WHO Meeting on Buruli ulcer Control and Research

25–27 March 2013

WHO Headquarters
Geneva, Switzerland

ABSTRACTS
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Epidemiological situation from endemic countries
Buruli ulcer in Australia, 2011-12

Presenter: Paul Johnson

Paul Johnson,1,2 Caroline Lavender,2 Richard Gair,3 Juliet Esmonde,3 Sushil Pandey,4 Carmel Cochrane,4 Chris Coulter,4 Christina Steffen,5 John McBride,5 Janet Fyfe.2

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2. Victorian Infectious Diseases Reference Laboratory, and WHO Collaborating Centre for Mycobacterium ulcerans, Victoria, Australia
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4. Queensland Mycobacterium Reference Laboratory, Brisbane, Australia
5. Cairns Base Hospital, Queensland, Australia

There has been a notable increase in the incidence of Buruli ulcer in Australia during 2011-2012 affecting both the southeastern and northeastern endemic areas.

Southeastern Australian endemic region

The mean number of notifications of Buruli ulcer per year in the Australian State of Victoria from 2004-10 was 34 (95% ci 25-44). There was a significant increase during 2011 (80 new cases) that was sustained during 2012 (75 new cases). The great majority were residents or visitors of the Bellarine and Mornington peninsulas near Melbourne (figure 1). The reason for the increase is not known but may be linked to variations in rainfall. Victoria had below average rain from November 2006 until September 2010. In January 2011 the rainfall total was the highest yet recorded for January and there was widespread flooding in the north and east of the state.1 Rainfall remained above or near the long term averages throughout 2011 and for most of 2012. However, from September 2012, there has been a shift back towards dry conditions. If transmission of Mycobacterium ulcerans is causally linked to rainfall and Victoria remains dry during 2013, we would expect to observe a reduction in cases from March 2013 (allowing for an incubation period of 4.5 months2, and delay of 1-2 months before definitive diagnosis and notification).

2 See companion abstract “Incubation period of Buruli ulcer” Trubiano JA et al.
Figure 1.

Sketch map showing the Bellarine and Mornington Peninsulas which flank Port Phillip Bay to the southwest and southeast of Melbourne. At least 140 cases of *M. ulcerans* infection notified during 2011-2012 were in residents or visitors to these coastal regions. Towns affected include Point Lonsdale, Queenscliff, Ocean Grove and Barwon Heads on the Bellarine Peninsula; Frankston, Blairgowrie and Sorrento on the Mornington Peninsula. A small number of cases continue to occur in the original Bairnsdale region 260 km to the east of Melbourne (not shown) and at Cowes on Phillip Island, but almost all cases now are linked to the emerging foci on the Bellarine and Mornington peninsulas. Buruli ulcer incidence in Point Lonsdale in 2011 was estimated at 770/100,000 of the permanent population.

Northeastern Australia endemic region

During 2011 there was an unprecedented outbreak of Buruli ulcer in the Australian State of Queensland in a small region between Mossman and just north of the Daintree River (see figure). The total permanent population of this region (formerly the Douglas Shire, now administratively part of the Cairns region) was ≈14,100 in the 2006 Census. The region has been recognized as an endemic area for Buruli ulcer since the 1950s but is usually only linked to 1-5 cases per year. Interestingly and unlike in Victoria, this endemic area has remained geographically fixed for over 50 years. During 2011 60 cases were with most diagnoses being made in the cooler months July to October. More than 40% of cases were from Wonga Beach but other locations in the region were also affected. The reason for this sudden increase is not known. Local clinicians noted that it had rained throughout the 2010 dry season and that the 2010-11 wet season was the biggest for many years. An increase in biting insect activity was recalled following the wet season particularly from March flies (*Tabanidae*). In support of these anecdotal observations the February 2011 rainfall total in Mossman (1194 mm) was more than 60% higher than in February 2010 (755 mm) and 2.5 times higher than February 2012 (478 mm). Cases appeared 6 weeks to several months after the “big wet”. Disease activity in 2012 was reduced but remained higher than the usual background rate with at least 17 of 25 Queensland cases linked to
the Northeastern endemic region. During January 2013 there has again been very heavy rainfall in the Cairns Region in the aftermath of tropical cyclone Oswald. If transmission of *Mycobacterium ulcerans* is causally linked to rainfall we would expect to observe an increase in cases diagnosed in the Northeastern endemic region during the 2013 dry season.

Table 1. Confirmed notified human cases by Australian State 2004-12.

