fikreab Hailemariam², Gail Davey³

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Background

Neglected tropical diseases (NTDs) resulting in lower limb lymphoedema are common in Ethiopia: recent nationwide mapping has demonstrated that leprosy accounts for approximately 12.8%, podoconiosis for 64.8% (approx. 1.5 million cases) and lymphatic filariasis (LF) for 0.3% of the total burden of lymphoedema. The WHO Global Program to Eliminate Lymphatic Filariasis’s 2013 ‘Aide-mémoire for programme managers’ recommends integrated morbidity management for people affected by chronic LF and podoconiosis, but precise guidance on how this might be achieved is not yet available. Three implementation projects in Ethiopia used here as examples of different approaches of:

1. Integrating lymphoedema morbidity management across NTDs; and
2. Integrating lymphoedema morbidity management into the existing health system.

Methods

Two projects piloted integration of disease management and disability prevention across NTDs: the first for patients with podoconiosis and leprosy and the second for patients with podoconiosis and LF. A further project illustrates four different approaches to delivery of care for patients with podoconiosis lymphoedema in four districts in northern Ethiopia. The key personnel charged with training patients in lymphoedema care/managing follow up varied in each district: District 1 – NGO health professionals/community-based government health workers; District 2 – NGO health professionals/patient-led groups; District 3 – NGO health professionals/faith-based communities; District 4 – health centre-based government health professionals (training and follow-up).

Results

The two cross-NTD projects demonstrated the feasibility of providing care for patients with lymphoedema of more than one cause. Of the four approaches to delivery of care, the District 4 approach reached and retained the most patients (229 vs 124, 137 and 213) and was the second most cost-effective (17.68 vs 25.01, 35.68 and 17.30 $/beneficiary). There were benefits to each approach which could be used to develop definitive guidance.

Conclusions

Important lessons can be learned for integration of disease management and disability prevention for lymphoedema from these small pilots across NTDs and of different approaches to delivery of care. Further formal implementation research is recommended to draw from and build on these pilot projects.
Opportunities for integrated control neglected tropical diseases that affect the skin

*Poster: Daniel Engelman*

Many neglected tropical diseases (NTDs) affect the skin, causing considerable disability, stigma and exacerbation of poverty. However, there has been relatively little investment into laboratory research, epidemiology, diagnostic tools or management strategies to control tropical skin diseases.

Integration may advance the control of skin disease across a range of domains, including mapping, diagnosis, clinical management and community control measures, such as mass drug administration. Examples of successful integration strategies include programs targeting scabies, impetigo, yaws and diseases causing lymphoedema. Future strategies should build on these experiences and the experience of integration of other NTDs, strengthen existing health systems, and contribute toward the attainment of Universal Health Coverage. Strong partnerships and political support and will be required to achieve these goals.
Community mobilization to scale up skin infections in the Ga West and South Municipalities of the Greater Accra Region of Ghana

Poster: Eric Koka

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Background

In our previous studies, we implemented a multidisciplinary project involving social scientist, microbiologists and clinicians to control Buruli ulcer in some endemic areas of Ghana. A striking observation made during community education, screening and surveys for BU was that other skin infections were often found in the endemic communities. Eight communities were identified as having high cases of skin infections especially among school children. Four out of these eight villages were randomly selected for a pilot study. Subsequently, screening for other skin infections, we identified leprosy, yaws and other skin conditions within these study communities.

Method

The study was conducted between July to December 2015 in the Ga West and South districts of the Greater Accra region of Ghana. The pilot study involved showing documentaries on Buruli ulcer and pictures of other skin infections, especially those depicting success stories of medical treatments in the night usually between 7 and 9 pm. The documentary was interspersed with questions and answers. Early morning mass screening for suspected skin lesions especially among school children were conducted. Samples were taken from all presumptive cases for laboratory confirmation at the Noguchi memorial Institute for medical Research.

Results

A total of 474 community members (including school children) were screened for all forms of skin infections in four villages. The children were 311 and adult community members were 163. The various cases of skin conditions identified and confirmed within the study communities from the pilot study were as follows: 27 cases of yaws, 18 cases of ring worm, 23 cases of eczema and 6 cases of leprosy. Fifteen (15) unidentified skin infections were also found in the study districts. 81.4% (22) of the yaws cases found were children between the ages of 5 to 17 years and 100% of the ring worm cases were also children. Treatment was provided for all cases of skin infections in their respective communities except leprosy which were referred to the Amasaman district hospital.

Conclusion

The data underline the need for a differential diagnosis system and expansion of our work beyond Buruli ulcer to include all other skin lesions including leprosy, ring worm and yaws for their etiology and appropriate care.
Screening for and treatment of neglected tropical diseases in school settings: The case of the Touboro health district in northern Cameroon

**Poster: Basiru Isa Manjo**

Authors: Manjo Basiru Isa and Dr Alphonse Um Boock

**Context**

Leprosy is a disease caused by *Mycobacterium leprae*, also called Hansen's bacillus. It is endemic in Cameroon. The fight against this disease began in the 1950s, in colonial times. The first campaigns were launched in 1956; at that time, the prevalence of leprosy was 42 new cases per 10,000 of population.

In 2002, the prevalence of leprosy crossed the threshold of 0.6 new cases per 10,000 of population. As a result, Cameroon has been classified among the countries that have eliminated leprosy as a public health problem. In 2006, the prevalence decreased to 0.34 new cases per 10,000 inhabitants. However, pockets of high prevalence of leprosy remain in the country.

Touboro in northern Cameroon is a district where active transmission of these diseases seems to be continuing. The influx of refugees could further aggravate the situation because of overcrowding. Indeed, during an exploratory mission to Touboro in 2015, 4 new cases of leprosy and 2 new cases of yaws were identified among refugees.

On 4-5 September 2016, an exploratory campaign to detect and treat leprosy and yaws was organized by the FAIRMED Foundation in Touboro.

**Objective and methodology**

The objective of the survey was to determine the prevalence of leprosy and yaws, and to treat all detected cases. It was a cross-sectional survey in the community, and bearing in mind that these diseases mostly affect children, we decided to target schools in the Dompta health area. The survey covered a population of 6879.

**Results and discussion**

Overall, 95 cases of yaws were detected during the survey, and 66% of these cases in schools. This observation shows that the school environment is ideally suited to neglected tropical diseases case-finding, particularly yaws. However, due to the low enrolment rate of children in this part of the country, such a strategy takes insufficient account of children. In our survey, children are the most affected, a finding borne out by the literature.

All the cases of yaws and leprosy have been recorded in the treatment registers of the health area concerned, which will ensure subsequent follow-up. A community intermediary has been assigned to monitor their treatment. The patient will be seen monthly at the health facility for an evaluation until completion of the treatment lasting 12 or 6 months, depending on the type of leprosy.

**Conclusion**

This study, although limited by insecurity in the region, has shown that neglected tropical diseases do exist in the health district of Touboro. It is important to integrate early detection and case management into the strategy for integrating health care in the national system.

**Keywords:** leprosy, yaws, screening, treatment, Touboro, school settings
A training guide for the recognition of NTDs through their skin signs.

Presented by Rod Hay

Many of the neglected tropical diseases present with visible changes to the skin that are distinctive and have long been identified by specialists as key diagnostic clues that provide an entry to both investigational and management pathways. At the suggestion of the WHO a group of subject specialists has produced a draft training manual to extend the diagnostic use of clinical signs of NTDs on the skin to front line Health Care Workers (HCWs). The diagnostic schemes are based on a syndromic approach using the common visible changes to the skin from ulcers to lumps on the surface. The manual is intended to help HCWs to identify suspicious lesions; these patients would be suitable for further investigation and complex management pathways. At the same time the guide is designed to help health workers to manage the very common skin diseases that they will encounter.
Training health professionals working on the field: Skin Neglected Tropical Diseases on-line course.

Presented by Carmen Carrion

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Universitat Oberta de Catalunya (UOC) together with World Health Organization (WHO) have implemented an interactive on-line course to train health professionals in cutaneous leishmaniasis (CL).

Thanks to the suggestion and advise of Dr. Jose Ruiz-Postigo from WHO an on-line specialization program has been organized. The e-learning Programme consists of 6 ECTS (European Credit Transfer System) targeted to clinicians, nurses or policy makers (150 study hours). The Faculty team is comprised of a tutor (Dr. Carme Carrion - UOC staff) and a consultant professor expert in dermatology (Dr. Mourad Mokni). The tutor follows the dynamics of the virtual classroom and defines activities and learning strategies. She accompanies all students in order to facilitate their learning and participation process. Student’s achievements are measured through continuous assessment activities together with a an initial and final multiple choice test to measure progress. At the end of the course students are asked to evaluate it through an on-line questionnaire that helps monitor the quality, in order to constantly improve the course.

Students receive up-to-date information on: natural history of the disease, epidemiology, diagnosis, treatment and surveillance. Teaching strategies are on-line, asynchronous and participatory, interacting with a dermatologist expert in the field (Dr. Mourad Mokni). It is based on scientific articles and WHO manuals and guidelines, as well as the study and the sharing of the different field experiences. The student’s achievements are measured through continuous assessment activities together with a final multiple-choice test, which is compared to pre-test at the beginning of the course. Once finished the students are asked to give their feedback of the course though an on-line questionnaire.

To date, three editions have already been made, two in 2014-15 (English and French) and one in French in 2016. The total number of people who enrolled for the courses was 47 from seven countries: Afghanistan, Algeria, Chad, Morocco, the Syrian Arab Republic, Tunisia and Yemen. The drop-out rate was 47% (21/47). The 92% (24/26) of the students who did the full course had a successful final assessment. When asked about their perceptions of the course, majority were very positive.

A new edition will be organized next autumn 2017 (October – February). A broader scope of the course content will be implemented in this future edition due to students and professors feedback. Next edition will be called: Skin Neglected Tropical Diseases clinical management, it will include skin diseases other than CL (Buruli Ulcer and Yaws among others) and it will be offered in English to worldwide students.

On-line training courses on clinical management of CL are a useful tool to train health professionals. Currently, n impact assessment of the Programme is being performed to better understand the mid-term and long-term outcomes of the course in terms of improving CL management and also the patients’ health outcomes in the areas where the students are developing their professional activities.
Skin4Lapp – a tool to support health care workers in dermatological care, specific in leprosy, Buruli ulcer and lymphatic filariasis

Presented by Anneke Taal

The ‘Skin4Lapp’ is a smartphone app on common skin diseases to support detection of not so common skin diseases, in specific leprosy, Buruli ulcer and Lymphatic Filariasis. Over the last 10 years, this has been a natural demand from supervisors and health workers in Mozambique. Common skin diseases are highly prevalent and there is a large scarcity of dermatologists: 10 dermatologists on a population of 27 million people. Many public health centers are run by clinical officers or nurses who have very limited training in dermatology. The Skin4Lapp includes a body map, symptom list and clear pictures of the disease to assist the health worker by early detection of common skin diseases, but the app also provides information on the treatment and follow-up.

The Netherlands Leprosy Relief (NLR) developed the ‘Skin4Lapp’ based on the adapted-from-Mahé’s algorithm for diagnosis and treatment of common skin diseases¹. A first version of the app was field tested for two weeks in Zambezia Province, Mozambique, both in urban and rural districts. Findings and feedback have led to an improved version of the ‘Skin4Lapp’ that now can be downloaded in Play Store. In February, NLR will field test a Portuguese version of the ‘Skin4Lapp’ in Mozambique to improve and distribute the ‘Skin4Lapp’ to a variety of health providers to enhance early case detection of skin-related NTDs through health systems strengthening.

At the WHO meeting on Skin NTDs, I would like to demonstrate the app, share some preliminary results and explore with the participants how to implement the ‘Skin4Lapp’ in other countries!

Telemedicine in wound care. A multi-disciplinary approach in low-resource settings developed by MSF

Presented by Sophie Delaigue

S Delaigue, E Comte, H Vuagnat, JJ Morand, R Wootton, T Aloudat

Introduction
Telemedicine has been used by Médecins Sans Frontières (MSF) for many years to support field health workers in low-resource settings, using both Store-and-Forward and real time services. The most common technique, Store-and-Forward, involves transmitting medical data such as medical clinical information and images to a medical specialist to be read at a later convenient time. In 2010, MSF launched a tele-expertise service with the aim of improving access to specialist medical advice for field workers. The MSF tele-expertise service, based on the Collegium Tele medicus system, has supported a total of 4647 clinical cases, to date, with the help of 306 active volunteer specialists in a wide range of specialties. Specialties which are heavily reliant on expert analysis of visual data, such as dermatology and radiology, are well suited to this model. Wound care expertise is another specialty with a strong visual component. In 2016, two wound care experts joined the MSF telemedicine network to assist its work.

Methods
We conducted a retrospective analysis of all cases managed by the wound care experts during their first year of operation. A descriptive analysis and a content analysis of these cases was performed by a single dermatologist with experience in telemedicine. The aim was to identify the benefits and limitations of telewound care in low-resource settings.

Results
During 2016 there were 19 clinical cases and 29 experts were involved in case management. Experts were specialized in orthopedic surgery (5), infectious diseases (5), pediatrics (5), general surgery (1), vascular surgery (1), dermatology (4), intensive care (2), wound care (2), cardiology (1), radiology (1), endocrinology (1) and neurology (1). The median time to provide the first specialist response was 2.9 hours (IQR 2.2 - 5.8) and the median number of experts by case was 3 (IQR 2-4). The most common topics treated were infectious diseases (11), diabetic foot (4), erythroderma (1), prevention and rehabilitation (1), surgical wound (1), trauma (1). The three main countries of origin of the cases were Central African Republic (8), Democratic Republic of Congo (3) and South Sudan (3). Tele-expertise had an important impact on wound care. In the tele-expertise cases, an additional significant diagnosis was made in 58%, 53% had their clinical management significantly altered by the consultation and 4 amputations were avoided. A request for information was sent to each referrer 21 days after the initial submission. The response rate was 26% (5/19). The responses were largely positive. All the referrers found the advice helpful.

Discussion
Telewound care is a useful tool: (1) To make a diagnosis. There are many possible diagnoses for patients with ulcerative lesions, which could be the manifestation of underlying illnesses, such as an infectious disease. Patients with Buruli ulcer, for example, have very long time delays to diagnosis and in five of the cases reviewed the diagnosis was not made prior to the teleconsultation, i.e. the initial request was about wound care treatment rather than diagnosis. (2) To assist caregivers in wound care treatment strategies. The treatment of skin ulcers requires frequent assessments of local wound status and adjustment of therapy. Embracing novel approaches to care can make wound care practices more efficient for all involved, such as nurses or general practitioners. (3) To assist with a multi-disciplinary team for difficult-to-treat cases (e.g. diabetic foot ulcer). (4) To prevent or rehabilitate is an important aspect of wound care.
Limitations of the study: (1) Limited number of cases and retrospective analysis. (2) Adapted advice to the limited field resources. In two of the cases reviewed the advice was considered by the field as not or only partially adapted to their limited resources. (3) Access to laboratory diagnosis can be a challenge. In a chronic wound without improvement, the risk of cancer requires a biopsy. Skin biopsies were not possible, which is a limitation. Buruli was discussed for 7 cases, direct microscopy was performed in 2 cases and was negative, none had PCR testing, whereas the WHO expectation is 70% of laboratory confirmation by positive PCR.

Conclusion
Management of wound care conditions in low-resource settings showed substantial benefits for all caregivers and was assisted by telemedicine. Tele-wound care expertise should be more widely available in the field.
Developing a Buruli ulcer community of practice in Bankim Cameroon: a model for Buruli ulcer outreach in Africa

Presented by Mark Nichter

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In the Cameroon, previous efforts to identify BU early through the mobilization of community health workers (CHWs) yielded poor results. In this presentation, we describe the successful creation of a BU community of practice (BUCOP) involving multiple stakeholders from clinic staff and former patients to CHWs and traditional healers. A community of practice is based on all stakeholders sharing a common objective, a common basic understanding of a focal problem, and mutual respect for what each stakeholder contributes to a process of problem solving. In the four year pilot project described, the creation of a BUCOP resulted in active and sustained community involvement in BU case identification, reduction in health care seeking delay, and a decline in treatment drop- out leading to better treatment outcomes. It also led to sustained partnerships between health staff, CHW, and traditional healers. CHW came to play a more active role in organizing BU outreach activities, and case follow -up resulting in a marked increase in their status within their community. Healers valued the partnerships established with health staff and found they gained more from collaboration than they lost from referral. An innovative outreach education also netted impressive results. Over the course of the project there was a shift from CHW and healers referring suspected cases of BU to clinics to self-referral. In addition to a significant rise in the number of cases of BU identified, treatment adherence rates rose and treatment dropout rates declined. The process of creating a BUCOP in a remote part of the Cameroon is described and lessons learned are highlighted.

Keywords: Buruli ulcer, community of practice, community outreach, culturally sensitive health education, health system strengthening
Integrating and strengthening the surveillance of Neglected Tropical Diseases with skin manifestation

Presented by Lise Grout

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Background

Effective disease control needs an effective disease surveillance. Early case detection, prompt treatment and surveillance are critical for the neglected tropical diseases requiring case-management (CM/NTDs). WHO has been working on the development and implementation of an integrated surveillance platform for CM/NTDs for a more efficient and sustainable health information system. Moreover, WHO promotes an integrated strategy for management and control of several NTDs sharing cutaneous manifestations – “skin NTDs”. The integrated platform for NTDs surveillance and control is the pillars of this integrated approach.

Methods

In 2016 the minimum data to be collected at health facility level and the indicators to be monitored have been defined for several skin NTDs. A paper-based patient form integrating information for four skin NTDs (Buruli ulcer, cutaneous leishmaniasis, leprosy and yaws) has been developed by WHO.

DHIS2 (District Health Information System, version 2), developed by the University of Oslo, has been chosen as the software to build the integrated platform. DHIS2 is a flexible, open-source information system, for reporting, analysis and dissemination of health data, with visualization features including GIS, charts, and tables. It has currently been implemented by around 50 countries, some of them being highly endemic for skin NTDs.

DHIS2 has been customized to reflect the paper-based integrated form into an online data collection tool, and computerize standardized indicators. Retrospective data has been imported in the online platform and dashboards have been created. Excel templates have also been developed to easily integrate data shared by countries that are not using DHIS2. Two other DHIS2 modules have been developed to enable good quality data collection during mass-drug administration of azithromycin against yaws and active case search activities in the community. These modules are available on several supports (computers, tablets and smartphones).