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Mycobacterium ulcerans in far north Queensland – The “Daintree” ulcer

Presenter: Christina Steffen

There are two main foci of M. ulcerans infections in Australia.

The Victorian focus, near Melbourne, is well known.

The other focus is in tropical north Queensland, about 100 km north of the regional centre, Cairns.

Between 1964 and 2008, 110 confirmed cases of “Daintree Ulcer” as it is called locally, were described. Most occurred in the vicinity of the village of Daintree on the Daintree River, giving rise to a concept that it was occurring only in the Daintree catchment.

In 2011 there was a six-fold increase in the number of cases, with 61 confirmed cases of which 25 were resident in a new main focus at Wonga and Wonga Beach, some distance from Daintree Village and the Daintree River. Many lived in the same streets.

The majority of cases were ulcerative. There were two oedematous presentations, both in small children, and two plaque presentations. Some patients reported their infection had started at the site of a March fly bite.

Of the 61 cases, 44 were managed by surgeons, in conjunction with infectious diseases specialists.

Local general practitioners managed the remainder. Exact numbers of cases has been difficult to ascertain due to issues with reporting of this disease.

As many lesions were small, excision alone or with a shorter course of antibiotics was the mode of treatment. Most patients preferred excision to an eight week course of antibiotics. Two patients required extensive debridement of necrotic skin, with skin grafting after full courses of antibiotics. Two patients with plaque-type presentations had lesser excisions of necrotic skin and skin grafting. One patient resolved spontaneously. Early presentation, antibiotic use, host factors and a less virulent strain may be reasons for the relative ease of management.

There were two recurrences – a neck lesion which recurred two months post-excision was re-excised and covered with a second course of antibiotics. The other recurrence was following inadequate excision by a local general practitioner unfamiliar with the infection.

The spike in cases in 2011 was not repeated in 2012, with the total number dropping back to ~12.

The years 2010 and 2011 were extremely wet years with continuous rain throughout eighteen months, giving rise to luxuriant foliage and increased numbers of biting insects. In one 2012 case a council worker who was taking a nap at Wonga Beach with a newspaper over his face woke to find a March fly biting his cheek and subsequently developed a positive lesion at the site.

The endemic area is clearly not confined to the Daintree catchment but is rather related to the cleared foothills and lowlands surrounding a large rainforested range, its runoff waters and inhabitants. Given the recent compelling evidence from Victoria of a possible mammalian reservoir in ring-tail possums, it is tempting to postulate a similar reservoir in the FNQ area, not necessarily possums but some other territorial marsupial such as the local “bandicoot”.

18
Buruli Ulcer in Japan: the current situation

Presenter: Rie Roselyne Yotsu

Rie Roselyne Yotsu¹*, Kazue Nakanaga²*, Yoshihiko Hoshino²*, Koichi Suzuki²*, Norihisa Ishii²*

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Buruli ulcer is commonly known as a disease confined to areas with tropical climate. However, interestingly, there are also, a few, but cases of Buruli ulcers in Japan. The first case was reported by Mikoshiba et al. in 1982 which was a case of a 19 year-old female who presented a chronic and necrotic ulcer on her left elbow. The case was considered to be an endemic infection due to lack of travel history outside the country. Later, Tsukamura et al. reported the mycobacterium obtained from this ulcer showed a close resemblance to M. ulcerans, but with some differences, in which with further research, he advocated this novel subspecies as “M. ulcerans subsp. shinshuense”. As of end of the year 2012, similar cases have accumulated to 36 in total diagnosed in different parts of Japan. In our presentation, we will introduce the newly diagnosed cases, 10 cases in 2011 and 4 cases in 2012. We will demonstrate the epidemiology and characteristics of these cases together with implications for better treatment of the disease from our experiences.
The situation of Buruli ulcer control in Cameroon in 2012

Presenter: Earnest Njih Tabah

Introduction

Cameroon is one of the endemic countries for Buruli ulcer (BU) in Africa. A total of 3433 new cases have been notified since 2001 primarily from five confirmed endemic foci, with an annual average of 286 cases. A number of new suspected foci are propping up year after year, and require confirmation through epidemiological surveys. In 2012, 160 new cases reported in the four major BU diagnostic and treatment centers came from 122 communities spread out in 27 health districts.

Main missions of the national control program

In order to reduce the morbidity and disability related to Buruli ulcer, the national control program set to following objectives:

- Ensure case detection and treatment in the major endemic foci
- Intensify advocacy for additional funding for program activities
- Develop and validate national guidelines for Buruli ulcer control
- Collaborate with research centers in the domain of diagnosis and treatment; community awareness, attitudes and practices;

Major activities included

- Community sensitization, case detection and treatment
- Capacity building of field actors
- Supportive supervision of treatment centers
- Active participation in the development and validation of the national NTD master plan
- Development of the national guidelines for BU control with the involvement of all major stakeholders

The activities were carried out with the collaboration and participation of the following partners: The FAIRMED Foundation, MSF-CH, WHO, CPC, and the Swiss TPH

Results:

Case detection, treatment and surveillance:

160 new cases were diagnosed and treated in the various BU-DTC. This number is far less that in the last three years. This could be explained by reduced active case finding activities especially in the Ayos, Ngoantet and Mbonge foci, following drastic reduction in support by FAIRMED as well as rapid turnover of trained personnel in these areas. The proportion of category 3 cases has increased from 9% in 2008 to 45% in 2012, meanwhile the proportion of cases confirmed by PCR has dropped from 56% in 2011 to 41% in 2012 (although some results are still pending).
Health system strengthening, development of norms and capacity building:

A national guideline for the management of Buruli ulcer in Cameroon and a supervision checklist for prevention of disability activities were developed in the course of 2012. This guideline shall be reviewed in order to integrate the new Buruli ulcer antibiotic treatment recommendations by WHO in the first trimester of 2013.

A brand new pavilion was constructed at the Bankim treatment centre by FAIRMED to host Buruli ulcer as well as the surgical wards.

An integrated training on diagnosis and treatment of Buruli ulcer, leprosy and yaws was carried out for 17 medical doctors and 72 nurses coming from 32 health districts in the country. A symposium on Buruli ulcer was also organized at the Faculty of Medicine in Yaoundé, which saw the participation of 135 medical students.

Treatment centers were supplied with dressing material and specific antibiotics as well as the new IEC material (booklets, posters, flyers, DVDs) acquired from the WHO during supervision visits.

Strategic planning and advocacy for additional resources:

The Buruli ulcer national control program actively participated in the development and validation of the national NTD master plan for 2012-2017, with Buruli ulcer featuring prominently. Advocacy was intensified at the level of the hierarchy of the ministry of public health, with a resulting allocation of funding for Buruli ulcer activities in the 2013 budget. Our performance shall certainly improve given this funding.

Challenges

Specific antibiotics:

Although we still have some little quantity of rifampicin in stock, we are totally out of stock of streptomycin. We welcome the new recommendation for the use of clarithromycine in place of streptomycin. We plead for an urgent supply of the drug consignment of Cameroon for 2013.

Funds for program activities:

The funds allocated by the two international partners supporting Buruli ulcer control in Cameroon have witnessed continuous attrition within last few years. This has negatively affected the performance of the control program. There is urgent need for new funding sources if the performance must be improved upon.
Report on Buruli ulcer control situation, Ghana 2011-12

Presenter: Edwin Ampadu

Introduction
National programme will continue to provide effective coordination and technical support to treatment centres in spite of the operational challenges.

Programme Objective
To minimise morbidity and disability associated with Buruli ulcer and also to strengthen awareness through advocacy

Strategy
- Early case detection and surveillance
- Capacity development in case management
- Collaborate with other stakeholders in control programme
- Prevention of disability
- Strengthen national office in Monitoring and evaluation
- Research collaboration
- Advocacy

Major activities carried out
- Extensive Preparation carried out to commence the WHO sponsored drug trial. This involved clearing and transporting all the medicines and other logistics for the trial, involved in the training of personnel in data management and documentations. This is a 2 year trial with another year of monitoring.
- A meeting was held to discuss setting up of a steering committee as a forum for Buruli ulcer collaborators and stakeholders to share and discuss issues on the disease control in the country. The committee when put in place will serve as the arena where major issues on the control programme will be shared and further inputs made to as a way of improving communications in Research interventions, advocacy and policy implementation actions. It will also serve as a pressure group to higher authorities for action to Buruli ulcer control in the country. Funding support is expected to come from partners and the Government of Ghana
- The ministry of health procured sizable quantities of surgical dressings, antibiotics to support the national programme. The amount was USD175, 000 in 2012. Furthermore, we continued to receive large quantities of educational materials from WHO to support education awareness and advocacy in the country. A distribution plan put in place and effected in the first year, 2012. The materials will last few years.
- Surgical interventions continue to take place in four treatment centres. This has been possible with the support of the Reconstructive and plastic surgery department from the two teaching hospitals in the country. The facilities included Amasaman hospital, Dunkwa hospital, Agogo hospital and Goaso hospital
The denominators are the total cases managed in the facility in the year.