Results

Aggregated retrospective data has been imported for all endemic countries for the four skin NTDs. Individual retrospective data has been imported for Buruli ulcer for Benin and Ghana. Dashboards have been created to monitor trends and share interpretation of computed indicators.

Two workshops has been organized in Q4-2016 in Benin and Ghana to discuss the concept of integration of skin NTDs. Disease-specific experts were consulted for inputs. The draft paper-based integrated patient form for skin NTDs and the online platform has been presented to get feedback on the tool before the finalization of the first version.

In 2017, the new tools will be tested and capacity will be built at all the levels of the surveillance systems, from health facilities to global level. The first countries planned to be trained for prospective data collection in the first semester of 2017 are Benin and Ghana. As soon as possible, the modules will be integrated into national DHIS2 instances.
Conclusion

At national level, this online tool will strengthen the national health information systems, the skin NTDs surveillance at health facility and community level, and therefore strengthen the estimation of burden of skin NTDs and the evidence-based decision making process. It promotes and improve the data standardization, collection, analysis and dissemination at local, national, regional and global level. At global level, this data warehouse eases the collection of good quality data, the trends analysis and the monitoring and evaluation all the NTDs in a single place. This platform will highly contribute to the integrated control and management of skin NTDs.
The overall objective of Buruli ulcer (BU) control is “to minimize the suffering, disabilities and socioeconomic burden”, through early detection and antibiotic treatment as main strategy. During the 5th WHO Advisory Group Meeting on BU, March 2002, cultural and socio-economic studies were selected as one of the six priority areas to provide “immediate direct benefit to BU patients in the medium term”.

Fifteen years later, the scientific outcome on this priority area has been modest, especially the (socio-)economic aspects. The marginal number of presentations covering this priority area at the previous WHO meetings on BU speaks volumes. For instance, the costs of treatment of various forms of BU disease as well as the economic impact of the disease management on the health system, the community and the country at large, remain poorly investigated. Some recent papers, however, explored the direct and indirect costs of management strategies, but mainly from a patient or household perspective.

Furthermore, the economic evaluation (e.g., cost-effectiveness) of different control strategies (e.g., decentralization of case diagnosis and management, implementation of point-of-care tests, integration of skin NTDs) is lacking, but paramount for a disease facing limited resources for (sustainable) control.

Finally, in the third report on NTDs, the WHO stressed that preventing severe BU disability will require increased public investment in early (active) case detection, treatment and care: about US$ 4.3 million (US$ 3.8–4.8 million) per year may be required during 2015–2030 covering direct medical costs only.

Therefore, we believe that the establishment of a working group on health economics within the existing organizational structure of technical groups on BU is not a luxury but rather an essential element in evidence based policy making, especially as part of the efforts to reach the WHO’s NTD roadmap targets.

In this presentation, the authors will (i) glance through the main concepts of health economics; (ii) review the scanty literature on health economic issues of BU; (iii) discuss the main current and forthcoming challenges of health economic issues and its investigations in BU control and research; and (iv) illustrate how a working group on health economics will contribute to evidence based decision making at decentralized, national, and international level.

Selected references:


2 http://www.who.int/buruli/research/priorities/en/


5 WHO. Investing to overcome the global impact of neglected tropical diseases: third WHO report on neglected diseases. WHO. 2015.
Control sessions

Case management
Wound Treatment: It Is A Serious Matter!

Presented by Terry Treadwell

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The management of wounds is generally not thought to be a priority in medical care. This is unfortunate because acute and chronic wounds are considered to be a new worldwide epidemic. Appropriate management of acute and chronic wounds is important because it can improve the quality of life for people with wounds and can return them to productive, self-sufficient lives. This will help protect every country’s most precious resource—their people. Many healthcare providers view wound treatment with disinterest and frustration, may treat wounds with outdated and ineffective therapies, or may pass the patients with wounds to anyone willing to help the patient. Lack of training in the effective treatment of wounds adds to the frustration of the providers. Teaching the concepts of modern wound treatment is a challenge that must be met if progress can be made in wound healing and limb salvage.

The treatment of any wound, whether it be an acute wound or a chronic wound, requires six basic steps which should be approached in an orderly fashion. The first involves recognizing and treating the underlying systemic conditions of the patient. The remainder involve the wound and include debridement of any necrotic or infected tissue in the wound, protection of the wound from additional trauma, maintenance of optimal moisture balance to promote healing, treatment of any infection or bacterial imbalance in the wound, and management of any swelling of the extremity whether it be due to edema or lymphedema.

Following these basic principles when faced with managing any wound will allow the healthcare provider to confidently move the wound toward healing. These basics of treatment and outcomes of healing will be discussed in further detail.
Integrating Care for Neglected Tropical Diseases within Health services and Communities

Presented by Linda Faye Lehman

American Leprosy Missions

Introduction

Neglected Tropical Diseases (NTDs) are a diverse group of often disabling diseases among poorest populations. Currently over 1 billion people live with one or more NTDs. Many NTDS can cause acute illness, long-term disability, depression, social exclusion and early death. They frequently have limited availability and accessibility to quality health services and self-care practices to manage their disease and chronic conditions, even after the disease has been detected and treated. Good impairment and disability data is needed to monitor and evaluate disease outcomes and provide information on best evidence-based practices.

There are many similar interventions across NTDs. An integrated strategy and approach to care can strengthen health systems, empower people affected and promote inclusive communities. This presentation will demonstrate similarities of interventions used within NTDs and the gaps that limit integrated approaches to planning and implementation.

Methods

The WHO ICF framework will be used to describe the “disabling effects” of NTDs. A summary table will be presented showing common issues across many NTD’s. The author will use the PRECEDE-PROCEED model to describe predisposing, enabling and reinforcing factors used to evaluate, develop and implement an integrated care plan for acute and long term interventions.

Conclusions

Successful disease management and outcomes require accurate documentation, integrated treatment approaches within health services, participation of the person affected, their family and community.
Former Buruli ulcer patients' experiences and wishes may serve as a guide to further improve Buruli ulcer management

Presented by Anita Velink


Background

Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, is a neglected tropical disease frequently leading to permanent disabilities. The ulcers are treated with rifampicin and streptomycin, wound care and, if necessary surgical intervention. Professionals have exclusively shaped the research agenda concerning management and control, while patients' perspective on priorities and preferences have not explicitly been explored or addressed.

Methodology/Principal findings

To get insight into patient perception of the management and control of Buruli ulcer a mixed methods research design was applied with a questionnaire and focus group discussions among former BU patients. Data collection was obtained in collaboration with a local team of native speakers in Ghana. A questionnaire was completed by 60 former patients and four focus group discussions were conducted with eight participants per group.

Former patients positively evaluated both the effectiveness of the treatment and the financial contribution received for the travel costs to the hospitals. Pain experienced during treatment procedures, in particular wound care and the streptomycin injections, and the side-effects of the treatment were negatively evaluated. Former patients considered the development of preventive measures and knowledge on the transmission as priorities. Additionally, former patients asked for improved accessibility of health services, counselling and economic support.

Conclusions

These findings can be used to improve clinical management and to guide the international research agenda.
Implementation of a neighbourhood treatment scheme to improve management of Buruli ulcer in Ouinhi commune, Benin. Lessons learnt

**Poster: Arnaud Amoussouhoui**

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**Introduction**

The commune of Ouinhi is located in Zou department, the only endemic department in Benin where management of Buruli ulcer was not decentralized until recently. All patients, regardless of the category of their lesion, were therefore immediately admitted to the Zagnannado treatment center. The socioeconomic difficulties faced by patients and their households during hospitalization have been documented and found to be sources of resistance to and delay in seeking medical treatment. To make treatment more accessible and minimize difficulties, a neighbourhood treatment experiment was set up at a health centre in Ouinhi. This study describes the process, from implementation to outcome, of decentralized care and the lessons learnt.

**Method**

A descriptive study of the process for implementing an intervention over two years. The process comprises four stages:

I. Design phase, based on a situation analysis, documentation of epidemiological data and selection of the intervention site.

II. Advocacy phase, consisting of negotiation with the management of the central hospital responsible for Buruli ulcer treatment in the region; administrative formalities involving health area officials.

III. Implementation phase comprising training of health workers, awareness-raising and information sessions, formation of community support groups, consultations and case management.

IV. The evaluation phase, during which 23 individual interviews and two group interviews were conducted. An analysis grid was used to collect epidemiological data and intervention outcome data.

**Results**

The management of UB patients in the Ouinhi peripheral health centre was effective 4 months after initiation of the intervention. During the preparatory phase, four health workers were trained in the Buruli ulcer management protocol; three patient support groups were set up to encourage community involvement. These groups consisted of former patients, women volunteers and community intermediaries. Information on the availability of decentralized care was disseminated through 32 awareness and education teams in the villages of the commune.
After two years of operation, 29 category 1 and 2 Buruli ulcer patients were treated in the health center until healed. 12 category 3 cases were referred to the hospital at Zagnannado. The fact that Buruli ulcer treatment was available at the peripheral health centre meant that 48 people consulted on account of chronic ulcers; 14 of these cases were treated until recovery. Before the intervention, only traumatic ulcer cases were treated at the health centre. The evaluation demonstrated high levels of satisfaction on the part of patients and their households, with fewer difficulties compared to hospitalization. However, the referral to Zagnannado hospital of certain cases requiring surgery was perceived by the population as a lack of fairness in the care offered by the health centre.

**Conclusion**

The lessons learnt from the community care experiment show the challenges that need to be overcome to improve health conditions in poor communities, especially in the context of chronic disease management. These challenges call for a better organization of the health system with an integrated approach to the management of chronic diseases and active community involvement.

**Keywords:** Buruli ulcer, decentralized care, chronic ulcers
Outcomes in category 3 patients treated in Benin: Analysis of socioeconomic integration challenges and needs

Poster: Arnaud Amoussouhoui

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Introduction

Treatment outcomes in Buruli ulcer screening and treatment centres in Benin are poorly documented. It is known that, in some patients, Buruli ulcer causes functional limitations that have adverse consequences in everyday life. This study aims to document outcomes in category 3 patients, the most vulnerable group, following scar formation.

Method

A retrospective, descriptive study was carried out in 14 communes covered by four Buruli ulcer screening and treatment centres (Allada, Lalo, Pobé and Zagnannado) in Benin. The targets were former patients in category 3 who had completed their treatment between 2011 and 2015. In all, 274 former patients of the 563 cases treated were located and interviewed in accordance with semi-structured interview guidelines. Data on 72 healed patients who had left the area were supplied by friends and family. The degree of disability and the needs of all the category 3 healed patients who were tracked down were assessed using a questionnaire.

Results

The data showed that the difficulties which patients experience after hospital treatment vary according to sex and age. Mobility limitations are the most frequent problem. Activities requiring maximum effort, such as agricultural work, become impossible. Men opt to emigrate to the cities in search of work in the tertiary sector. After long stays in hospital, children drop out of school and are directed towards learning a trade. Scar management is another challenge for these treated patients. In approximately 32 cases, the scar was found to have reopened and become superinfected; this makes people in the community think that the disease is incurable. Twenty-two (22) cases of death (3.24%) after scarring were identified.

Conclusion

The analysis of outcomes in category 3 patients highlights issues with the definition of health in the management of Buruli ulcer in Benin. Patients treated in Benin are cicatrized but do not regain health, i.e. "a state of complete physical, mental and social well-being, and not merely the absence of disease or infirmity". Appropriate efforts are still required in the case of persons rendered vulnerable by the disease.

Keywords: chronic ulcers, treatment, scar formation, participation
**Description of Buruli ulcer cases originating in Nigeria and treated in Benin, 2006—2016**

*Poster: Gilbert Ayelo*

Gilbert Ayelo, Ange Dossou, Jean Gabin Houezo, Ghislain Sopoh, Esaï Anagonou, Yves Barogui, Anita Wadagni, Julia Aguiar, Roch Johnson, Arnaud Amoussouhoui

**Introduction**

Buruli ulcer is an infectious skin disease caused by a mycobacterium occurring in the environment, *Mycobacterium ulcerans*. It is managed in Benin through 4 specialized centres which, because of their proximity to Nigeria, also admit cases from that country. The aim of this study is to describe the epidemiological, clinical, biological and therapeutic characteristics of these cases of Buruli ulcer originating in Nigeria and treated in Benin in the period 2006-2016.

**Method**

A retrospective, descriptive study of the 174 confirmed cases of Buruli ulcer originating in Nigeria and treated at the 4 Buruli ulcer screening and treatment centres in Benin in the period 2006-2016. The data were analysed using IBM SPSS Statistics Version 20 statistical software.

**Results**

224 suspected cases of Buruli ulcer from Nigeria were treated at centres in Benin between 2006 and 2016. Specimens were taken from 188 (84.0%) of patients for laboratory confirmation; 174 (78%) of these cases were confirmed as Buruli ulcer by PCR.

69.0% (120/174) of confirmed Buruli ulcer patients were from Ogun state and 51.0% (89/174) were male. The median age of the patients was 15 years (IQR: 8-30 years). 80% (139/174) of the patients had ulcers and 66.1% (115/174) were category 3 cases.

**Conclusion**

The study found that the majority of Buruli ulcer cases originating in Nigeria and treated in Benin were ulcerative and category 3. This is contrary to the trend observed in recent years in Benin and calls for collaboration between Buruli ulcer control stakeholders in Benin and Nigeria.
Ozone Therapy after surgical skin graft of Buruli ulcer

Poster: Antonella Bertolotti

Antonella Bertolotti; Annunziata Izzo; Luca Papetti (Intermed Onlus) M.L. Iabichella. (Eliosmed Onlus)

During the last five years of our work in Benin, Ghana and Ivory Coast, Intermed Onlus has faced several cases of Buruli ulcer which we have treated with “ozone therapy”.

The genesis of employing such treatment lies in our research and review of studies which confirm the bactericide and virus static properties of ozone.

Based on such research, Intermed Onlus applied ozone on various patients suffering from a Buruli ulcer; it would appear that ozone therapy is effective in eliminating mycobacterium ulcers, as published in a British Medical Journal report on our work.

Additionally, a common problem associated with one of the traditional treatments for Buruli ulcer, being skin transplants, is that post-operative wounds do not heal properly and continue to produce fibrin and exudate.

Given the aforementioned properties of ozone, Intermed Onlus has applied ozone to surgically treated wounds (which have been re-examined using the Ziehl-Neelsen stain test and PCR) and results indicate that ozone therapy causes the immediate disappearance of fibrin and, after just three applications, the appearance of granulation tissue, indicating the beginning of a complete recovery.
Prevention of disability programme at the Allada Buruli Ulcer Detection and Treatment Centre: organization and results 2006-2011

Poster: Joseph Capo-Chichi

Authors: Capo-Chichi S.J.M.H.1, Sopoh G.E.1,2, Dossou DA1, Houézo JG2, Agossadou D3, Johnson RC4

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Introduction

Treatment for Buruli ulcer includes specific antibiotic therapy, surgery, and also the prevention of disabilities. The essential physiotherapy component of prevention of disability is indispensable for scar formation without limitation of activities. This article describes the experience of the Allada Buruli ulcer detection and treatment center in implementing this component.

Method

This retrospective study focused on confirmed cases of Buruli ulcer that had undergone a complete course of physiotherapy and had a full medical file, treated at the Allada Buruli ulcer detection and treatment centre between 1 January 2006 and 31 December 2011. The data were extracted from patient records in a special file recorded in Excell® and analysed using Epiinfo 7.

Results

Of the 333 patients admitted to the treatment centre in this period, 102 met the inclusion criteria. The largest cohort was from 2006 (32/102), or 31% as against 7% in 2009 (8/102). The cohorts from 2006 to 2011 comprised 72 patients (70.59%), and those from 2007 to 2011 comprised 30 patients (29.41%).

57.33% (42/72) of the patients admitted between 2006 and 2008 had tissue retractions, 16.67% (12/72) had joint stiffness and 25% (18/72) were treated from a preventive care perspective. In contrast, 56.67% (17/30) of patients admitted between 2009 and 2011 had tissue retractions, 6.67% (2/30) had joint stiffness and 36.67% (11/30) were treated from a preventive care perspective.

Patients treated between 2006 and 2008 benefited from fewer physiotherapy techniques than those treated between 2009 and 2011. The time spent in hospital (median, Q1-Q3) did not differ significantly in the period 2006-2008 (89.5, 58-143.5) compared with the period 2009-2011 (104; 53.5-146) (p = 0.87). Despite a downward trend, the proportion of patients discharged with an activity limitation was not significantly different in patients admitted between 2006 and 2008 (22%) and patients admitted between 2009 and 2011 (10%) (P = 0.150).

Conclusion

Weaknesses in the statistical tests did not make it possible to draw clear conclusions regarding the role of physiotherapy in the management of Buruli ulcer. But there have been many advances in the field, which have helped to improve treatment. Despite the progress, however, it is important to develop validated prevention of disability protocols for the management of Buruli ulcer so as to ensure continuous improvement in management of the disease.

Keywords: Buruli ulcer, prevention of disability, physiotherapy
Preliminary data about wound care management in Buruli ulcer — Evidence from lesions complicated by Topical (T-MC) and non-Topical Microbial Colonization (nT-MC)

Poster: Maria Isabella Iabichella

Iabichella ML*, Fusari V**, Topolinska M*; Amaku Anzako C***, Pediliggieri C*, Iabichella A*
* Helios Med Onlus – International Health Cooperation
** University of Pavia
*** Ariwara Hospital, R. D. Congo, Africa Centrale

Background

Since 2012, in DR Congo and Benin, Helios Med Onlus has drawn up a form to collect demographic and clinical data for the treatment of skin lesions. Helios Med Onlus detects infections in acute and chronic skin lesions due to Buruli Ulcer (BU) or other aetiology (n-BU), which influence not only the healing time of the trophic lesions but also the patients’ quality of life. The aim is to provide training in Wound Care Management with Ozone Therapy and Hyperoil to local health workers, in order to meet the local needs.