- The National programme carried out some monitoring support visits. The centres included issues identified were the differences in facilities approach to use of dressing materials and wound care in general.
- No guidelines for wound care.
- Low morale to the health staff in such rural areas working on conditions such as Buruli ulcer. Staff attrition was high and capacity building was irregular.
- Limitation of resources and how to overcome them in utilization bearing in mind professional commitment. Prevention of disability associated with the disease and how to integrate the affected ones into the society [schooling, gainful employment] took centre stage. 2 physiotherapists have been posted to two centres [Amasaman in Ga West and Dunkwa in Upper Denkyira districts].
- The proposed drug trial sites were also visited and assessed for the commencement of the trial. All logistics have been provided ready to use.

Laboratory results

Noguchi and KCCR continue to support case confirmation in the country.

<table>
<thead>
<tr>
<th>Laboratory Confirmed cases 2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facility</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>KCCR</td>
</tr>
<tr>
<td>Noguchi</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
Surveillance and case management

Between 2011 and 2012, there was a drop in the overall cases detected by almost 40%.

Disability prevention continues to pose challenges as very few staff accept postings to the endemic areas.

Two physiotherapist have been posted to Upper Denkyira and Ga West districts.

The above picture demonstrates gradual decline, however, there is the need to strengthen and support community case detection to reduce high percentage of Cat 111 and increase Cat 1 and 11.

Collaborations [research and others]

- **Stop Buruli project** over the past three years has and continues to support the programme with capacity development in the confirmation of Diagnosis
- **Inter Flemish University Council – Noguchi** - in capacity development towards transmission clues for the past 4 years
- **European Foundation initiative for NTD**- Case detection and treatment in Ashanti Akim North
- **Hershy FHI 360** – Supporting BU control in Cocoa growing areas of Ashanti
- **Rotary club Sunyani**- Strengthening case control in Asunafo Districts

**The way forward, 2013**

- Support drug trial implementation in the country
- Expand stakeholders’ involvement in the disease control activities
- Put wound care and prevention of disability on top agenda of the programme
- Attract stakeholders to invest in Buruli ulcer control
Development of Buruli ulcer treatment in Togo (2008–2012)

Presenter: Basile Kobara

Basile Kobara¹, Denis Gadah²

¹Coordinator, Togolese National Buruli ulcer, leprosy and yaws control programme
²Project director, Handicap International

Introduction
The first cases of Buruli ulcer in Togo were described in 1996 by F. PORTAELS, M. MEYERS et al. In 1999 Togo established its National Buruli Ulcer Control Programme (PNLUB) following the international conference on Buruli ulcer held at Yamoussoukro in 1998. Owing to various constraints, the Programme was unable to pursue its full range of activities and thus could not obtain the expected results. It was only in October 2006 that it was able to resume its work in earnest with the appointment of a new coordinator; in 2007, it relaunched its activities with renewed support and commitment from its partners (WHO, DAHW and Handicap International). The Ministry of Health, with support from Handicap International and other partners including DAHW and WHO, developed and adopted a policy document and a national strategic plan for Buruli ulcer control for the period 2008-2012. The strategic plan was piloted in the Maritime region, the most highly endemic, and was subsequently extended to the Central region. As a result, Togo now has reliable data on Buruli ulcer. The Buruli ulcer control activities that have been implemented in the context of the partnership between Sanofi, Anesvad, Handicap International and other donors and the Togolese Government over the past four years have yielded satisfactory results; these are illustrated graphically and commented on in the summary below. However, work still needs to be done in the area of early diagnosis and use of antibiotics at the earliest signs of the disease, through increasing access to early detection and treatment at the peripheral level of the health system.
I. Map of intervention area, HI and DAHW project
II. Principal Buruli ulcer control interventions with financial and technical support from Handicap International, DAHW and WHO

Table 1: Strengthening the capacity of community intermediaries to pursue awareness-raising and early detection activities