Helios Med Onlus has developed a method to topically clean wounds using ozone and Hyperoil. The best administration is 3 times in the first week, positioning a bag around the lesion and insufflating an O₂-O₃ mixture at a concentration of 20-30 μg/ml. The inflated bag is sealed just above the lesion to avoid gas leakage. Then, the bag is positioned to let the gas mixture in contact with the ulcer for around 20 minutes. When the bag is removed, the wound will be medicated as usual, applying a few drops of Hyperoil in the ulcer bed with a gauze every two days until complete healing. An uncompressed bandage protects the wound from dust.

The properties of ozone and Hyperoil avoid wound worsening, reduce the risk of infection and stimulate the tissue repair. Therefore, its use in chronic BU was effective. The extract of Hypericum flowers (Hypericum perforatum) and Nimh oil (Azadirachta indica) due to its antiflammatory, antiedemigen, bacteriostatic and natural healing properties, has been successfully used in complicated wounds. Furthermore, it is easy to be managed by the caregivers, above all far away from Buruli Ulcer Depistage Centre, and in absence of equipments and power to produce ozone.

Methods

BU's have been diagnosed according to WHO guidelines. Processed data concern 96 lesions complicated by topical microbial colonization (T-MC) due to different causes (BU, non-BU like post-cathether, post-intramuscular injection, post-snake bite, post-mastitis, post-c section, post-operation): 26 acute (1 new BU: 1F; 25 n-BU: 20F and 5M), and 70 chronic (39 BU: 20F and 19M; 31 n-BU: 13F and 18M) followed until complete healing or drop out. Moreover, processed data include 96 lesions without T-MC (nT-MC) due to different causes (BU, n-BU): 39 acute (8 new BU: 6F and 2M; 31 n-BU: 24F and 7M), and 57 chronic (28 BU: 10F and 18M; 29 n-BU: 10F and 11M) followed until complete healing or drop out.

The first week the treatment was performed with ozone bag and with a few drops of Hyperoil applied in the lesion. Hyperoil was applied in the wound every two days, after cleaning with saline water, until complete recovery. No systemic antibiotics has been given to patients during the topic treatment of their lesions.
Results

As Table 1 shows, T-MC acute lesions are 27.1% (1% BU and 26.1% n-BU) and nT-MC acute lesions are 40.6% (8.3% BU and 32.3% n-BU). Whereas, M-TC chronic lesions are 72.9% (40.6% BU and 32.3% n-BU) and nT-MC chronic lesions are 59.4% (29.2% BU and 30.2% n-BU). BUs are many among chronic lesions, but n-BUs are more among acute lesions.

A higher number of women (20.8%) looks for health care in case of acute lesions, both T-MCs and nT-MCs, above all in case of n-BUs due to post c-section lesions. No difference between sexes is registered in case of T-MC BUs (19.8% M; 20.8% F) and T-MC n-BUs (18.8% M; 13.6% F). When men have nT-MC chronic lesions, they look for treatment more than women (M: 18.8% BU and 19.8% n-BU; F: 10.4% BU and 10.4% BU and 10.4% n-BU). Conversely, women are more careful in case of nT-MC acute n-BUs (25.0%) than men (7.3%), as well as in case of nT-MC acute BUs (2.1% M; 6.3% F).

Table 1

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Type</th>
<th>Sex</th>
<th>New/Acute (%)</th>
<th>Chronic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-MC</td>
<td>BU</td>
<td>M</td>
<td>1 (1.0)</td>
<td>39 (40.6)</td>
</tr>
<tr>
<td></td>
<td>n-BU</td>
<td>F</td>
<td>25 (26.0)</td>
<td>31 (32.3)</td>
</tr>
<tr>
<td>nT-MC</td>
<td>BU</td>
<td>M</td>
<td>8 (8.3)</td>
<td>28 (29.2)</td>
</tr>
<tr>
<td></td>
<td>n-BU</td>
<td>F</td>
<td>31 (32.3)</td>
<td>29 (30.2)</td>
</tr>
</tbody>
</table>

As Table 2 shows, in case of T-MC the debridement time and the healing time in both acute and chronic lesions is longer in BUs than in n-BUs, but it does not differ in nT-MC lesions in both BUs and n-BUs.

Debridement time in T-MC and nT-MC acute and chronic lesions is higher in BUs (T-MC: 18±0.0 new and 9.6±0.0 chronic; nT-MC: 5±1.0 new and 6.2±3.1 chronic) than in n-BUs (T-MC: 3.7±1.4 acute and 7.2±2.1 chronic; nT-MC: 3.0±2.1 acute and 5.0±1.9). Also healing time is higher in T-MC and nT-MC new and chronic BUs (T-MC: 65.0±0.0 new and 51.7±10.5 chronic; nT-MC: 10.8±7.3 new and 28.0±10.0 chronic) compared to n-BUs (T-MC: 10.8±7.3 acute and 44.4±24.7 chronic; nT-MC: 15.0±7.1 acute and 21.0±14.1 chronic).

Table 2

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Debridement time (days)</th>
<th>Healing time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>New/Acute</td>
<td>Chronic</td>
</tr>
<tr>
<td>T-MC</td>
<td>BU</td>
<td>18.0 ±0.0</td>
</tr>
<tr>
<td></td>
<td>n-BU</td>
<td>3.7 ±1.4</td>
</tr>
<tr>
<td>nT-MC</td>
<td>BU</td>
<td>5.0 ±1.0</td>
</tr>
<tr>
<td></td>
<td>n-BU</td>
<td>3.0 ±2.1</td>
</tr>
</tbody>
</table>

Only 67.90% of patients follow the treatment until healing. As Table 3 shows, drop-out is higher for chronic ulcers, both T-MCs (BU 2.0%; n-BU 14.6%) and nT-MCs (BU 2.1%; n-BU 8.3%). Drop out is higher for men having chronic T-MC (9.4% M; 5.2% F) and nT-MC (8.3% M; 0.0% F) n-BUs than T-MC (1% M; 1% F) and nT-MC (2.1% M; 0.0% F) BUs. There is no drop out for new BUs for both sexes in T-MCs, and it is minimal in nT-MCs (M 1.0%; 0.0% F), and it is lower than in T-MC (1.0% M; 1.0% F) and nT-MC (2.1% M; 0.0% F) acute n-BUs.
**Table 3**

<table>
<thead>
<tr>
<th>Aetiology</th>
<th>Drop out</th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td></td>
<td>M</td>
<td>F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T-MC</td>
<td>BU</td>
<td>1 (1.0)</td>
<td>1 (1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-BU</td>
<td>1 (1.0)</td>
<td>1 (1.0)</td>
<td>9 (9.4)</td>
<td>5 (5.2)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>nT-MC</td>
<td>BU</td>
<td>1 (1.0)</td>
<td>2 (2.1)</td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n-BU</td>
<td>2 (2.1)</td>
<td>8 (8.3)</td>
<td></td>
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</tbody>
</table>

**Conclusion**

BUs and n-BUs without microbial colonization have shorter debridement and healing times. Non-surgical debridement in BUs and in n-BUs seems to be complicated by skin microbial colonization (T-MC).

As is known, microbial colonization can lead to lesion chronicization and, when it does not affect new and chronic BUs, their healing time is higher than the one of lesions having other aetiology. Instead, when BUs are complicated by microbial colonization, they need significantly longer healing time than n-BUs, and also they require a longer debridement time, above all new BUs.

Topical microbial colonization significantly affects the lesion healing time compared to the debridement time of acute and chronic infected ulcers, irrespective of their aetiology. Therefore, data suggest that topical treatment of BUs necessarily requires antiseptic dressings able to reduce the risk of local infection and effectively stimulate the granulation tissue, and the complete tissue repair.

Microbial colonized acute BUs and n-BUs are more frequent for women than for men, who show more BU and nBU chronic ulcers and look more frequently for medical care. Conversely, topically microbial colonized chronic BUs show a similar percentage for men and women, but chronic BUs without topical microbial colonization are higher for men than for women. Thus, training local health workers is relevant to prevent topical microbial colonization, in order to avoid chronicization and complications related to management.

Therapeutic compliance is optimal at 1-2 weeks and reduces to 1-3 months. An effective treatment in a short time is respected, but if it lasts more than 2 weeks, although it improves the lesion, it is not completed by the majority of people and promotes drop out, with slight differences between the sexes. This figure is attributable to several factors, such as lack of confidence in foreign therapies new for the local cultural environment; difficulties to bear the costs; improving the health status or re-employment; lack of immediate improvement or deterioration. All these factors can lead to drop out. Patients having BU seem more adapt to follow the treatment than patients suffering from other chronic ulcers. Probably this data depends on the awareness raising campaigns on BU promoted by WHO through the local Centres de Dépistage et de Traitement de l’Ulcère de Buruli.
Integrated management of Buruli ulcer and leprosy wounds and complications in Kimpese region, Democratic Republic of the Congo

Poster: Désiré Imposo

by D. H Imposo B.B., B. Dunda, D. Phanzu

Evangelical Medical Institute General Referral Hospital

Introduction

For several decades, leprosy control (neglected tropical disease/case management) has been conducted in a number of tropical and subtropical countries under the auspices of WHO with support from governmental partners and non-governmental organizations. Likewise, efforts to control Buruli ulcer, a neglected tropical disease similar to leprosy that involves a case-management approach, have now been under way for nearly two decades.

Notwithstanding the good result obtained in all cases, it must be noted that almost all the programmes involved in these control efforts have hitherto used a vertical approach. In order to pool resources and make control efforts more efficient, WHO has for many years advocated an integrated approach to controlling several neglected tropical diseases. This was reflected in World Health Assembly resolution WHA66.12 of May 2013 adopted by Member States, which calls on Member States to step up and integrate control measures to improve the health of populations affected by neglected tropical diseases. However, until now, integration has been slow to materialize in a number of countries, including in the Democratic Republic of the Congo.

Context and explanation

Leprosy and Buruli ulcer have been deliberately taken as a model for integration in this presentation, not only because of their close resemblance as diseases with cutaneous manifestations but also because of the presence in Kimpese region of established structures and real opportunities for integration. (Kimpese region comprises two health districts, called health zones, one large General Referral Hospital at the Evangelical Medical Institute and another, more modest hospital, with a catchment area of approximately 100km, accommodating patients from adjacent health zones and neighbouring Angola). Yaws, a disease with cutaneous manifestations, has not been taken into account because the condition has not yet been diagnosed in our region. Nor have we taken into account other neglected tropical diseases requiring case management which do not have cutaneous manifestations but may lead to complications requiring specific management at the General Referral Hospital, owing to a lack of data (unknown prevalence).

Opportunities conducive to integration

• Presence of two general referral hospitals in the region
• Presence of health workers qualified to treat both diseases, although seldom at the same time
• The traditional partners supporting the two conditions are identical, etc.

Integration per se

At the Evangelical Medical Institute General Referral Hospital, about 10 patients suffering from Buruli ulcer complications are treated by the resident specialist team, whereas at the hospital’s former leprosarium three kilometers from the main hospital another team that looks after a dozen people with leprosy complications. Both teams should now work together to integrate activities such as wound care, prevention of disability, surgery, etc. This would also be the case at the peripheral level in the areas of detection, awareness-raising, supervision and other activities, not only for leprosy and Buruli ulcer but also for other neglected tropical diseases requiring a case management approach. Only complicated cases will be referred to the General Referral Hospital.
Conclusion

The Kimpese region has major strengths in terms of integrating the satisfactory management of leprosy and Buruli ulcer wounds and complications, bearing in mind that some challenges will need to be overcome such as the distance between the main hospital and the former leprosarium and other facilities.
Benefits of including morbidity and disability data within unified skin NTD electronic data systems

Poster: Linda Faye Lehman
American Leprosy Missions

Introduction
Neglected Tropical Diseases (NTDs) are a diverse group of often disabling diseases among poorest populations. The lack of data on morbidity and disabling consequences of NTDs limits our knowledge about the true size of the problem, as well as the quality of disease outcomes. Inclusion of data allows monitoring and evaluation of activities and outcomes, providing valuable information for evidence base practices.

Methods
Discussion on key impairments and disabling issues across skin NTDs and Lymphatic Filariasis will be presented. Examples of data will be presented for reflection and discussion.

Conclusions
Resources are needed to fund interventions for morbidity management and disability that may be lifelong. This information needs to be part of monitoring and evaluation for adequate planning of services and interventions. Furthermore, data can be used to help with advocacy and development of inclusive health and community services.

Recommendation
Inclusion of morbidity and disability documentation and data within a unified electronic data systems permits us to identify and measure the size of NTD impairment and disability as well as provide evidence based data about outcomes from interventions. The author recommends the development and inclusion of care data within a unified electronic data system across skin NTDs that also includes Lymphatic Filariasis.
Improving limitations of movement evaluation and interventions in Buruli ulcer

*Poster: Linda Faye Lehman*

American Leprosy Missions

**Introduction**

The inconsistent and inaccurate measurements to identify Limitations of Movement (LOM) in Buruli Ulcer contribute to a delay in interventions to improve LOM. Comparison of LOM documented on BU form with patients, demonstrates that between 20-30% of people with LOM are not identified. As a result interventions are not done or are started late, which can affect outcomes.

**Methods**

This presentation will show examples of LOM not identified in BU and demonstrate methods for evaluating and documenting LOM in Buruli Ulcer. Key interventions to improve LOM will be demonstrated.

**Conclusions**

Early identification of LOM and immediate actions to improve mobility can prevent or minimize long-term mobility limitations. Best results are observed when there are combined interventions done during wound management, hospital and community health education programs to empower the person affected the their family to do self-care and physiotherapy rehabilitation activities.

**Recommendation**

Accuracy in identifying LOM can be improved by comparing affected and non-affected sides together. Teamwork and the empowerment of the affected person and their family/caregiver to take needed daily actions to manage and care for these problems is key to preventing and sustaining mobility during and after antibiotic treatment.
Antibiotic complications during the treatment of *Mycobacterium ulcerans* disease in Australian patients

**Poster: N. Deborah Friedman**

Daniel P O’Brien¹,²,³#, N Deborah Friedman¹, Andrew Hughes¹, Aaron Walton¹, Eugene Athan¹.

Department of Infectious Diseases, Barwon Health, Geelong, Victoria, Australia¹; Department of Medicine and Infectious Diseases, Royal Melbourne Hospital, University of Melbourne, Melbourne, Victoria, Australia²; Manson Unit, Médecins Sans Frontières, London, United Kingdom³

**Introduction**

Antibiotics are the recommended first-line treatment for *M. ulcerans* disease. Antibiotic toxicity is common in Australian patients, yet antibiotic complication rates and their risk factors have not been determined.

**Methods**

A retrospective analysis of antibiotic complications was performed using data from a cohort of all *M. ulcerans* cases managed at Barwon Health from 1/1/1998-30/6/2016. An antibiotic complication was defined as an antibiotic adverse event that required its cessation. A Poisson regression model was used to assess antibiotic complication rates and their associations.

**Results**

The study included 337 patients; 184 (54.6%) were male and median age was 57 years (IQR 36-73 years). Median antibiotic treatment duration was 56 days (IQR 49-76 days). Overall, 75 (22.2%) patients experienced an antibiotic complication after a median 28 days (IQR 17-45 days) at a rate of 2.71 per 100 person-years (95% CI 2.16-3.40). Individual antibiotic complication rates per 100 person-years were: rifampicin 1.76 (95% CI 1.34-2.33), clarithromycin 1.95 (95% CI 1.34-2.84), ciprofloxacin 2.21 (95% CI 1.57-3.11), ethambutol 4.61 (95% CI 1.92-11.06), moxifloxacin 3.33 (95% CI 1.07-10.32) and amikacin 12.21 (95% CI 3.09-37.86). Eleven patients required hospitalization.

In a multivariable analysis, the complication rate was significantly increased with reduced estimated glomerular filtration rates (aHR 2.65, 95%CI 1.25-3.76 for EGFR 60-89 mls/min and aHR 1.43, 95%CI 0.54-3.76 for EGFR 30-59 mls/min compared with EGFR ≥90 mls/min, p=0.01) and female gender (aHR 1.94, 95%CI 1.23-3.07, p<0.01).

**Conclusions**

Antibiotic complications during *M. ulcerans* treatment are high with increased rates independently associated with reduced renal function and female gender.
Mycobacterium ulcerans disease management in Australian patients: the re-emergence of surgery as an important treatment modality

Poster: Daniel O’Brien

Daniel P O’Brien1,2,3#, N Deborah Friedman1, Eugene Athan1, Peter Callan4, Anthony McDonald4.

Department of Infectious Diseases, Barwon Health, Geelong, Victoria, Australia1; Department of Medicine and Infectious Diseases, Royal Melbourne Hospital, University of Melbourne, Melbourne, Victoria, Australia2; Manson Unit, Médecins Sans Frontières, London, United Kingdom3, Department of Plastic Surgery, Barwon Health, Geelong, Victoria, Australia1

With the advent of effective antibiotic treatment, the management of Mycobacterium ulcerans disease has changed from a mainly surgically to a mainly medically treated disease. However, in Australian patients, antibiotic treatment alone is associated with prolonged wound healing times, high rates of treatment toxicity, and significant tissue destruction associated with severe paradoxical reactions. We will present the current state of Mycobacterium ulcerans management in Barwon Health, Australia, where a close working relationship exists between the infectious diseases and plastic surgical units, and where treatment has evolved based on nearly 20 years of experience gained from managing a Mycobacterium ulcerans epidemic on the nearby Bellarine and Mornington Peninsulas. In our experience, surgery has re-emerged to play an important role in the treatment of Mycobacterium ulcerans in improving the rate of wound healing, minimising antibiotic associated toxicity and preventing further tissue loss associated with severe paradoxical reactions. For selected lesions surgery without antibiotics can also be an effective treatment option.
Trial use of modern dressings in the treatment of Buruli ulcer at Kimpese Evangelical Medical Institute

Poster: Delphin Phanzu


1Kimpese Evangelical Medical Institute, Buruli Ulcer Project, Kongo Central, DR Congo
2American Leprosy Missions, Greenville, South Carolina, United States of America.

Summary. Buruli ulcer is a necrotizing disease of the skin, subcutaneous tissue and bone, of infectious origin, the causative agent of which is the mycobacterium Mycobacterium ulcerans which occurs in the environment. The onset of the disease is usually characterized by a painless nodule that evolves in the absence of treatment towards extensive ulceration, followed by severely disabling sequelae. In Africa, the majority of cases are still diagnosed at a late stage and in some countries about 70% of cases are ulcerative. To a large extent, these ulcers are still managed using traditional dressings based on disinfection by antiseptics. To date, the use of modern dressings is confined to a handful of health centres on the African continent.