<table>
<thead>
<tr>
<th></th>
<th>NUMBER OF COMMUNITY INTERMEDIARIES TRAINED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2008</td>
</tr>
<tr>
<td>Nurses</td>
<td>08</td>
</tr>
<tr>
<td>Community health workers</td>
<td>30</td>
</tr>
<tr>
<td>Teachers</td>
<td>-</td>
</tr>
<tr>
<td>Traditional healers</td>
<td>14</td>
</tr>
<tr>
<td>Women’s groups</td>
<td>06</td>
</tr>
<tr>
<td>NGOs/local associations</td>
<td>03</td>
</tr>
<tr>
<td>TOTAL</td>
<td>61</td>
</tr>
</tbody>
</table>

To ensure greater impact of awareness-raising activities and better community involvement, the National Buruli Ulcer, Leprosy and Yaws Control Programme (PNLUB-LP), supported by its partners, has proceeded to strengthen the capacity of the various stakeholders in the community health sphere. A total of 90 and 270 community intermediaries were trained in 2011 and 2012, respectively. They have carried out early detection activities through door-to-door and home visits, targeted awareness-raising and school visits.
### Table 2: Data on awareness-raising activities

<table>
<thead>
<tr>
<th>Mass awareness-raising (MAR) and home visits (HV)</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAR</td>
<td>660</td>
<td>482</td>
<td>496</td>
<td>376</td>
<td>1 272</td>
</tr>
<tr>
<td>HV</td>
<td>688</td>
<td>581</td>
<td>474</td>
<td>418</td>
<td>1 486</td>
</tr>
<tr>
<td>Number of awareness-raising sessions per community health worker</td>
<td>55 910</td>
<td>36 937</td>
<td>31 653</td>
<td>39 395</td>
<td>97 338</td>
</tr>
<tr>
<td>Number of persons reached by awareness-raising</td>
<td>85 264</td>
<td>41 884</td>
<td>63 975</td>
<td>139 988</td>
<td></td>
</tr>
<tr>
<td>Number of persons concerned</td>
<td>55 910</td>
<td>29 354</td>
<td>18 377</td>
<td>10 231</td>
<td>42 650</td>
</tr>
<tr>
<td>Number of persons concerned</td>
<td>55 910</td>
<td>29 354</td>
<td>18 377</td>
<td>10 231</td>
<td>42 650</td>
</tr>
</tbody>
</table>

### Table 3: Referral of suspected cases of Buruli ulcer through the community channel

<table>
<thead>
<tr>
<th>Public</th>
<th>Number of films projected and MAR events</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communities/Schools</td>
<td>- - 1 2700 18 6 467 41 23 900 47 37 873</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Traditional healers</td>
<td>- - - - - - 2 1 100 01 500</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Referral of suspected cases of Buruli ulcer through the community channel

<table>
<thead>
<tr>
<th></th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of referrals</td>
<td>86</td>
<td>20</td>
<td>31</td>
<td>140</td>
<td>433</td>
</tr>
<tr>
<td>Number of refusals</td>
<td>82</td>
<td>12</td>
<td>4</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>Referral rate (%)</td>
<td>51.19</td>
<td>62.5</td>
<td>88.57</td>
<td>98.59</td>
<td>95.16</td>
</tr>
<tr>
<td>Refusal rate (%)</td>
<td>48.81</td>
<td>37.5</td>
<td>11.43</td>
<td>1.41</td>
<td>4.84</td>
</tr>
</tbody>
</table>
When the project was launched in 2008, patients were very reluctant to visit health centres for consultations. Almost half of all suspect cases refused to seek medical assistance owing to their magical way of thinking about the disease. But awareness-raising efforts have now borne fruit. The rate of refusal to seek medical assistance was virtually zero in 2011 (1.41%) and 2012 (3.33%). As activities were scaled up throughout the Maritime region, it became possible to visit the remotest areas where beliefs and customs constitute a psychological impediment to health-care access in general. And given that behaviours cannot be changed overnight, more time will be needed to further raise the awareness of populations that remain wedded to their traditions.
Table 4: Number of cases of Buruli ulcer detected and treated in the period 2007-2012

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of cases treated</th>
<th>Number of cases confirmed by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>24</td>
<td>10</td>
</tr>
<tr>
<td>2008</td>
<td>83</td>
<td>38</td>
</tr>
<tr>
<td>2009</td>
<td>56</td>
<td>33</td>
</tr>
<tr>
<td>2010</td>
<td>52</td>
<td>28</td>
</tr>
<tr>
<td>2011</td>
<td>52</td>
<td>41</td>
</tr>
<tr>
<td>2012</td>
<td>51</td>
<td>43</td>
</tr>
</tbody>
</table>