We report our experience on the use of modern dressings for the treatment of Buruli ulcer in a resource-constrained context. Eight patients admitted to the general surgery department of the hospital of the Kimpese Evangelical Medical Institute in the Democratic Republic of the Congo between February and June 2005 were able to benefit from an absorbent Vacutex®-type dressing and the results were satisfactory. Compared to local treatments based on ordinary compresses in common use, the cleansing and granulation processes appeared to be faster, thus reducing the time required before the skin graft could be performed. In less than three weeks on average the wounds were ready for grafting, with obvious consequences for the healing time and the duration and cost of hospitalization.

Our study highlights the potential benefit to patients, health services and the community of using this type of easy-to-use dressing. It stresses the importance of promoting their wider use and training health workers in endemic areas to adopt modern methods.
Direct medical costs of in-patient care of Buruli ulcer in the Democratic Republic of the Congo: an exploratory case study

Poster: Patrick Suykerbuyk

Patrick Suykerbuyk*, Delphin Mavinga Phanzu§, Blanchard Diengidi§, Jaume Puig-Junoy*

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Background
Since the end of 2004, general reference hospital of the Institut Médical Evangélique Kimpese (GHRIMEK) launched a specialized BU program sponsored by American Leprosy Missions (ALM), aimed to (i) improve the patient care of BU patients admitted at GHRIMEK offering in-patient treatment free of-charge and supplementary aid; and (ii) promote early community-based detection of suspected BU cases. Furthermore, the launch of the BU control project coincided with the introduction of antibiotic therapy as additional or sole treatment to wound care, surgery and scar management.

From 2010, the first phase of this BU control project was expanded with community-based control and management activities of BU cases in two health zones in Songololo Territory.

Objective
We explored the effect of the implementation of this program on the direct medical costs associated with in-patient care of Buruli ulcer patients in a General Rural hospital

Methods
The study was a retrospective study analyzing the financial cost (as opposed to the economic cost) from the perspective of the funding agency (ALM) who is financing the BU control project at GHRIMEK and the decentralized BU management program in Songololo Territory. We limited our analyses to the direct medical cost covered by ALM, including hospitalization costs, medical treatment and nutritional aid. These financial costs correspond to the hospital bill that the hospital charges the BU control project funded by ALM. We analyzed both clinico-epidemiological characteristics and median total hospital fees from Buruli ulcer patients admitted at the hospital before (2005-2009) and after (2010-2015) decentralization.

Results and conclusions
We have shown that median direct medical costs for in-patient care of BU patients varied significantly, i.e., 913 USD (IQR=1175) and 1319 USD (IQR=1398), respectively before and after the implementation of the decentralized activities. Our findings have indicated that the increased cost of the hospitalization component played a significant role in the increase of the median direct medical costs. Furthermore, our data suggest that this increase in hospitalization costs after decentralization is related to the more complicated clinical profile of BU patients at admittance. This resulted in (i) more complicated (and expensive) treatment strategies; (ii) a significant increase in the length of stay of BU patients; and (iii) more treatment outcomes with limitations of movement at any joint.

The decentralized program increased the admission of patients with functional disabilities, challenging treatment outcomes and raising the median total hospital fee significantly.
Modern wound care for resource limited settings: teaching the basics

Poster: Hubert Vuagnat

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Since the 1960s, wound care for chronic wounds has made huge advances through better knowledge and material. Despite this, limited resource settings did not benefit of it. Cares are still based on heavy disinfection and drying of the wound not respecting its physiology. Not only for those suffering Buruli, we believe that better wound care can be provided to millions through basic training in modern adapted wound care for local caregivers.

For this, six basic principles are used:

1) Evaluate and correct:
   a) The specific cause of the wound
   b) The patient’s general condition
2) Maintain a moist wound environment
3) Protect wound from any additional trauma
   a) Physical, chemical
   b) Protect peri wound skin
4) Promote a clean wound bed without infection
5) Control the peri wound Lymphedema
6) Prevent or correct secondary disability by active mobilization and good positioning


A second revised, increased and more practical edition is in the process of being published this year. The basic ideas are to work in partnership with communities worldwide to advance sustainable wound and lymphedema management in limited resource settings through teaching basics in wound physiology and care.

Current experiences in various African countries and Haiti over the last years show us that this concept is useful.
Report on wound management practices in two Buruli ulcer treatment centers in Benin

Poster: Anita Wadagni

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Background

Buruli ulcer (BU) is a chronic cutaneous disease caused by Mycobacterium ulcerans. Although the issue is not usually fatal, BU can cause severe disabilities, especially in the rural areas of West Africa where treatment options are limited. Wound care is a major part of the treatment that requires eight weeks of antibiotic therapy, surgery and physiotherapy.

Objectives

This study aims to describe the current wound-management practices in two CDTUBs (Allada and Lalo) in Benin in order to improve this important component of the treatment.

Method

We conducted, in three stages, a cross-sectional qualitative study in two CDTUBs in Benin: 1. Literature review to identify the current guidelines and elements relating to the context; 2. Assessment of the wound management practices in the study sites; 3. Analysis of the strengths, weaknesses, opportunities and threats (SWOT) of wound management at the CDTUBs, in comparison to the current standards and recommendations identified in the literature review.

Results

The literature review helped to identify the different types of wounds, the various wound healing phases, the treatment phases of a chronic wound, the basic principles of the management of BU wounds, and the recommended treatment for each type of wound.

The assessment of the CDTUBs reveals an adequate environment, equipment, and hygiene practices, but a weakness in terms of updating knowledge of health workers, a lack of standardized protocol leading to a wide variation in practices between workers and centers. Pain management is limited to psychological accompaniment. Most of the patients or caretakers expressed their satisfaction about treatment received and hospitalization conditions, but complained about the excessively long hospitalization. The major opportunity for a quality of wound dressing is the commitment of partners, which could also be the major threat in the case of a lack of more involvement of the government in funding of wound care consumables.

Conclusion

Adequate wound management remains a weak point and a major concern for the PNLLUB. This study will be used as the basis for designing and implementation of a standardized dressings protocol tacking into account the recommended procedures adapted to local environment. It will be support continuous training to the nursing staff. The medical treatment combined with such adequate local treatment will accelerate healing, thereby resulting in a reduction in surgical procedures and the associated risks and social problems.
Experiences of pain and expectations for its treatment amongst former Buruli ulcer patients

Poster: Rebecca J. Woolley

Authors: Rebecca J. Woolley1, Anita Velink1, Richard O. Phillips2, William A. Thompson3, K. Mohammed Abass3, Tijp S. van der Werf4, Janine de Zeeuw4, Ymkje Stienstra4*

Buruli ulcer (BU) is one the 17 Neglected Tropical Diseases for which the World Health Organization (WHO) has adopted resolutions to improve treatment. BU was previously described as a relatively painless condition; however recent research has indicated that some patients experience substantial pain. The objective of this study was to explore patients’ experiences of pain and their expectations for its treatment.

Semi-structured interviews were conducted in a BU endemic region of Ghana. Interviews were held with former BU patients (n=20) and community controls (n=19). Former patients were asked about BU related pain and their expectations for its treatment. The interviews were conducted in October 2014 and were audio-taped, translated and transcribed into English and then qualitatively analyzed.

Of the 20 former BU patients interviewed, 19 (95%) reported experiencing pain, with patients reporting pain as a consequence of the ulcer and wound management. Some participants expressed pain through crying, while others did not openly express pain, sometimes because they feared the repercussions of doing so. Patients would like to receive pain relief; however, many were unable to name a medication. Non-pharmaceutical options were cited as being an alternative.

Many BU patients experience pain, however former patients and community members alike appear to have a limited knowledge about available pain relief. A low-cost alternative to medication may be the use of non-pharmaceutical means for pain relief. Routine pain assessment may reduce patients fear and unwillingness to express pain. Awareness of such issues will be valuable when implementing a BU pain relief guideline.
Research sessions

Diagnostics
Validation of a LAMP test for *Treponema pallidum*, the diagnostic tool for the last mile in Yaws eradication

*Presented by Oriol Mitjà*

**Authors:** Mohammed Bakheit, Charmie Godornes, Sieghard Frishchmann, Sheila Lukehart, Oriol Mitjà

**Background**

The final stages of yaws eradication require a test with high sensitivity and specificity to detect residual cases following mass treatment. Serology is unable to differentiate patients with active yaws vs. patients with latent yaws plus a different cause of the current skin lesion (eg *Haemophilus ducreyi*), and persistent low nontreponemal titers are common in patients after treatment. PCR- based techniques are able to overcome these drawbacks but . We evaluated the sensitivity and specificity of Loop-mediated isothermal amplification (LAMP) compared to gold-standard PCR tests.

**Methods**

124 lesional swab specimens for evaluation were collected from children (aged 1 – 18 years) with a presumptive clinical diagnosis of yaws during a yaws eradication program in Lihir Island (Papua New Guinea) between May 2013 and October 2016. We calculated the sensitivity and specificity of the LAMP test for *T. pallidum pertenue* (TP; Mast Diagnostica) and compared values with those obtained with standard laboratory PCR (University of Washington).

**Results**

Of 124 specimens tests 68 (48.3%) were positive for TP-PCR. The LAMP test had a sensitivity of 81% (95%CI 70 - 89) and specificity of 95% (85 – 98). In subgroup analysis, sensitivities and specificities did not differ according to the type of specimen (extracted DNA vs direct swab). For extracted DNA specimens, the sensitivity was 85% (71 – 93) and specificity was 97% (82 – 99), for direct swab specimens, 74% (95 - 87) and 93% (77 – 98), respectively.

**Interpretation**

LAMP is a field deployable device that permits sensitive and specific testing for yaws cases, it is rapid and cost-effective (4USD /test). The LAMP test for yaws needs to be validated in field conditions so that it can be implemented in yaws eradication programs.
Pilot study on e-nose detection of Buruli ulcer

Presented by Stan Chudy

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Introduction

Microscopy, which is the cheaper, easier and most available diagnostic method for Buruli ulcer (BU) in endemic areas, is far from perfect with a low sensitivity. And although qPCR for the IS2404 target has excellent sensitivity and specificity, it requires sophisticated laboratories with strict quality control as it is prone to false positives. This restricts the availability of qPCR and patients in remote areas seldom have rapid access to molecular diagnostics. Thus, a reliable point-of-care test would save valuable time and allow for confirmation of BU during active case finding activities.

For years, physicians in Sub-Saharan Africa have reported on a characteristic odour of BU lesions, leading to its inclusion as a parameter in clinical studies. A recent study in Cameroon found that the characteristic odour was the strongest clinical predictor of a highly likely Buruli ulcer. These findings provided the basis to attempt a smell-based approach towards a non-invasive point-of-care diagnostic test with an electronic nose.

Materials and Methods

We analysed the smell of BU and non-BU wounds in specialised wound treatment centres in Allada and Lalo, Benin, embedded in a study on the differential diagnosis of BU. To assess smell samples, the e-nose uses a set of gas array sensors coupled to a pattern-recognising chip to analyse Volatile Organic Compounds (VOCs) in the air. All sensors respond to different VOCs, so different odours produce different patterns. In the present study we used the Cyranose 320, a hand-held e-nose with 32 nanocomposite sensors. Besides directly smelling ulcerated wounds, used gauzes were sampled in a glass jar with an in- and output tube in the lid. The e-nose was connected to the output and a carbon filter to the input to filter environmental VOCs.

Results

Using Principal Component Analysis (PCA), the e-nose data was reduced to 3 PCs that best describe the variability within the dataset of sampled gauzes. The separation of the BU patients and the control group based on two PCs was statistically significant with a p-value of respectively 0.048 and 0.007. We applied linear discriminant analysis and constructed a model with the ‘leave-one-out’ method that allocated 66.7% of cases correctly after cross-validation. The results were compared to the clinical diagnosis as the IS2404 qPCR results, as gold standard for classification of BU vs non-BU, are pending. Cohen’s κ was run and demonstrated an agreement of κ = 0.485 between our model and the clinical diagnosis.

Conclusion

Despite e-noses being used for a growing group of medical purposes in recent years, they have primarily been used for breath analysis. To our knowledge, this pilot study is the first in which Buruli ulcer lesions are analysed by an e-nose.
While this proof of concept demonstrates that the e-nose can discriminate between BU and non-BU, a better model is required before this device is developed into a point-of-care test. A high sensitivity is desirable unless reliable qPCR can be used as a confirmation test without great delay, but not at the cost of a great loss of specificity as antimicrobial treatment of BU can come with serious side effects.

The methods used were suboptimal and the signal-to-noise ratio of the e-nose might have been decreased by a faint non-specific smell that was left in the jars and tubes after repetitive use and the smell of disinfectants. Improved methods and a larger population of non-treated patients, ideally compared to PCR for IS2404, could increase the quality of evidence and precision of the e-nose as a point-of-care test for Buruli ulcer.
Physical characterization of mycolactone A/B for rapid detection applications

Presented by Jessica Z. Kubicek-Sutherland

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Buruli ulcer, a chronic necrotizing skin and soft tissue infection, is caused by the bacterial pathogen Mycobacterium ulcerans. The lipid-like toxin mycolactone produced by M. ulcerans is responsible for both the tissue necrosis (cytotoxicity) as well as the painlessness (immunosuppression) characteristic of Buruli Ulcer disease. Due to its direct role in the pathogenesis of M. ulcerans, mycolactone is a promising diagnostic and prognostic biomarker for Buruli Ulcer disease. However, several challenges have deterred progress in developing diagnostic strategies to detect mycolactone. There is limited availability of purified mycolactone to study, there is limited physical characterization of the toxin due to its inherent “stickiness”, and its immunosuppressive activity has hampered antibody development. In order to address these challenges, we started to study the physical characteristics of mycolactone A/B. First, we utilized a thin layer chromatography (TLC)-based purification protocol to separate mycolactone A/B from bacterial culture extracts. Then, we developed a mass spectrometry analysis protocol that was used to analyze the level of purity of our purified mycolactone, and degree of labeling of our biotinylated mycolactone, relative to a synthetic version of the toxin. Next, we identified optimal handling and storage conditions of mycolactone A/B, which included a comparative analysis of the synthetic, purified and biotinylated forms of the toxin. Mycolactone is an amphipathic molecule, which by its very nature will aggregate into micelles in aqueous solution at a threshold concentration. We have determined this concentration for biotinylated-mycolactone A/B as well as synthetic unlabeled mycolactone A/B in water to be 0.4 µM and 14 µM, respectively. Interestingly, biotinylated mycolactone A/B forms micelles at a concentration 35-fold lower than the natural toxin, which may explain the observed reduction in cytotoxicity of biotinylated mycolactone A/B. In light of these findings, we have started exploring the inherent differences in cytotoxicity of the synthesized, biotinylated and culture-purified mycolactone using a L-929 cytotoxicity model and hope to provide a comprehensive repository of methods and strategies to study mycolactone to the research community. Finally, in an effort to develop a rapid point-of-care diagnostic tool for the detection of mycolactone directly in patient tissue samples, we have explored diagnostic strategies utilizing the innate biochemistry of the toxin by examining the interaction of mycolactone A/B with supported lipid bilayers, which could be used to immobilize the toxin using its amphiphilic biochemistry without the need for a specific antibody. Understanding the lipid-like properties of mycolactone can enhance diagnostic strategies by incorporating host-pathogen interactions that have so far only prevented its detection in Buruli ulcer patients.

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The selection of recombinant antibodies against mycolactone using display methods

Presented by Andrew Bradbury

Leslie Naranjo\textsuperscript{1}, Caroline Demangel\textsuperscript{2}, Yoshito Kishi\textsuperscript{3}, Basil Swanson\textsuperscript{1}, Andrew Bradbury\textsuperscript{1},

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\end{itemize}

It has proved impossible to generate antibodies recognizing mycolactone using traditional immunization methods. This talk will discuss our efforts to select antibodies recognizing mycolactone alone and in complex with Wiskott Aldrich Syndrome Protein (WASP) using in vitro display methods. Building on our success in selecting hundreds of antibodies against Ag85\textsuperscript{1}, a TB protein, we applied the same approach to mycolactone and the mycolactone-WASP complex. Biotinylated mycolactone was interacted with a large naive human phage antibody library\textsuperscript{2} (comprising \(\sim 3 \times 10^{11}\) clones). After two rounds of phage selection the selected antibody genes were transferred to a yeast display vector and two rounds of sorting by flow cytometry, carried out. Individual antibodies selected from this population had a low affinity (1-5µM) for mycolactone. In vitro affinity maturation of the antibody population has allowed us to select antibodies with higher affinities. The results of attempting similar selections against complexes between mycolactone and host proteins will also be presented.

This approach will likely be successful in other cases where traditional immunization is ineffective, either because the immunogen is toxic, immunosuppressive, conserved or hidden from the immune system. Furthermore, this is an important step towards immuno-based diagnostic for BU via detection of mycolactone.

Buruli ulcer (BU) is a severe, slow progressing necrotizing skin infection caused by the environmental mycobacterium, *Mycobacterium ulcerans*. BU is characterized by nodule, papule and plaque formation which ultimately develops into large painless ulcers with undermined edges. The WHO's Global BU Initiative has identified the development of simple diagnostic tools as one of the priority areas to control BU. In a pilot study, funded under the Cambridge-Africa Partnership for Research Excellence, we sought to identify key *M. ulcerans* metabolic markers that can be found only in Buruli ulcer patients, with the ultimate aim of identifying potential targets for further development as a diagnostic tool. We also sought to determine the best sample matrix (lesion biopsy, swab or fine needle aspirate) for the extraction and identification of metabolites using gas chromatography mass spectrometry based metabolomics. We collected tissue biopsy, swabs and fine needle aspirates collected from 28 Buruli ulcer confirmed patients and 21 patients with tropical ulcers that were not Buruli ulcer. Interesting metabolites identified in both groups of patients included cadaverine, putricine, pinitol, palmitate, naphthalene, chlopyrifos and oxaspiro. The former two metabolites are interesting because they classify all the samples as containing degenerating tissue. The fatty acid palmitate is a common metabolite present in human tissue. Pinitol and the later three identifies metabolites are chemical residues that may have been present in the treatment poultice used as unorthodox remedies by the patients. Unfortunately, due to our inability to obtain enough materials from the lesion swabs which were in the majority, we could not identify unique metabolites differentiating the Buruli ulcer patients from the control patients based on the data we obtained. We are presently performing a more detailed analysis of the metabolome of the host and mycobacterium in BU confirmed lesions. To gain more insight into the novelty of the biomarkers we identify, we have included another phase of experimentation which involves the characterization of the lipid and aqueous metabolome of *M. ulcerans* and other mycolactone producing mycobacteria including *M. pseudoshottii*, *M. liflandii* and *M. marinum DL*. 
The location of Australian Buruli ulcer lesions – implications for unravelling disease transmission

Presented by Paul Johnson

Arvind Yerramilli, Ee Laine Tay, Andrew J Stewardson, Peter G Kelley, Emma Bishop, Grant A Jenkin, Mike Starr, Janine Trevillyan, Andrew Hughes, N Deborah Friedman, Daniel P O’Brien, Paul DR Johnson

Background
Buruli ulcer (BU), caused by Mycobacterium ulcerans, is increasing in incidence in Victoria, Australia. To improve understanding of disease transmission, we aimed to map BU lesions on to templates of the human body from our region.

Methods
Using notification data and clinical records review, we conducted a retrospective observational study of patients diagnosed with BU in Victoria from 1998-2015. We created electronic density maps of lesion locations using spatial analysis software and compared lesion distribution by age, gender, presence of multiple lesions and month of infection.

Findings
We examined 579 patients with 649 lesions; 32 (5.5%) patients had multiple lesions. Lesions were predominantly located on lower (70.0%) and upper (27.1%) limbs, and showed a non-random distribution with strong predilection for the ankles, elbows and calves. When stratified by age, upper limb lesions were more common (OR 1·97, 95% CI 1·38-2·82, p<0·001) while lower limb lesions were less common in men than in women (OR 0·48, 95% CI 0·34-0·68, p<0·001). Patients aged ≥ 65 years (OR 3·13, 95% CI 1·52-6·43, p=0·001) and those with a lesion on the ankle (OR 2·49, 95% CI 1·14-5·43, p=0·02) were more likely to have multiple lesions. Most infections (71.3%) were likely acquired in the hottest 6 months of the year.

Interpretation
Comparison with published work in Cameroon, Africa, showed similar lesion distribution and suggests the mode of M. ulcerans transmission may be the same across the globe. Our findings also aid clinical diagnosis and provide quantitative background information for further research investigating disease transmission.
Figure 1: Density map of the distribution of Buruli ulcer lesions on front and back templates of the human body generated using ArcGIS software version 10.3.1.
Collaborative efforts to improve access to diagnosis of Buruli ulcer

Poster presented by Isra Cruz

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In 2013, FIND and WHO convened a meeting of Buruli ulcer (BU) experts to review unmet diagnostic needs for the disease. The outputs of the meeting were refined further and used to develop the FIND strategy on BU diagnostics. Since then, FIND has been collaborating with WHO and other partners in the development and evaluation of diagnostic tests for BU. In March 2016 FIND and Anesvad signed a Memorandum of Understanding to collaborate in support of the WHO integrated strategy on skin NTDs that require intensive disease management, such as BU. The aim of the partnership between Anesvad and FIND is to support the development, evaluation and introduction of diagnostic tools and strategies for early detection of BU, thus contributing to improved access to diagnosis at the primary healthcare level. This will result in improved management of BU and other skin diseases. To achieve these goals, collaborations are being established with country-level governments, research institutions from Africa and Europe and implementing agencies in BU endemic countries in Benin, Côte d’Ivoire, Democratic Republic of the Congo, Ghana and Togo. The specific activities related to BU, which are also supported by UBS Optimus Foundation and the Swiss Agency for Development and Cooperation, include:

i. Development and evaluation of an antigen detection test for diagnosis at the primary healthcare level. In collaboration with SwissTPH, Alere/Standard Diagnostics (SD, Korea) and IME, FIND is working to develop a rapid diagnostic test (RDT) for BU that can be deployed at the primary healthcare level. Monoclonal antibodies specific to the MUL-3720 protein of Mycobacterium ulcerans have been developed by Swiss TPH and used to develop an ELISA test whose sensitivity is comparable to current reference methods. These reagents are transferred to SD for use in developing the RDT. Prototype RDTs are under evaluation.

ii. Development and evaluation of a dried reagents-based loop mediated isothermal amplification (DRB-LAMP) test for detection and confirmatory diagnosis of M. ulcerans DNA (IS2404) at the microscopy laboratory level. A partnership with DITM/KUM and NMIMR supported by FIND is developing a LAMP test for BU that, coupled to a simple sample processing method, will overcome the requirement for well-equipped facilities and cold-chains for transport and storage of reagents, making highly specific and sensitive confirmatory diagnosis more accessible.

iii. Confirmation and treatment monitoring at the district hospital level. With FIND support, the WHO in collaboration with Harvard University (USA) are working with a number of laboratories in Ghana, Benin and the DRC to evaluate fluorescence thin layer chromatography (f-TLC) for the detection of M. ulcerans mycolactone and its use in diagnosis and monitoring the efficacy of treatment. The results obtained are comparable to those obtained by the IS2404 PCR, and further evaluation at district level evaluation is planned for 2017.

In the frame of this partnership FIND and Anesvad will seek to support these efforts, as well as strategies for early detection of BU in Benin, Côte d’Ivoire, DRC, Ghana, and Togo; in collaboration with national BU programmes and research institutions, as part of unique programmes that Anesvad is promoting in these countries in collaboration with the ministries of health.
Dermal leishmaniasis comprises of a group of infections caused by Leishmania parasites. Between 0.7 and 1.2 million cases of cutaneous leishmaniasis (CL) are reported worldwide per year. Although not lethal, CL causes chronic and disfiguring skin lesions, and is an important cause of morbidity and social stigma. Post-kala-azar dermal leishmaniasis (PKDL), a complication of visceral leishmaniasis (VL) that is common in areas where *Leishmania donovani* is endemic, could also have a potential role in the transmission of VL. Microscopy of Giemsa stained samples, including skin scrapings, fine needle aspirates, or slit-skin smears, remains the reference test for diagnosis of the different forms of dermal leishmaniasis. However, microscopy has a low and variable sensitivity and requires trained and experienced personnel. The technical complexity of the more sensitive molecular techniques (e.g., PCR) limits their application as routine diagnostic tools in endemic areas. Thus new point-of-care (POC) tests are needed. The use of these tests should improve access to treatment, which will benefit patients and communities by reducing the risk of both sequelae and Leishmania transmission. To ensure that patient, control programs, and market needs are met, the requirements of such a test should be specified in a target product profile (TPP).

To identify minimal/optimal characteristics for a POC test for dermal leishmaniasis, a draft TPP was first reviewed and discussed with experts from academia and international organizations with expertise in CL diagnosis and management, during a RedLeish meeting (DNDi-Latin America) focused on clinical research. Consensus characteristics were defined in a second step with a Delphi method, in which CL experts and stakeholders from different Leishmania endemic regions were invited to participate.

We present a TPP for a POC test to diagnose the different forms of dermal leishmaniasis, including localized CL, mucocutaneous leishmaniasis, diffuse CL, CL recidivans and PKDL. This TPP includes 29 features of the test, which refer to specific requirements or specifications of the diagnostic tool to be developed.

This TPP identifies the requirements for a POC test for dermal leishmaniasis diagnosis, and will guide efforts by the academic institutions and product developers to achieve the ideal test, which will have a strong impact on patient management and disease control.
External validation of a clinical score for the diagnosis of *Mycobacterium ulcerans* (MU) infection in Cameroon

*Poster presented by Yap Boum II*

MSF, Fairmed, Cires, National Yaws, Leishmaniasis, Leprosy and Buruli Ulcer Control Programme, Epicentre.

**Background**

Access to laboratory diagnosis can be a challenge for individuals suspected of Buruli Ulcer (BU). After developing a decisional algorithm based on a clinical score assessing BU probability, our objective now is to perform an external Validation.

**Methodology**

In a previous study done between 2011 and 2013, we enrolled individuals presenting at Akonolinga District Hospital, Cameroon with chronic wounds. We collected clinical data, performed laboratory tests including ZN microscopy, culture and PCR for detection of *Mycobacterium ulcerans*. Based on a latent class model using laboratory test results, patients were categorized into high, or low BU likelihood. Variables associated with a high BU likelihood in a multivariate logistic model were included in the Buruli score. The variables identified for the Buruli score were: characteristic smell (+3 points), yellow color (+2), female gender (+2), undermining (+1), green color (+1), lesion hyposensitivity (+1), pain at rest (-1), size >5cm (-1), locoregional adenopathy (-2), age above 20 up to 40 years (-3), or above 40 (-5). This score had AUC of 0.86 (95%CI 0.82-0.89), indicating good discrimination between infected and non-infected individuals. The cut-off to reasonably exclude BU was set at scores <0 (NPV 96.5%; 95%CI 93.0-98.6). The treatment threshold was set at a cut-off ≥4 (PPV 69.0%; 95%CI 49.2-84.7). We now plan to validate the Buruli score in Akonolinga, Ayos and Bankim, three districts with the highest prevalence of Buruli ulcers in Cameroon. We also aim to assess the performance of point of care test for diagnosis of Buruli Ulcer. The study aim to enroll 280 participants in a period of two years.
First reported cases of *M. Leprae* detection by PCR in Côte d’Ivoire

**Poster presented by David Coulibaly N’Golo**

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Leprosy is an infectious and contagious disease caused by *Mycobacterium leprae*. This bacterium has a special affinity for skin and peripheral nerves (WHO, 2006). It is among the neglected tropical diseases listed to eradicate in the long term (WHO, 2012). Globally, leprosy is the 2nd mycobacteria disease after tuberculosis and still forming today a public health problem in many countries. With the advent of multidrug therapy (MDT) in 1982 (WHO, 1982), much progress has been made in the fight against this disease which causes severe socio-political consequences (physical disabilities, social rejection, ...).

Côte d’Ivoire like some African countries reached the elimination threshold of disease and MDT is available throughout the country (WHO, 2004). However, Côte d’Ivoire hasn’t managed to break permanently the chain of transmission of *M. leprae*. Thus, in the country where leprosy is endemic, we went from 1,169 cases detected in 2013 to 891 cases reported in 2015 with the number of level II handicaps observed evolving. It was 3% higher in 2015 than in 2013. On the other hand, the diagnosis of infection is often taken to default and based only on clinical arguments and microscopy. The eradication of a disease being conditioned by the performance of diagnostic measures implemented; we have undertaken to implemented PCR, a non-existing diagnostic to contribute in the confirmation of suspected cases of the National Eradication Leprosy Program.

45 samples from patients clinically diagnosed as leprosy cases were collected. Those samples consisted of 12 nasal mucus and 33 slits skin smears that have been successfully confirmed by PCR. The genetic material was extracted with an homemade method using guanidine thiocyanate and amplified twice; first by conventional PCR on ABI 9700 (Applied Biosystem) targeting *M. leprae*’s repeated elements RLEP and second by real-time PCR on StepOne plus (Applied Biosystem) targeting the 16sRNA of the bacterium. Results showed positivity rates respectively of 49% and 33% for realtime and conventional PCRs. Sequences of 6 PCR products of the repetitive elements RLEP have been done on ABI 3500 XL genetic analyzer (24 capillaries) and BLASTn analysis (NCBI) confirmed that PCR products obtained were to related *M. leprae* genome with 100% sequence identity

Molecular technology (PCR) is available for confirmation of leprosy cases in Côte d’Ivoire. This would help to reduce the number of level II observed handicaps and the leprosy elimination.

**Keywords**: Côte d’Ivoire, *M. leprae*, Leprosy elimination, Molecular diagnosis, PCR.
Nanobodies for the detection of mycolactone

Poster presented by Thomas Laval

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Mycolactone, a toxin unique to Mycobacterium ulcerans among human pathogens, is essential for Buruli ulcer disease pathogenesis, and as such represents an attractive target for diagnosis. However, the isolation of anti-mycolactone antibodies has been challenged by the poor immunogenicity of this immunosuppressive macrolide. The variable heavy-chain domain of camel homodimeric antibodies, called nanobodies, are small proteins which maintain the binding properties of immunoglobulins as monomers. Nanobodies offer an attractive alternative to conventional antibodies because of their small size and easy production in bacteria, allowing large and fast screenings of antibody libraries on an antigen. By screening large naïve phage-displayed libraries from non-immunized camelids, we isolated anti-mycolactone nanobodies. The binding capacity of those new nanobodies was confirmed through ELISA assays. Once conjugated to a fluorophore, the nanobody was able to detect the mycolactone naturally produced by M. ulcerans in immunofluorescence.
Research sessions

Transmission
Environmental transmission of *Mycobacterium ulcerans* drives dynamics of Buruli ulcer in endemic regions of Cameroon

*Presented by Benjamin Roche*

Andrés Garchitorena, Calistus N. Ngonghala, Gaëtan Texier, Jordi Landier, Sara Eyangoh, Matthew H. Bonds, Jean-François Guégan and Benjamin Roche (presenting author)

Buruli Ulcer is a devastating skin disease caused by the pathogen *Mycobacterium ulcerans*. Emergence and distribution of Buruli ulcer cases is clearly linked to aquatic ecosystems, but the specific route of transmission of *M. ulcerans* to humans remains unclear. Relying on the most detailed field data in space and time on *M. ulcerans* and Buruli ulcer available today, we assess the relative contribution of two potential transmission routes –environmental and water bug transmission– to the dynamics of Buruli ulcer in two endemic regions of Cameroon. The temporal dynamics of Buruli ulcer incidence, which is surprisingly highly seasonal, are explained by estimating rates of different routes of transmission in mathematical models. Independently, we also estimate statistical models of the different transmission pathways on the spatial distribution of Buruli ulcer. The results of these two independent approaches are corroborative and suggest that environmental transmission pathways explain the temporal and spatial patterns of Buruli ulcer in our endemic areas better than the water bug transmission.
Porosity of the underground layer and *Mycobacterium ulcerans* transmission: the missing link — Exploratory analysis of 20 years well documented data in Victoria, Australia

*Presented by Isabelle Jeanne*


GCEID/Barwon Health Hospital /Deakin University, Department of Health and Human Services, Victoria.

**Background**

In 1997, the World Health Organisation has recognised Buruli ulcer (BU) due to *Mycobacterium ulcerans* (Mu) infection as a re-emerging disease and one of the 17 neglected tropical infectious diseases. It was first described in 1948 from Gippsland, Victoria. For the last 20 years BU incidence increases in the Victoria state and displays a seasonal pattern. The clinical cases spread westwards, mainly in coastal regions and they increase after anthropic or natural environmental changes involving water.

*Mycobacterium ulcerans* has been found in various environments (vegetation, soil, water) and multiple hosts, vertebrate and invertebrate animals. Despite this, its transmission remains a mystery. The objective of the study was to define spatial and temporal transmission risk factors and to build a species distribution model for *Mycobacteria ulcerans* in Victoria.

**Methods**

From 1994 to 2014, there were 769 bacteriologically confirmed clinical cases of BU notified to the Department of Health and Human Services in Victoria. Nearly 80% of them were mapped at the smallest geographic unit for which Census data are available, (Mesh block scale*), in a geographic information system integrating climate and environment data to allow exploratory spatial analysis with two different targets in the same environment: patients and bacteria. To define the geographical range of Mu ecological niche, we searched for environment patterns of endemic areas. Only the positive mesh blocks where patients have been infected locally were used to determine environment risk factors. Some parameters were selected after known microbiological characteristics, like salinity and pH. Surface and hydrogeological parameters were used.

**Results**

We have determined clusters of BU occurrence in space and in time. 427 out of 613 patients got infected locally at their residence or nearby and the others have visited endemic areas. The spatial niche species analysis showed a strong association of Mu presence with aquifers characteristics: not one positive mesh block was found associated with limestone environment and positive mesh blocks were significantly found in sand and gravel aquifers areas (89%), with high porosity and within a high range of salinity.

**Conclusions**

Our study allows us to build up a suitability map of Mu acquisition risk for Victoria. This model could be tested in the West Africa BU context. The geographical patterns of Mu in Victoria lead to a new hypothesis of the role of anthropological activities (mainly water use) as bacteria acquisition risk and explain the climatic link with both flood and drought.

* Mesh Blocks have been designed by the Australian Bureau of Statistics to be small enough to aggregate accurately to a wide range of spatial units and thus enable a ready comparison of statistics between geographical areas, and large enough to protect against accidental disclosure. Most residential Mesh Blocks contain approximately 30 to 60 dwellings (source: ABS.gov.au).
Chitin does not only promote *Mycobacterium ulcerans* growth but also increases its tolerance to acidic environments

**Presented by Daniel Sanhueza**

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The natural life cycle of *Mycobacterium ulcerans* - the causative agent of Buruli ulcer (BU) in humans - is not fully identified given the wide biodiversity of aquatic organisms reported as natural carriers of this bacillus. However, field surveys performed in BU endemic areas reported that high abundance levels of *M. ulcerans* were associated with human-made perturbations and with coordinated changes in several abiotic environmental parameters. To disentangle which parameters actually define the most favorable habitats, we experimentally manipulated the 7H9 Middlebrook culture broth to test in vitro their effects on *M. ulcerans* growth. In a first set of experiments, we manipulated the concentrations of five chemical elements within the ranges observed in BU endemic areas. This showed the limiting effects of iron, sulfate, phosphate and zinc as well as the slight stimulating effect of calcium on the bacillus growth. We also investigated the growth effect of two ubiquitous polysaccharides using the same concentration ranges (0.2 – 5000 mg/l). Chitin was showed to strongly promote *M. ulcerans* growth while starch had no effect. We then analyzed how the presence or absence of chitin in growth medium interplay with the variation ranges in **pH** values observed in BU endemic areas (4.5 \(\leq \text{**pH**} \leq 7.5\)). The stimulating effect of chitin on *M. ulcerans* growth was confirmed not only in control (\(\text{**pH**} = 6.7\)) but at seven other \(\text{**pH**}\) values (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5). Moreover, the strongest stimulating effects of chitin on *M. ulcerans* growth were observed in the least suitable \(\text{**pH**}\) conditions (4.5 \(\leq \text{**pH**} \leq 5.5\)), indicating thus that chitin may increase *M. ulcerans* tolerance to acidic environments. Altogether these results congruently support the view that the availability of chitin plays a key role in the biology and metabolism of *M. ulcerans*, pinpointing on the multifactorial, complex relationships that exist between this bacillus and its natural environment. From then, using a new RNA sequencing approach we discuss on the bacterial genes that are differentially expressed in presence or absence of chitin, and that would help in clarifying the physiological bases of roles of chitin in the biology of *M. ulcerans*.
Examination of Australian Mycobacterium ulcerans disease family clusters suggests a short-duration exposure risk for infection and argues against human to human transmission

Presented by Daniel P. O’Brien

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Introduction

The environmental reservoir and mode of transmission of Mycobacterium ulcerans (M. ulcerans) remain unknown, however the examination of disease family clusters may provide important new information about disease epidemiology.