Figure 2: Number of cases treated annually and case confirmation by PCR

About 50 cases of Buruli ulcer are treated every year in Togo. However, the adoption of a highly effective system of diagnosis delivers a high rate of confirmation by PCR of treated cases. Togo considerably exceeds the WHO indicator for case confirmation; this rate was 78.8% in 2011 and 84.3% in 2012.
Table 5: Distribution of case management between referral hospitals and peripheral health facilities

<table>
<thead>
<tr>
<th>Year</th>
<th>Referral Hospitals</th>
<th>Peripheral Health Facilities</th>
<th>Total Number of Cases Treated</th>
<th>Proportion of Cases Treated at Referral Hospitals</th>
<th>Proportion of Cases Treated at Peripheral Health Facilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>24</td>
<td>0</td>
<td>24</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>2008</td>
<td>60</td>
<td>23</td>
<td>83</td>
<td>72.28%</td>
<td>27.7%</td>
</tr>
<tr>
<td>2009</td>
<td>23</td>
<td>33</td>
<td>56</td>
<td>41%</td>
<td>58.9%</td>
</tr>
<tr>
<td>2010</td>
<td>24</td>
<td>28</td>
<td>52</td>
<td>46%</td>
<td>53.8%</td>
</tr>
<tr>
<td>2011</td>
<td>17</td>
<td>35</td>
<td>52</td>
<td>32.6%</td>
<td>67.30%</td>
</tr>
<tr>
<td>2012</td>
<td>8</td>
<td>43</td>
<td>51</td>
<td>15.68%</td>
<td>84.32%</td>
</tr>
</tbody>
</table>

Figure 3: Efficacy of early detection of cases of Buruli ulcer
Thanks to the results of the awareness-raising activities, the project receives increasing numbers of category-I and category-II cases that do not require hospital treatment. These are principally nodules and lesions of less than 5 cm requiring pharmaceutical management (combination of 2 antibiotics, rifampicin and streptomycin). Until the end of 2012, almost 84.32% of patients were treated as outpatients at peripheral facilities. Disabling sequelae, stigma and school abandonment, which used to be common features of this disease, have been reduced through successful early case detection. The exorbitant costs associated with the inpatient management of complex cases of Buruli ulcer have been significantly reduced. Of the 51 cases treated in 2012, only 8 were inpatients (treated at the Tsévié referral hospital); the remaining 43 were treated at peripheral health facilities. Of the 8 inpatients, 4 were children and 4 were adults. The rate of school abandonment by children with Buruli ulcer has thus been significantly reduced.

III. Challenges encountered and the outlook for the future

The issue of differential diagnosis remains a worrying problem in the implementation of treatment activities. WHO advocates that all persons with Buruli ulcer should be treated completely free of charge. Mobilization campaigns for early case detection are careful to mention this fact in their messages to communities. But for local people, there is no difference between a wound due to Buruli ulcer and any other kind of chronic wound. In the local dialect, there is no way of distinguishing between Buruli ulcer and a chronic wound, so it would be unethical not to treat the non-ulcerative form of a Buruli ulcer wound. Following the awareness-raising campaign, patients visit the community health worker with wounds that could have any of a number of different causes (diabetic, cancerogenous, sickle cell, secondary infection following injury, etc.), and the community health worker is unable to make a diagnosis. The test for Buruli ulcer is therefore performed and the patient is treated free of charge if the test is positive.

To ensure an ethical and more efficient approach, it was decided that the National Programme, with technical and financial support from Handicap International provided through Sanofi Espoir and Anesvad, should implement a comprehensive care strategy for all chronic wounds at community level in Maritime region. The implementation of this pilot strategy has yielded reliable data on every type of non-ulcerative wound that could be encountered. It remains to be seen to what extent this strategy can be scaled up to the whole of the Maritime region on a sustainable basis.

Conclusion

It should be stressed that the largely satisfactory results of the initiatives in the areas of awareness-raising, early case detection, and outpatient, surgical and orthopaedic treatment have been achieved thanks to the effective mobilization of development partners such as Handicap International and DAHW, and through the financial and technical support of Sanofi Espoir, Anesvad and WHO. Medicines are provided by WHO; DAHW is responsible for financing supervision, logistics, health worker training, patient screening and follow-up, and surgical treatment; HI is responsible for funding and extensive involvement in awareness-raising and early case detection, training of community intermediaries and the treatment and prevention of disabilities following surgical intervention. The Togolese Government has contributed by establishing the control programme, providing equipment for facilities and strengthening the health workforce.
Buruli Ulcer (BU) Pilot Project in Nigeria: A Progress Report

Presenter: Anthony Meka

Authors: Meka AO\(^1\), Alphonsus C\(^2\), Chukwu JN\(^1\), Nwafor CC\(^1\), Oshi DC\(^1\), Madichie NO\(^1\), Ekeke N\(^1\), Anyim MC\(^1\), Ikpoti O\(^3\), Obasanya JO\(^4\).

1. German Leprosy and TB Relief Association, Enugu.
2. St Benedict Hospital Ogoja, Cross River State.

Introduction

Nigeria is a BU endemic country as established by the WHO assessment mission in November 2006. Nothing significant in terms of decreasing the burden was done since then. The BU Pilot Project is an initiative of German Leprosy & TB Relief Association (GLRA) Nigeria. It is aimed at providing evidence to inform policies in BU Control in Nigeria. It is a one year Project implemented at St. Benedict Hospital, Ogoja, Cross River State, Nigeria.

Methodology

A total of 31 communities located within three BU endemic Local Government Areas (LGAs) around Ogoja area were purposively selected for advocacy and outreach activities. Trained health workers carried out awareness campaigns to inform and educate community members on Buruli Ulcer including the symptoms and opportunities for free diagnosis and treatment. BU suspects were identified and laboratory specimens collected for examination. Confirmed cases were managed according to WHO guidelines at St Benedict Hospital, Ogoja. Relevant data were collected and analyzed, aimed at informing programmes and policies at the national level.

Result

So far, a total of 31 BU suspects were identified, out of which 26 were confirmed using the polymerase chain reaction (PCR) method. Of the 26 confirmed new cases, 6(23%), 7(27%) and 13(50%) were Categories I, II and III lesions respectively. There was a preponderance of females: 17cases (65%) while the rest were males (35%). Among all cases, 10 (38.5%) were children aged 0-14 years. See Table below. Twenty five (25) of the confirmed cases were put on treatment while one case declined treatment. Of the number put on treatment, 15 have so far been discharged with hospital stay ranging from 56 to 244 (median of 135) days. A total of 20 cases required and got surgery in addition to antibiotic management. A case was lost to follow-up. Of the discharged cases, 6 had their wounds healed without limitation of joint movement.

Conclusion

While validating/reaffirming Nigeria’s status as BU-endemic country, this project clearly demonstrates the effectiveness and feasibility of a programmatic approach to the control of this neglected disease.

The final report of the subject will be shared with the national programme and the WHO with a view to charting/resourcing a plan for nation-wide scale up based on the evidence generated in Cross River.
Table showing Age and Sex Distribution of New BU cases in various Categories in BU Pilot Project in Cross River State, Nigeria 2012-2013.

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>CAT I</th>
<th>CAT II</th>
<th>CAT III</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children (0-14Years)</td>
<td>Male</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Adults (Above 14years)</td>
<td>Male</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

Figure 1. Gender distribution of BU patients in various Categories in BU Pilot Project in Cross River State, Nigeria 2012-2013.

Figure 2. Age Distribution of BU Patients in BU Pilot Project in Cross River State, Nigeria 2012-2013.
Buruli ulcer in Nigeria: Description of a series of cases at four Buruli ulcer detection and treatment centres in Benin, 2006–2012

Presenter: Gilbert Ayelo

Gilbert AYELO, Ghislain SOPOH, Ange DOSSOU, Jean Gabin HOUEZO, Esaï ANAGONOU, Yves BAROGUI, Annick CHAUTY, Julia AGUIAR, Roch Christian JOHNSON, Didier AGOSSADOU, Kingsley ASIEDU

Introduction
In Benin, Buruli ulcer (BU) is treated at specialized centres that also receive cases from other countries, specifically from Nigeria, where active surveillance is not yet effective. The purpose of this study is to describe the epidemiological, clinical and biological aspects of the cases originating in Nigeria and treated in Benin in three Buruli ulcer detection and treatment centres (CDTUB) between 2006 and 2012.

Method
This is a retrospective, descriptive and analytical study which has included all BU cases originating in Nigeria that have subsequently been treated in the CDTUB at Allada, Lalo et Zagnanado in the past seven years (2006-2012).