Methods

An analysis of family clusters from a comprehensive data set of a prospectively collected cohort containing all confirmed M. ulcerans cases managed from 1/1/1998-12/4/2016 at a tertiary hospital in an endemic area in south-eastern Australia was performed. Additionally, whole genome sequencing and single nucleotide polymorphism (SNP) analysis of six isolates derived from three family cluster pairs was undertaken.

Results

Analysis included 324 patients with a median age of 57 years (IQR 34-74 years) and a median duration from initial diagnosis until study analysis of 4.7 years (IQR 2.8-9.7 years). Twenty-one (6.5%) patients were part of a family cluster. The rate of new diagnoses of an M. ulcerans lesion in another family member was 5.69 per 1,000 person years (95% CI 3.15 -10.29 per 1000 person years) and the median time between diagnoses of lesions in family members was 2.8 months (IQR 1.1-20.6 months). Pairwise genomic comparisons of family cluster isolates revealed one genetically identical pair and two pairs with small genetic differences.

Conclusions

The analysis revealed the incidence of M. ulcerans disease in family members was increased, however they are closely temporally related suggesting a short-term risk of exposure and infection. Epidemiological and genetic evidence suggests human to human transmission of M. ulcerans disease does not occur.
Population genomics aligns the spread of *Mycobacterium ulcerans* and Buruli ulcer with the scramble for Africa

**Presented by Koen Vandelanoote**

Koen Vandelanoote¹, Conor J. Meehan¹, Miriam Eddyani¹, Françoise Portaels¹, Timothy P. Stinear², Delphin Mavinga Phanuzu³, Kapay Kibadi⁴, Bouke C. de Jong¹

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After almost 70 years of study in Africa, the mode of transmission and the non-human reservoir(s) of *M. ulcerans* are still largely unknown. The detailed resolution offered by genomics to distinguish between different strains of *M. ulcerans* is opening up new possibilities to explore the pathogen’s cryptic epidemiology and disease ecology. Here, we used comparative second and third generation genomics to reconstruct the evolutionary history of *M. ulcerans* and to explore its molecular epidemiology at the continental scale, and at the smaller geographical “village scale” in a Buruli ulcer (BU) endemic region of the Democratic Republic of Congo.

The genetic diversity of African *M. ulcerans* was found to be restricted due to the bacterium’s slow substitution rate coupled with its relatively recent origin. We identified two specific *M. ulcerans* lineages within the African continent, and showed that *M. ulcerans* lineage Mu_A1 existed in Africa for several hundreds of years, unlike lineage Mu_A2, which was introduced much more recently during the 19th century. Additionally, we observed that specific *M. ulcerans* epidemic Mu_A1 clones were introduced during the same time period in the three hydrological basins (Congo, Nyong, and Oume) that were well covered in our panel. The estimated time span of the introduction events coincides with the Neo-imperialism period, during which time the European colonial powers divided the African continent among themselves. Using this temporal association, and in the absence of a known BU reservoir or vector on the continent, we postulate that the so-called “Scramble for Africa” played a significant role in the spread of the disease across the continent through the displacement of BU-infected humans.

Furthermore, we identified a relationship between the mycobacterial population dynamics of *M. ulcerans* from the Songololo Territory and the timing of health policy changes in managing the BU epidemic in that region. The human *M. ulcerans* reservoir was inversely associated over time with BU control activities, suggesting an impact. Conversely, the human reservoir appears to be important to sustain new infections.

These combined observations suggest that humans with open, discharging BU lesions can indirectly cause new BU infections.
*Mycobacterium ulcerans* infection found in domestic animals in the Buruli ulcer endemic area of Sedje-Denou, Southern Benin: From basic molecular identification to sequences confirmation

*Presented by Francis Zeukeng*
AgroEcoHealth Platform, West and Central Africa, IITA- Benin.

**Background**

*Mycobacterium ulcerans* (MU) has been identified in several environmental reservoirs including invertebrates and vertebrates. However, the trophic relatedness within these identified matrices has been poorly investigated and remains seminal to understanding the unknown modes of transmission of buruli ulcer (BU). MU infections characterized by opened sores and ulcerations have been described in domestic and wild animals in Australia. To date, the disease has been described in wild animals in Africa and is suspected to be present in domestic environment. In this study, we screened MU presence in domestic animals from BU endemic villages of Sedje-Denou.

**Methods/Expected findings**

We conducted a molecular screening of MU in animals (cattle, carnivorous and birds) from three BU endemic villages (Agongbo, Agodenou and Agbahounsou) in Southern Benin. Swabs and lesions were aseptically collected from animal carrying opens or active wounds. In addition, animal feces and saliva were systematically collected from surveyed animals. Twenty five animals out of 359 (6.96%) carried active wounds. When these wounds were analyzed by qPCR for the MU (DNA targets IS2404, IS2606 and KR-B), three of them (1 from a dog and from 2 goats) were positive to MU DNA with two wounds having the typical characteristics of Buruli ulcer disease. One animal feces (1/322) and one saliva (1/322) were positive to the three MU DNA targets. Source-tracking of MU isolates between animals was performed using MIRU-VNTR genotyping and length polymorphism and results obtained further confirmed with sequencing. One MU genotype named Z was successfully typed from both animal swabs and feces. The evolutionary history inferred from sequenced data revealed that isolated strains of MU are closely related.

**Conclusion/Significance**

The fact that MU resides in the domestic environment and infects animals highlights potential risks of transmissions associated with contact between human and domestic animals.

**Keywords:** Buruli ulcer, *M. ulcerans*, Commensal animals, MIRU-VNTR typing, Sedje-Denou.
Whole-genome comparative analysis of *Mycobacterium ulcerans* subsp. *shinshuense* and other mycolactone-producing mycobacteria

**Presented by Mitsunori Yoshida**

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*Mycobacterium ulcerans* is a causative agent of Buruli ulcer (BU), and its first case in Japan was reported in 1980. The causative agent was isolated and identified as *M. ulcerans* subsp. *shinshuense* (hereafter referred as *M. shinshuense*) because it was closely related to *M. ulcerans* but certain phenotypic differences were observed in conventional methods. The first clinical isolate was listed as a reference strain of *M. shinshuense* (strain ShT-P). Of note, all BU cases to date reported in Japan have been caused by *M. shinshuense*. We thus assume that *M. shinshuense* is an endemic species of *M. ulcerans*. To characterize *M. shinshuense*, here we generated whole-genome sequence of ShT-P and 26 clinical isolates and compared them with that of other mycolactone-producing mycobacteria.

The chromosome of ShT-P is 5.9 Mbp in length with a 65.6% G+C content, and the plasmid pShT-P is 167 Kbp in length with a 62.8% G+C content (Yoshida et al., Genome Announcements, 2016). The average nucleotide identities were 98.9% to *M. ulcerans* (strain Agy99) and 98.8% to a fleg pathogen *Mycobacterium lflandii* (strain 128FXT), and 97.8% to a fish pathogen *Mycobacterium marinum* (strain M), indicating that these stains closely resembled at the nucleotide level. The chromosome and pShT-P contains 5,015 and 72 predicted protein coding sequences (CDSs) respectively, and functional classification could be assigned to 71.4% of these. By comparing all CDSs of ShT-P with those of Agy99, 128XFT, and M, we found that they shared over 2700 CDSs at the amino acid level, whereas there were 236 *M. shinshuense*-specific CDSs. The total number of insertion sequences was 233, among which we counted 2 copies of IS2606, this is significantly less than those of *M. ulcerans* (91 in Agy99). We also counted 209 copies or fragments of IS2404 which is comparable to that of *M. ulcerans* (235 in Agy99). We performed phylogenic analysis of all IS2404 sequences of ShT-P and Agy99 and found that IS2404 sequences of ShT-P were tightly clustered and distinct from those of Agy99. These results suggested that IS2404 expansion occurred in both *M. ulcerans* and *M. shinshuense* after the two species were derived from a common ancestor but IS2606 expansion occurred only in *M. ulcerans*. Additionally, our single nucleotide polymorphism (SNP) comparison of 26 clinical isolates suggested localized clonal expansion in 5 regions of Japan, suggesting that most BU in Japan result from local transmission of a single circulating clone in each region.
Evidences of the non-implication of mosquitoes in the transmission of MU in Benin, West Africa

Poster presented by Rousseau Djouaka

By: Rousseau Djouaka et al., 2017
IITA- Benin, AgroEcoHealth Platform for West and Central Africa

Background
Buruli ulcer (BU) continues to be a serious public health issue in endemic regions. The reservoirs of Mycobacterium ulcerans (MU) the etiological agent of BU, as well as its mode of transmission remain poorly understood. In Australia, MU has been identified in several environmental samples including larvae and adult mosquitoes. However, there is no clear information linking mosquitoes to BU transmission in Africa, the continent with the highest endemicity of this disease. In this research, we screened the presence of MU in mosquito species collected in BU endemic villages in Benin. In addition, we investigated in the laboratory the potentials of mosquito’s larvae to pickup MU from their breeding environment and host it throughout larval developmental stages.

Methodology/Principal findings
Adults and larvae of mosquitoes were sampled from three BU endemic villages in Southern Benin and one non-endemic village in the Northern region of the country. Six thousand two hundred and seventy height samples of adults and larvae respectively were pooled (pools of 10 mosquitoes) and screened for MU DNA targets (IS2404, IS2606 and KR-B) using quantitative real time PCR. Positive and negative controls were analyzed under similar conditions. The analysis of these samples did not show any presence of MU in collected mosquitoes. Further investigations based on the monitoring of artificially infected larvae of Anopheles kisumu (a laboratory strain of Anopheles mosquitoes) with MU strains revealed that mosquito larvae are capable to ingested and host MU during L1, L2, L3 and L4 developmental stages. However we noticed a total absence of this bacteria at both pupae and adult stages certainly showing the inability of infected mosquitoes to vertically transmit MU to their offspring.

Conclusion/Significance
The refractory effect of mosquito larvae to host MU throughout developmental stages coupled with the absence of MU in mosquito samples collected in areas with reported cases of Buruli ulcer highlights the fact that this group of invertebrates (mosquitoes) are unlikely to be MU replicative reservoirs in BU endemic villages of Benin.

Keywords: Buruli ulcer, Mycobacterium ulcerans, mosquitoes, vertical transmission, Benin.
Comparative genomics suggest *Mycobacterium ulcerans* population expansion is aligned with rise of Buruli ulcer in south east Australia.

*Poster presented by Tim Stinear*

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Over the past five years, cases of Buruli ulcer have increased dramatically in specific areas of south east Australia. The reasons for this increase are unclear. Here we have used whole genome sequence comparisons on more than 150 *M. ulcerans* isolates obtained primarily from human clinical specimens spanning 70 years to model the population dynamics of this pathogen in south east Australia. Using phylogeographic and Bayesian approaches, we found that there has been a westward migration of the pathogen from the Bairnsdale region, beginning in the 1980s, 300km west to the major human population centre around Melbourne. This move has then been followed by a significant increase in *M. ulcerans* population size. These analyses inform our thinking around Buruli ulcer transmission and control, indicating that *M. ulcerans* is introduced to a new environment and then expands, rather than awakening a quiescent pathogen reservoir.
Research sessions

Treatments
Evaluation of new intermittent oral treatments in a murine model of Buruli ulcer

Presented by Jérôme Robert

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Introduction

The reference antibiotic treatment of Mycobacterium ulcerans infection (Buruli ulcer, BU), relies on 2 months of a daily regimen of injectable Streptomycin (STR) and oral Rifampicin (RIF). Daily fully oral treatment by Clarithromycin (CLR) – RIF is promising but still under evaluation. Intermittent oral treatment (e.g. 2/7) would greatly simplify treatment organization on the field. This goal will likely be reached with new antibiotics active against M. ulcerans.

Objectives

Our first objective was to evaluate in a murine model of BU the activity of a fully intermittent oral regimens (2/7 or 3/7) based on Rifapentine (RPT) a long lasting ansamycin, associated with either CLR, Moxifloxacin (MXF) or Bedaquiline (BDQ), recent drugs with known antimycobacterial activities, as compared to the reference RIF-STR 5/7 regimen.

A second objective was to evaluate, also in a murine model of BU, the bactericidal activity of several new antimycobacterial agents: tedizolid (TDZ) an oxazolidinone compared to Linezolid (LNZ), selamectin (SEL) an avermectin compared to Ivermectin (IVE), Q203 an imidazopyridine amine and PBTZ169 a benzothiazinone. Tested groups were compared to the reference RIF-STR 5/7 regimen.

Methods

For both objectives, four weeks old balb/c mice were inoculated in footpad with M. ulcerans Cu001 strain (≈ 4 log10/footpad). After an incubation time of 5 weeks, mice were randomly allocated into the following groups for testing fully intermittent oral regimens: RPT-MXF-2/7, RPT-CLR-2/7, RPT-CLR-3/7, RPT-CLR-5/7 and RPT-BDQ-2/7; and for testing new antibiotics, into the following: TDZ-5/7, LNZ-5/7, SEL-1/7, IVE-5/7, Q203-5/7, PBTZ-5/7, RIF-5/7. RIF-STR-5/7 (standard treatment) and no treatment were used as positive and negative controls, respectively in the two experiments. Bactericidal activity was assessed by negativation of footpad cultures at the end of 2 months treatment for both tests. Sterilizing activity was monitored only in the first experiment by clinical and bacteriological relapses during a 28 weeks post-treatment observational period.

Results

In the fully intermittent oral treatment experiment, all treated mice were culture-negative at the end of the 2 months treatment period, except 3/10 in the RIF-STR group, while cultivable bacilli increased in the untreated group. During the 28 weeks of the observational period after the end of treatment, relapses occurred in 3 groups: 1/39 in RPT-MXF-2/7, 1/40 in RPTCLR-5/7 and 4/40 in RIF-STR-5/7. The mean CFU count at the end of the follow-up was not statistically significant in the RPT-MXF-2/7 group from that in the RIF-STR-5/7 control group (p = 0.17), whereas it was lower in all other intermittent oral regimens (p = 0.04 for all comparisons).

In the new antibiotics experiment, Q203 and, to less extent LNZ, were the only drugs showing
significant bactericidal activities after 2 months of treatment and treated mice were culture-negative only in the Q203-5/7 group.

**Conclusion**

Fully intermittent oral regimens based on only 16 or 24 doses of RPT associated with either CLR, MXF or BDQ, appear as effective as the conventional RIF-STR regimen based on daily injections (40 doses) in the BU mice model. Such regimens would allow fully supervised treatment in the field.

Among new antibiotics tested, only Q203 showed promising results with significant antimycobacterial activity. Sterilizing activity, monitored by clinical and bacteriological relapses during a 28 weeks post-treatment observational period, will be conducted to confirm these promising results.
Activity of Linezolid and newer Oxazolidinones in combination with Rifampicin against *Mycobacterium ulcerans* in the mouse model of Buruli ulcer

*Presented by Paul Converse*

Deepak V. Almeida, Paul J. Converse, Till F. Omansen, Si-Yang Li, Jacques H. Grosset and Eric L. Nuermberger

Johns Hopkins University Center for Tuberculosis Research, Baltimore, MD, USA

**Background**

Linezolid (LZD) was the first oxazolidinone to reach market. It has been effectively used to treat cases of MDR and XDR tuberculosis. LZD is also active against most rapid and slow growing nontuberculous mycobacteria including *M. ulcerans* and is bactericidal in the mouse footpad model of Buruli ulcer (BU). However, concerns over toxicity limit the use of LZD beyond 2 weeks (wks). Newer oxazolidinones include tedizolid (TZD) and sutezolid (SZD). TZD was recently approved for treatment of acute bacterial skin and skin-structure infections (ABSSSI). It is active against *M. tuberculosis* in macrophages and in mice. SZD has undergone a phase II clinical trial and has more potent anti-TB activity in mice when compared to LZD. To our knowledge, neither TZD nor SZD have been tested against *M. ulcerans* in vivo. Their potentially better toxicity profile makes them ideal candidates to be considered for an improved, all-oral, potent and possibly shortened treatment of BU.

**Methods**

170 BALB/c mice were infected in the right hind footpad with an autoluminescent *M. ulcerans* 1059 (Ghanaian) strain. Treatment began after 40 days (D0) when all mice had footpad swelling with average lesion index (ALI) of ≥2. Mice were randomized to one of the following treatment groups: (1) rifampin alone (RIF, R10), (2) R10 + streptomycin (STR, S150), (3) R10 + clarithromycin (CLR, C100), (4) R10SZD50, (5) R10LZD50, (6) R10LZD100 and (7) R10TZD10 (doses in mg/kg are shown in subscripts). All drugs were given orally by gavage except for STR, which was injected subcutaneously. Mice were treated 5 times a wk, for a total of 8 wks. Efficacy of each regimen was evaluated by weekly ALI and relative light unit (RLU) readings. At wks 2, 4, 6 and 8, five mice from each treatment group were sacrificed to determine the reduction in CFU counts.

**Results**

At the start of treatment (D0) the average log10 CFU/footpad was 5.5 ± 0.66 and the mean RLU had increased from 0.48 ± 0.25 at baseline (D-39) to 26 ± 9. After 2 wks the CFU in all treatment groups, except for R alone, was reduced significantly compared to day 0 (p ≤ 0.001), with a corresponding decrease in footpad swelling. Among the oxazolidinones, R-SZD was the most active at 2 wks and was significantly better than R alone (p = 0.001) but not different than RC. The RLU count in all treatment groups also showed a rapid decline and was below the baseline (RLU = 0.1) by the end of 2 wks indicating the activity of all regimens. At 4 wks the CFU counts were as follows: R = 1.68 ± 0.38, RS = 0.59 ± 1.3, RC = 1.67 ± 0.43, R-SZD 1.86 ± 1.34, R-LZD50 =1.50 ± 0.59, R-LZD100 =2.14 ± 0.94, R-TZD = 2.06 ± 0.92. At 4 wks all treatment groups showed similar reduction in CFU except for RS which was significantly better than all oxazolidinone-containing regimens except R-LZD50. The ALI for all mice was below 0.5 except for the untreated, R-TZD and R-LZD100 groups. Mice were rendered culture negative in all treatment groups after 8 wks of treatment.
**In-vivo testing reveals high-dose rifamycins as best improvement for Buruli ulcer treatment**

*Presented by Till F. Omansen*

Till F. Omansen¹ ², Paul J. Converse¹, Deepak Almeida¹, Si-Yang Li¹, Jin Lee¹, Ymkje Stienstra², Tjip van der Werf² ³, Jacques Grosset¹, Eric Nuermberger¹

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**Background**

Buruli ulcer (BU) is a toxin-mediated infection with *Mycobacterium ulcerans* that presents as destructive and potentially disabling skin lesions. The current treatment is an 8-week course of streptomycin and rifampin. The treatment is effective but toxicity and intramuscular administration of streptomycin as well as the long treatment duration are problematic. A short, all oral treatment is needed. Here we tested whether avermectins or verapamil could be repurposed for BU or if high-dose rifampin or rifapentine in combination with oral clarithromycin are at least as effective as streptomycin and rifapentin in a BU mouse model. Avermectins are anti-parasite drugs that kill *M. ulcerans* in-vitro. Verapamil is a calcium channel blocker, suspected to affect export of the bacterial toxin mycolactone. A dose-finding study of daily rifampin or rifapentine has never been conducted.

**Material/methods**

Six week old, female BALB/c mice (n=5 per group) were infected in the hind footpads with 4.5-6.2 log₁₀ CFU autoluminescent *M. ulcerans*. After incubation for 4-5 weeks, mice were treated 5 times per week via oral gavage with escalating doses of the test compounds. As an effect on toxin export was suspected with verapamil, a preventive model was chosen in which treatment began 1 or 5 days post-infection instead. Clinical footpad swelling, relative light units (RLU) emitted from the bacteria, as well as colony-forming units (CFU) per footpad from sacrificed animals were obtained at the start, after two weeks and at the end of treatment to measure efficacy.

**Results**

While mice treated with ivermectin and selamectin showed elevated rates of RLU proportional to the drug dosage, there was no significant difference in clinical swelling or CFU per footpad between treated mice and untreated controls at the end of experiment. Verapamil increased footpad swelling after 4-5 weeks but likewise did not reduce CFU. Escalating the dosage of rifampin or rifapentine with clarithromycin resulted in dose-dependent reductions in RLU, swelling and CFU per footpad. All mice receiving 40mg/kg rifampin and 5-20 mg/kg rifapentine plus clarithromycin were culture-negative at week 4.

**Conclusions**

While avermectins have been shown to kill *M. ulcerans* in-vitro, they failed to show activity in mice. Inadequate drug exposures or counter-productive local immune-modulatory effects might explain the lack of efficacy. Verapamil promoted pathology without affecting CFU. Promotion of toxin production or export is suspected. No ceiling effect was observed with the maximum tested dose of rifampin 40 mg/kg. In humans, 35mg/kg daily is equally safe as lower doses. High-dose rifampin or possibly rifapentine containing regimens are therefore promising candidates for shortened oral treatment for Buruli ulcer.

All oxazolidinones tested showed activity against *M. ulcerans* and their activity was additive to RIF over the first 2 wks of treatment, but not thereafter. SZD was the most active at the end of 2 wks. Overall, RS was the most potent regimen. No oxazolidinone improved the activity of R-containing regimens to a greater extent than CLR, but they may be useful in oral regimens in the event that CLR is not well tolerated.
Discovery of new therapies for Buruli ulcer treatment

Presented by Santiago Ramón-García¹,² and Alfonso Mendoza-Losana²

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Standard treatment of Buruli ulcer (BU) involves 8 weeks of combination therapy with rifampicin and streptomycin. The daily painful injection of streptomycin together with the side effect associated, i.e. ototoxic, nephrotoxic, and hepatotoxic, in addition to issues related to pregnancy and treatment to young infants made the scientific community look for alternatives to streptomycin. Clarithromycin or moxifloxacin have now been proposed, although their effectiveness still needs to be clinically demonstrated. No alternatives for rifampin are, however, currently available, thus making it the cornerstone drug for BU therapy; the emergency of rifampicin resistance in Mycobacterium ulcerans would virtually bring BU to the pre-antibiotic era.

Drug development for neglected diseases such as BU, mainly affecting developing countries, is especially complicated due to lack of interest from the main scientific community and, as a consequence, lack of investment. To overcome these limitations and speed up the discovery and development process of a much-needed new BU therapy we are applying knowledge gathered in tuberculosis (TB) R&D programs.

A collaborative effort between the University of British Columbia (Canada) and GlaxoSmithKline at the Diseases of the Developing World (GSK-DDW) campus in Spain has used two innovative and complementary approaches to identify new anti-BU therapies:

- **Repurposing clinically approved antibiotics.** We have identified promising antibiotics active alone and in synergistic combinations with rifampicin against *M. ulcerans* with a clinical pedigree, low toxicity, and orally and pediatric available.
- **Screening a GSK in-house library of advance anti-TB drug candidates.** We have identified promising compounds with activities in the nano molar range that are currently in pre-clinical development for TB therapy.

Our work here presented open new therapeutic avenues, both in the short term by optimizing current BU drug therapy and in the long term by identifying new alternatives to rifampicin treatment, that promise to be much more effective and less toxic that currently available treatments.
Paradoxical reactions in *Mycobacterium ulcerans* antibiotic therapy: related to bacterial load?

*Presented by Richard Phillips*


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**Introduction**

Buruli ulcer (BU) caused by *Mycobacterium ulcerans* infection is effectively treated with rifampicin and streptomycin or rifampicin and clarithromycin for 8 weeks. Paradoxical reaction with new inflammation and significant enlargement of a healing lesion during or after antibiotic treatment is a common complication causing diagnostic and management difficulties. We have investigated the relationship between bacterial load in BU lesions and the development of paradoxical reaction following initiation of antibiotic treatment.

**Methods**

In 2013–2016 patients with active BU were recruited at Tepa, Agogo, Dunkwa and Nkawie Government Hospitals in Ghana. Fine needle aspirates (FNA) and swabs were obtained to establish the diagnosis of BU by microscopy, culture and PCR. Samples obtained at baseline, during treatment (week 4 and 8) and after treatment (week 12 and 16) if the patient had an unhealed lesion were placed in RNA protect. In order to detect live *M. ulcerans*, DNA and RNA extracted using the AllPrep DNA/RNA mini kit were processed by the combined 16S rRNA reverse transcriptase / IS2404 qPCR assay as described (Beissner et al., 2012). Patients were followed up 2 weekly until complete healing.

**Results**

Among 131 patients with PCR confirmed BU, paradoxical reactions developed in 19 patients (15%) between 2 and 42 weeks after treatment initiation (median 6 weeks). There was no association between PR and age, sex, category of lesion or lesion site.

Before antibiotic treatment the bacterial load based on qPCR for IS2404 was significantly higher in patients who had a PR compared to those who did not (p=0.02). *Mycobacterium ulcerans* culture was positive in 11 of 19 patients (58%) that developed a PR compared with 27 of 112 (24%) in the no-PR group and the number of copies of 16S rRNA was significantly higher in the PR group. After antibiotic treatment for 4 weeks, 4 of 18 patients (22%) in the PR group had a positive culture compared to 8 of 75 patients (10%) in no-PR group. Healing was delayed during the first 20 weeks in patients who had a paradoxical reaction but time to complete healing was comparable for the two groups.

**Conclusion**

BU patients that develop a paradoxical reaction are characterised by high bacterial load in lesion samples taken at baseline, a higher chance of positive *M. ulcerans* culture at baseline and persistently positive culture or 16S rRNA assay during antibiotic treatment, all of which suggests a higher initial burden of bacterial infection.

**References**

Gallium targets mycobacterial iron-uptake mechanisms: A potential treatment for Buruli ulcer?

*Presented by Arthur Baca*

Gallium has been used in medicine as both a diagnostic (imaging) and a therapeutic agent. The activity of gallium relies largely on its ability to compete with ferric iron (Fe\(^{3+}\)). Ga binds to the iron transport protein transferrin, and is then taken up by cells that express transferrin receptor, where it can inhibit iron-dependent enzymes. Because ferric iron is required by ribonucleotide reductase, which is essential for DNA synthesis, gallium can impair a cell’s ability to reproduce. Cancer cells and bacteria are preferential gallium targets due to their fast growth and consequent overexpression of transferrin receptor. In most healthy tissues, however, iron is heavily recycled, so there is little need for new iron, and gallium has very little effect. In bone marrow, only ferrous iron (Fe\(^{2+}\)) is taken up, so Ga doesn't enter this system. The antiproliferative effects of gallium therapy have been observed in infectious disease models (viral, bacterial, parasitic) and in cancer patients (rapidly growing cancer cells). Several research groups continue to explore gallium as a potential antibiotic using *in vitro* and animal models of infection, including models incorporating intracellular bacteria such as mycobacteria. Gallium maltolate is a new, orally and topically administrable gallium compound that has completed Phase I clinical trials in the United States, demonstrating a safety profile superior to that of the FDA-approved intravenous gallium nitrate formulation. Based on previous data demonstrating inhibitory effects of gallium on other mycobacterial species in a variety of *in vitro* and *in vivo* models, it is hypothesized that gallium maltolate could serve as a safe and convenient treatment for *Mycobacterium ulcerans* infection.
Long-term efficacy of single mass azithromycin treatment for yaws

Poster presented by Oriol Mitjà

Authors: Oriol Mitjà, Charmie Godornes, Wendy Houinei, August Kapa, Raymond Paru, Sivauk Bieb, James Wangi, Eric Mooring, Camila González-Beiras, Sergi Sanz, Kingsley Asiedu, Quique Bassat, Sheila Lukehart

Background
Yaws is an important cause of chronic disfiguring ulcers in children in the tropics. The long term efficacy of the WHO strategy for yaws eradication needs to be determined.

Methods
In an initial study, we implemented the yaws eradication strategy on a Papua New Guinea island (16092 population) on which yaws was endemic. Mass azithromycin treatment was administered at 0 months with a coverage rate of 84%, and followed every 6 months by a targeted treatment program to treat all persons with active clinical cases and their contacts. The participants were initially followed for 12 months; in this extended follow-up study, clinical, serological and PCR surveys were conducted at 18, 24, 30, 36 and 42 months. In addition to the prevalence of serologically confirmed active and latent yaws, we measured active yaws confirmed by PCR as a primary-outcome indicator.

Results
The prevalence of PCR-confirmed active yaws infection fell from 1.8% before mass treatment to a minimum of 0.1% at 24 months (difference from baseline, -1.7%; 95%CI, -1.9 to -1.4; P<0.001), but began to re-emerge after 2 years and there was evidence of a significant increase to 0.4% at 42 months (difference from 24 months, 0.3%; 95%CI, 0.2 to 0.4; P<0.001). At each time point after baseline, about 40% of new cases had been absent from initial mass treatment. The prevalence of high titre latent yaws in children aged 1 – 5 years fell from 13.7% before mass treatment to 1.5% at 24 months, and 0 cases at 42 months. At months 36 and 42, two cases and three cases, respectively, revealed mutations at the 23S ribosomal DNA conferring resistance to azithromycin.

Conclusions
The WHO strategy did not achieve yaws eradication in a high-endemic setting, perhaps due to the incomplete coverage of the resident population. Azithromycin-resistant T. pertenue has emerged in Papua New Guinea.
Research sessions

Pathogenesis
The bacterial diversity in Buruli ulcer skin lesions

Presented by Conor Meehan

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Background

Buruli ulcer lesions are often associated with secondary bacterial infections, which can cause pain and soft tissue infection beyond the ulcer, and result in delayed healing. Thus understanding the diversity of bacteria, termed the microbiome, in these open lesions is important for proper treatment.

Methods/Results

In a pilot project, using 16S rRNA sequencing, we determined the microbial composition of 5 BU lesions, 3 non-BU lesions and 3 healthy skin samples. Our results showed a lower bacterial diversity in both the BU and non-BU lesions compared to the healthy skin. Although no significant differences in diversity were found between BU and non-BU lesions, the former were characterized by an increase of Bacteroidetes compared to the non-BU wounds and the BU lesions also contained significantly more obligate anaerobes. With this molecular-based study, we were also able to detect bacteria that were missed by culture-based methods in previous BU studies, primarily a high abundance of strict anaerobes.

Conclusions

Our study suggests that BU leads to changes in the bacterial community within the lesions and proposes a pipeline for future microbiome analyses, including appropriate controls to include in the design of such studies. However, further microbiome studies are necessary, involving sufficient sample sizes and lesions from the same body site in many patients, in order to confirm these findings and tease apart the underlying differences in bacterial compositions.
The clinical presentation of Buruli ulcer (BU) is very heterogeneous and ranges from pre-ulcerative nodules to ulcerative lesions. In Africa more advanced stages are commonly seen and the histopathological knowledge of BU lesions is mainly based on studies conducted on such advanced ulcerative lesions from African patients. In the BU endemic areas of Australia the infection is frequently diagnosed in its early stages and then often treated by excision of the entire small lesions, providing access to excised early tissue specimens from patients who are not under antibiotic treatment.

Our in depth analysis of 12 early ulcerated BU lesions (max. 12 weeks old) from patients originating from Queensland, Australia between 2000 and 2015, revealed a strong early cellular infiltration of the infected tissue. The necrotic lesion core containing the acid fast bacilli was surrounded by a belt of infiltrating cells composed of macrophages, neutrophils, T-cells and clusters of B-cells. The necrotic core itself showed all characteristic histopathological hallmarks of BU, including necrosis and fat cell ghosts. It contained clusters of extracellular acid fast bacilli (AFB), but no viable mammalian cells. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining revealed the presence of apoptotic cells throughout the entire belt of infiltration, indicating gradual diffusion of mycolactone from the necrotic core to the periphery of the lesion.

Results from our pig model studies support these results. Six weeks after subcutaneous injection we observe a necrotic core which is surrounded by a thick belt of infiltrating neutrophils, macrophages and T-cells [1].

Taken together these results indicate that a strong inflammatory response is induced by the infection with Mycobacterium ulcerans, but that the infiltrating leukocytes are not able to reach and eliminate the bacteria, once a necrotic core containing extracellular clusters of mycolactone producing M. ulcerans is established.

References
Antibody-mediated neutralisation of the exotoxin mycolactone prevents cells from undergoing apoptosis

Presented by Jean-Pierre Dangy

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Introduction

Mycolactone, the macrolide exotoxin produced by \textit{Mycobacterium ulcerans}, causes extensive tissue destruction by inducing Bim-dependent apoptosis. In this study, we aimed at the production of anti-mycolactone antibodies that could neutralize the cytotoxic activities of mycolactone.

By applying the B cell hybridoma technology, we were able to generate a series of monoclonal antibodies (mAbs) with specificity for mycolactone from spleen cells of mice immunized with synthetic mycolactone-protein conjugates. L929 fibroblasts were used as a model system to investigate whether these antibodies can inhibit the biological effects of mycolactone.

By measuring the metabolic activity of L929 fibroblasts, we found that the cytotoxic activity of mycolactone can be completely neutralized by some of the mAbs.

Based on these findings, the \textit{in vivo} functionality of anti-mycolactone mAbs will now be evaluated by performing active immunisation experiments in infected mice.

This project was supported by the Medicore Foundation and by the Stop Buruli Initiative funded by the UBS-Optimus Foundation.

Mycolactone membrane permeation and aggregation from the lens of simulations

Presented by Jessica Swanson

Jessica MJ Swanson, Rui Sun

Mycolactone is the exotoxin produced by Mycobacterium ulcerans that acts as the key virulence factor behind Buruli ulcer disease. It kills host cells via a myriad of interactions with cytosolic and membrane-bound targets. Although it is thought to passively permeate through host membranes, its interactions with membranes is unclear given its amphiphilic structure. It has been shown to be carried in outer membrane vesicles in the extracellular matrix of M. ulcerans, and to have increased cytotoxicity when delivered in such vesicles. In this work we demonstrate how transition-tempered metadynamics can be used to characterize membrane permeation and aggregation of this large toxin in atomistic molecular dynamics simulations. We find that the exotoxin has a strong affinity for the lipid bilayer, even greater than that of a cholesterol molecule. Although the permeation mechanisms differ for the two dominant isomers, both show complete submersion in the bilayer environment. These findings suggest that lipids play an integral role in the transport and delivery of mycolactone to and within host cells.
Introduction to our major new collaborative Wellcome Trust Investigator project “Investigating the role of coagulation in the pathogenesis of Buruli ulcer”

Presented by Rachel E. Simmonds*

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In my presentation, I hope to introduce to the Buruli ulcer community a major new project that I am leading, funded by the Wellcome Trust, to understand the role of coagulation in the pathogenesis of the disease. This started with an initial investigation into the effect of mycolactone on endothelial cells. No longer considered inert cells that line the blood vessels, endothelial cells are known to be extremely dynamic and also to play a vital role in maintaining intravascular fluidity. The motivation here was not only the work we had been doing to uncover the molecular mechanism of mycolactone function, but also my many years of experience in the field of haemostasis and endothelial cell biology in my PhD and early postdoctoral labs.

What we found was that human dermal microvascular endothelial cells are exquisitely sensitive to mycolactone, requiring ~100-fold lower concentrations to affect Sec61 (the molecular target). This is important because it puts them on the front-line during the establishment phase of an infection. More specifically the critical anticoagulant protein thrombomodulin was shown to be rapidly depleted from the cells in a Sec61-dependent manner. Furthermore, similar depletion was found in BU patient lesions, and this was shown to be associated with a local coagulation defect commonly characterised by fibrin deposition. However, this is certainly still not the complete picture and we know remarkably little about how endothelial cell function is affected by mycolactone, or how this might alter local haemostasis. The important question of whether fibrin deposition is a cause or effect of tissue necrosis remains open.