Findings
In total, 134 patients from Nigeria have been treated at the four CDTUB between 2006-2012, or 19 cases a year on average. Most were received between 2008 and 2012 (100/134), i.e. the years following the evaluation of BU in Nigeria (Chukwuekezie et al, 2007). Most of the patients came from Ogun state (83/134) or Lagos (38/134) and were mostly referred by former patients who had previously been treated in Benin. 50% were female. Ulcers were the predominant clinical forms (44%) and mixed forms (38.8%), mostly in category III (73.9%).

Conclusion
This study shows that the majority of cases of Buruli ulcer originating in Nigeria that are treated in Benin are category-III ulcers. This is contrary to the trend observed in recent years in Benin and action needs to be taken to ensure more active surveillance in Nigeria, especially in areas along the border with Benin.
Disease presentation and outcome of Buruli ulcer: descriptive and analytical results from the largest cohort of laboratory-confirmed cases worldwide

Presenter: Alexandre Alcaïs

Vincent Q¹, Ardant MF²,³, Adeye A²,³, Agossadou A⁴, Johnson C⁵, Goundote A²,³, Marsollier M⁶, Chauty A²,³, Alcaïs A¹,⁷ for the PAP (Pobè-Angers-Paris) Buruli ulcer Consortium

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Buruli ulcer (BU), caused by Mycobacterium ulcerans, is the third most common mycobacterial disease worldwide, after tuberculosis and leprosy. It has been identified as a neglected emerging infectious disease by the WHO. An estimated 25% of those infected — particularly children — become permanently disabled and endure permanent developmental, economic and social problems. However, our understanding of the disease, based on analytical epidemiology, remains incomplete. We report here the results of an analysis of the largest cohort of laboratory-confirmed cases worldwide. Prospective clinical and laboratory data were collected for 1,357 laboratory-confirmed cases diagnosed over the last 10 years at the Centre de Détention et de Traitement de l'Ulcère de Buruli in Pobe, Benin. We assessed and modeled the impact of age and sex on 11 aspects of BU clinical presentation. We also investigated critical predictors of BU severity and the risk of sequelae.

Typical BU lesions were an ulcer (67%) on the lower limb (60%) measuring more than 15 cm in diameter (38%) and occurring at a median age of 13 years. Osteomyelitis occurred in 6% (n=83) of patients, about 20% of whom presented with no history of BU skin lesions. Age at diagnosis was significantly lower in male than in female patients (p < 0.0001). The proportion of individuals with plaques, edema or osteomyelitis decreased significantly with age, whereas the proportion of patients with ulcers increased significantly with age. Osteomyelitis was more frequent in male patients than in female patients. Finally, the occurrence of permanent sequelae was strongly associated with the severity of initial presentation (odds ratio of 8.50, 95% CI [5.69-13.08], p <0.0001).

BU may be more frequent than TB and leprosy and should be considered a potential etiology of chronic ulcers in children living in endemic areas. BU patients presenting with osteomyelitis, edema, multifocal or large lesions should receive special attention, such as enhanced surveillance, intensive physiotherapy and timely reconstruction surgery, to prevent disabilities. Our results also highlight the critical need for microbiological characterization of the causal agent in cases of isolated osteomyelitis in young boys living in zones in which BU is endemic.
Antibiotics
Mycobacterium ulcerans disease: Experience with exclusive medical treatment in an Australian cohort

Presenter: Deborah Friedman

Friedman ND¹, Hughes AJ¹, Athan E¹, Khajehnoori M², McDonald A³, Callan P³, Rahdon R³, O’Brien DP¹,⁴

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Background

Mycobacterium ulcerans (MU) is responsible for disfiguring skin lesions and is endemic on the Bellarine peninsula of south-eastern Australia. Antibiotics have been shown to be highly effective in sterilizing lesions and preventing disease recurrences when used alone or in combination with surgery. Our practice has evolved to using almost exclusive medical therapy.

Methods

We describe a consecutive cohort of our MU patients planned for exclusive antibiotic therapy. Exclusive medical therapy was defined as treatment of a M. ulcerans lesion with either antimicrobials alone or antimicrobials in conjunction with limited surgical debridement.

Results

From 1/10/2010 through 31/12/11, there were 54 patients with MU infection managed at Barwon Health. Forty-two patients were treated with exclusive medical therapy, of which 5 patients (12%) also underwent limited surgical debridement. The median age of patients was 50.2 years, and the median duration of therapy was 56 days. Seven of 42 patients (17%) received less than 56 days of therapy, and 41 patients were