Thus the project will examine how the different facets of coagulation-control might work together to initiate clinical disease, with a particular focus on defining the endothelial dysfunction and examining wound formation and healing. Success in the project will rely on key collaborations in the BU community including Dr Richard Phillips (KCCR, Ghana), Dr Mark Wansbrough-Jones (St Georges, London, UK) and Prof Gerd Pluschke (STPHI, Basel, Switzerland) to attack this problem from in vitro, in vivo and clinical perspectives. We hope to provide a firm pathogenic basis from which to develop enhanced BU treatment strategies that target this mechanism in the future, and welcome comments and further collaboration within this community of dedicated researchers.

The biochemical mechanism by which mycolactone inhibits the Sec61-dependent translocation of proteins into the endoplasmic reticulum

Presented by Belinda S. Hall

Michael McKenna*, Belinda S. Hall‡, Rachel E. Simmonds‡ and Stephen High*

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All mammalian cells have sub-compartments that help the cell to function correctly. These include the outer cell membrane, the nucleus, and the endoplasmic reticulum (ER). The main way in which proteins enter the ER is via a specialised pore-like machine known as the Sec61 translocon. In 2014 we identified that the major cellular target of mycolactone was the blockade of protein translocation via Sec61 [1]. Our previous studies have shown that mycolactone prevents cells from making many of the secretory proteins that normally make a vital contribution to both cellular defence and communication and showed that this was due to a mycolactone-induced change in conformation of the major subunit of the Sec61 translocon, Sec61α [2]. The only secretory proteins that escape this inhibition are an extremely rare type of short secretory protein that has a specific mechanism of crossing the Sec61 translocon [2].

We will present our most recent and extensive biochemical assays studying the translocation of different classes of single pass transmembrane protein (TMP) into the ER lumen and their incorporation into membranes. Such proteins are sub-divided into three main classes based on whether or not they possess a cleavable signal peptide, and their final orientation in the ER membrane. Type I TMPs have a signal sequence as well as an internal transmembrane domain that anchors the protein in the ER membrane with its amino-terminus in the ER lumen and its carboxy-terminus in the cytosol. Type II and type III TMPs lack the signal peptide and end up in inverse orientations in the membrane. Our studies show that mycolactone strongly inhibits the translocation of type I and type II TMPs, but that type III TMPs are completely resistant to mycolactone’s effects. Furthermore, the assays provide new evidence into the general mechanism by which translocation via Sec61 takes place – a process for which much of the molecular detail is still a mystery. For instance, in cases where type I TMPs (the most common form of single-pass TMP in the genome) have a large amino-terminal domain >100bp then translocation blockade is complete. However, in cases where this domain is smaller (such as the subunit of the T cell receptor CD3δ) a small amount may escape blockade due to the way the elongating polypeptide folds within the translocon pore. Likewise, the (rare) type III TMPs seem to display mycolactone resistance because they also utilise this so-called “headfirst” orientation rather than a “hairpin” type insertion. This latter arrangement, which requires inversion of the polypeptide within the translocon, is restricted by mycolactone.

The implications of these biochemical and molecular findings for BU, as we continue to seek to explore the cellular effects of pathogenic mycolactone exposure, will be explained.

Mycolactone prevents immune cell signaling and communication by inhibiting the Sec61 translocon

Presented by Jean-David Morel

Jean-David Morel*, Ludivine Baron*, Anja Paatero*, Francis Impens†, Laure Guenin-Macé*, Sarah Saint-Aure†, Nicolas Blanchard†, Rabea Dillmann‡, Fatoumata Niang*, Sandra Pellegrini*, Jack Taunton§, Ville O Paavilainen‡, Caroline Demangel†

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The virulence of M. ulcerans, the causative agent of Buruli ulcer disease, relies on the production of mycolactone, a polyketide-derived macrolide with tissue-specific properties. In addition to inducing local skin ulceration and analgesia, mycolactone diffuses in infected organisms to dampen cellular immune responses at the systemic level. Previous work by Hall and col. has shown that mycolactone blocks the co-translational translocation of inflammatory mediators into the endoplasmic reticulum (ER), with subsequent degradation of these proteins by the ubiquitin:proteasome system. In eukaryotes, this process is mediated by a heterotrimeric channel called the Sec61 translocon. I will present recent work showing that mycolactone operates by binding to the alpha subunit of Sec61 (Sec61α). Quantitative proteomic analysis of mycolactone-exposed T cells showed that key signal-transmitting receptors and adhesion molecules are affected by Sec61 inhibition during T cell activation. Interestingly, a range of IFN-γ-inducible cytoplasmic proteins was also impacted, due to mycolactone-induced inhibition of the production of IFN-γ receptor. We describe an amino acid mutation near the luminal plug of Sec61α, which confers total resistance to mycolactone-mediated immunosuppressive effects in vitro and in vivo. Over-expression of mutant Sec61α in mycolactone-treated T cells rescued both their homing potential and effector functions. In macrophages, it restored IFN-γ receptor-mediated anti-microbial responses. In addition to describing the molecular mechanism underpinning the immunosuppressive effects of mycolactone, our work therefore uncovers a novel mechanism of immune evasion evolved by M. ulcerans.
Mycolactone effects on the central nervous system

*Presented by Laure Guenin-Macé*

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*Mycobacterium ulcerans* is unique amongst human pathogens in its capacity to produce mycolactone. In addition to suppressing the development of immune responses at the systemic level, this diffusible macrolide causes the painless destruction of the skin that characterize Buruli ulcers. While the mechanism underpinning the immunosuppressive effects of mycolactone have been elucidated, our understanding of those contributing to analgesia remain incomplete. In particular, whether mycolactone can access the central nervous system (CNS) to affect the viability and functions of tissue-resident cells is unknown. Our investigations using an *in vitro* model of the human blood brain barrier suggest that mycolactone can cross the brain endothelial layer in a transcellular manner. Using a representative panel of mouse primary cells, we also show that mycolactone inhibits the development of pro-inflammatory responses in the CNS at non-cytotoxic concentrations. Together, these data thus suggest that inhibition of neuroinflammation may contribute to the analgesic properties of mycolactone in patients with Buruli ulcers.
Mycolactone, the lipid virulence factor of *Mycobacterium ulcerans* causes an atypical stress response which it underlies its cytotoxic effects

**Presented by Joy Ogbechi**

Joy Ogbechi¹, Belinda Hall¹, Kirsti Hill², Thomas Sbarrato², Anne E. Willis², Ronald C. Wek³ and Rachel Simmonds¹

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Despite the fact that mycolactone is known to be responsible for the tissue necrosis associated with Buruli ulcer lesions, the precise molecular mechanism involved in mycolactone-mediated cell death is still not completely resolved. During our discovery of Sec61 as the major cellular target of mycolactone (1), we showed that it also causes a change in the polysome profile of macrophage-like RAW264.7 cells. This was characterised by a pronounced increase in the size of the 60S peak and a decrease in the area under the peaks associated with heavy polysomes, without affecting the translation of cytokine and other immune-mediated mRNAs. This change in the polysome profile suggested that mycolactone was causing a remodelling of some aspects of the translatome and prompted us to identify the transcripts whose translation were affected by mycolactone.

Translational microarray profiling carried out on RAW264.7 cells exposed to mycolactone resulted in the identification of only 140 genes whose translation appeared significantly altered, with 111 of these being up-regulated while 29 were down-regulated. Gene Ontology analysis suggested that several proteins of the integrated stress response pathway may be activated in these cells. The integrated stress response is an adaptive response activated by cells to enable them recover from noxious stimuli. With prolonged exposure to stress however, activation of this pathway can also lead to apoptosis. We validated the findings of the microarray and found that the ISR pathway is commonly activated in cells exposed to mycolactone including dermal fibroblasts. Using detailed molecular analysis, including investigation of cellular signalling pathway analysis, CRISPR gene knockout, stable cell lines and cellular inhibitors we have unpicked this pathway and investigated the cause and effect of ISR activation, and the fate of cells exposed to mycolactone. Our findings indicate that mycolactone induces an atypical stress response which underlies its cytotoxic effect.

Genetic variation in autophagy-related genes influences the eisk and phenotype of Buruli ulcer

*Poster presented by Carlos Capela*

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Buruli ulcer (BU) is an infectious disease caused by *Mycobacterium ulcerans*. *M. ulcerans* is unique among mycobacteria due to its slow growth, its optimal growth temperature of 32°C and the fact that it produces an exotoxin, mycolactone. BU presents as necrotising skin and soft tissue disease, which can progress to bone damage. Emerging evidence in the literature has suggested a major role for genetic factors in the development of BU. Indeed, not all individuals develop clinically overt disease, even after sustained exposure to *M. ulcerans* in endemic wetlands; the disease presents a wide spectrum of clinical manifestations; cases of spontaneous healing have been reported; BU cases tend to cluster within families; and frequencies of antibodies to *M. ulcerans* in serum samples from affected and unaffected subjects were shown to be similar. In that sense, we hypothesized that several polymorphisms, already described to be associated with tuberculosis or leprosy, can also be associated with BU, being responsible not only for susceptibility or resistance, but also for the inter-individual differences of disease phenotypes.

The function of specific components of the autophagic process has been associated with resistance to several intracellular pathogens, including *M. tuberculosis*. Additionally, mycolactone was shown to impair the autophagic process, resulting in an accumulation of autophagosomes within the cytoplasm. Given this evidence, we explored genetic variants in the autophagy-related genes *NOD2*, *PARK2* and *ATG16L1* given their previous association with susceptibility to other mycobacterial diseases. We constitute a Beninese genotyped cohort constituted by 208 patients with BU and 300 healthy controls. We analysed their association with risk to develop BU, the progression to an ulcerative form and the progression to severe phenotypes. We observed that the rs1333955 SNP in *PARK2* is significantly associated with increased susceptibility to develop BU [OR, 1.43; P = 0.05]. In addition, both the rs9302752 and rs2066842 SNPs in *NOD2* genes significantly increased the predisposition of patients to develop category 3 lesions (OR, 2.23; P = 0.02; and OR 12.7; P = 0.03, respectively), whereas the rs2241880 SNP in *ATG16L1* was found to significantly protect patients from presenting the ulcerative phenotype (OR, 0.35; P = 0.02). Our findings indicate that specific genetic variants in autophagy-related genes influence susceptibility to the development of BU and its progression to severe phenotypes.
Murine infection with bioluminescent *M. ulcerans* shows late onset of immune response and lack of gastro-intestinal colonization

**Poster presented by Till F. Omansen**

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**Background**

*Mycobacterium ulcerans* causes the debilitating skin infection Buruli ulcer - a neglected tropical disease. Buruli ulcer is mainly prevalent in rural foci in West Africa but also in Australia. While *M. ulcerans* DNA has been recovered from various environmental samples and puncturing injury with contaminated material produces infection, its niche and mode of transmission are not yet entirely understood. In Australia, possums have clinical lesions and excrete *M. ulcerans* in their faeces. *M. ulcerans* DNA has also been found in mosquitoes, suggesting a transmission cycle involving mammals and a mosquito vector. Here, we characterize two murine subcutaneous and gastro-intestinal infection models with an auto-luminescent Australian *M. ulcerans* to simulate transmission and study immunology and possibly vaccination against the disease.

**Material/methods**

Ten six weeks old, female, BALB/c mice were infected with 5.5 log10 CFU autoluminescent *M. ulcerans* into the dorsal aspect of the upper third of the tail. During 17 weeks, mice were imaged with an IVIS® (in-vivo imaging) camera, detecting luminescence. At given time-points, antibody titres against *M. ulcerans* and at the end of the experiment, the cytokine profile of culled mice were recorded. To study the gastro-intestinal infection with *M. ulcerans*, 10 mice were infected with 5.6 log10 CFU via oral gavage. During 25 weeks, mouse faeces were collected weekly from and subjected to qPCR analysis for excreted *M. ulcerans*. At the end of the study (week 25), consecutive samples of the gastrointestinal tract were analysed for *M. ulcerans* presence and pathology via qPCR and histology.

**Results**

Electroporation of pMV306 hsp16+luxG13 produced viable, auto-luminescent *M. ulcerans*. Virulence was demonstrated by the development of clinically apparent lesions in tail-infected mice from week 4-5 post-infection. After 7 weeks of infection, a transition from exponential increase of light-signal detected in the IVIS®, into a more stationary phase of infection was observed. At this time-point antibody titres against *M. ulcerans* heat-shock protein 17 and whole cell lysate rose. Gastro-intestinal inoculation with *M. ulcerans* resulted in low, transient qPCR signal and neither GI, nor cutaneous pathology.

**Conclusions**

We demonstrated and characterized the subcutaneous infection of the mouse tail with auto-luminescent *M. ulcerans*. Unlike other models, our model follows the *M. ulcerans* infection over a prolonged timeframe of 17 weeks and correlates in-vivo images with the IVIS® system with immune parameters. The IVIS® technology allows to not only quantify bacteria by emitted light but also track their position within the living organism; in this case, the bacteria stayed highly localized. This model will be applied for further transmission and vaccinology studies. We furthermore showed that *M. ulcerans* neither causes pathology nor colonizes the rodent gastro-intestinal tract. If possums lack GI-pathology like mice, it is probable that they acquire *M. ulcerans* from another environmental niche.

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What are the histopathological criteria for Buruli ulcer: a Study on 33 cases of confirmed Buruli ulcer in Cameroon

Poster presented by Yasmine Lucile Ibrahim

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Introduction

Buruli ulcer positive diagnosis remains a challenge in resource-limited countries. Both Ziehl Neelson stain used to detect the alcohol resistant bacilli and culture have limited sensitivity. The PCR for the IS2404 is specific to M. ulcerans DNA and is a highly sensitive method to detect M. ulcerans in human tissue. PCR on 3 mm punch biopsies tissue specimen proved to be the best diagnostic tool for non ulcerated lesion with a sensitivity rate of 85% and PCR assessment of swab samples was the best diagnostic tool for ulcerated lesion (1). Histology performed on excised lesions is more sensitive than culture and ZN (63-90%), but surgical excision is often not required when patients are treated with antibiotic therapy. Histological description of true Buruli ulcer has been mostly done on case reports and only one study offers a systematic description of the infected tissue on excisional skin-biopsy (2): in this paper, we aim to clarify the main histopathological features of cutaneous BU based on skin punch biopsies of 4 mm.

Methods

Between 2011 and 2013, a prospective cohort study was conducted in Akonolinga Health District, central Cameroon. Dry swabs from ulcerative and fine-needle aspirates of non-ulcerative lesions were examined after Ziehl Neelson staining, followed by PCR targeting IS2404 and culture performed in the reference laboratory in Yaounde. Two 4 mm punch skin biopsies were performed. On ulcerative lesion, the biopsies were taken one at the edge and one in the center of the lesion, and for non ulcerated lesions in the middle of the nodule (although biopsy was optional for these cases). The biopsies were fixed, stained analyzed in Yaounde (Centre Pasteur Cameroon, CPC), after which slides and remaining paraffin blocks were sent to Geneva University Hospitals for a second reading. Staining by Hematoxylin/Eosin and Ziehl-Neelsen was performed for all samples. Every section was assessed for the presence or the absence of Buruli ulcer type: coagulative dermal and, or subcuticular necrosis with or without ghost cells, panniculitis, signs of vasculopathy with vasculitis +/- vascular parietal necrosis, acute neutrophilic inflammation or chronic inflammation with lymphocytes, plasma cells or eosinophils and the presence or not of granulomas. Patients with a final diagnosis of BU corresponded to one of the following criteria (3): 1) at least two positive tests among ZN done in Akonolinga, or in CPC, PCR, culture, or positive acid-fast bacilli (AFB) on histology; 2) being the most likely diagnosis based on both expert reviews of photographs; 3) BU agreed upon as the most likely diagnosis during consensus meetings where clinicians, dermatologists, and histopathologists reviewed cases without a definite diagnosis. In this analysis, we focused on patients with a final diagnosis of BU which also had histologically confirmed BU, defined as either the presence of AFB on histological sample or a definite BU diagnosis on histology (presence of Buruli ulcer-type coagulative necrosis of the dermis or the subcutis with or without granulomas) with other positive laboratory tests.
Results

The 364 patients included in the study had 422 lesions, of which 381 were ulcerative. 357 lesions were biopsied and analysed in Centre Pasteur Cameroon (CPC). 353 were analysed in Geneva. Among the 87 cases with a final diagnosis of BU, histology criteria for a definite BU were fulfilled in 33 lesions biopsied from 32 patients (sensitivity 37.9%) 31 patients had PCR positive swabs. 27 presented alcohol resistant bacilli confirmed by Ziehl-Neelson stain on histological examination. The 6 cases without alcohol resistant bacilli by Ziehl-Neelson stain had at least 2 positive laboratory tests for M. ulcerans. All fulfilled some histological criteria of M. ulcerans infection: as epidermal regeneration and subcutis necrosis was seen in all lesions (33/33), as well as an acute neutrophilic inflammatory infiltrate (33/33). Ghost cells could be seen in 72.7% of BU lesions (24/33). Most showed signs of vasculopathies (28/33), some with thrombi (12/33) and many with vasculitis (28/33). 30/33 showed vascular parietal necrosis. More than half of the punch biopsies had a final diagnosis of non-specific ulcer (190/353 = 53.8%).

Discussion

Based on the systematic histological examination of 33 definite BU cases, we retain three main histologic criteria for ulcerated type of Buruli ulcer: 1) Necrosis of subcutaneous tissue, 2) Inflammatory infiltrate and 3) Presence of acid fast bacilli detected by Ziehl Neelson stain. These 3 criteria can be considered the most reliable histopathologic features for the histologic diagnosis of Buruli ulcer disease. When PCR access lacks, histological analysis of the clinically suspected Buruli ulcer can be one of the confirmatory laboratory method for diagnosing M. ulcerans infection, although diagnosis of BU requires correlation with clinical data and other laboratory results. Histopathological analysis has some inconvenient as it requires well trained personnel in a sophisticated laboratory. It is also quite expensive and requires an invasive procedure. Diagnostic value of punch biopsies is sometimes limited when samples are too superficial, not allowing for examination of the hypodermis.

Conclusion: Although histopathology is not as specific as PCR in diagnosing BU, histopathological analysis of suspected skin specimens by using punch biopsies of 4 mm in the center and in the periphery of the lesion and adding the Ziehl Neelson stain can help in providing the correct diagnosis of BU.

References:

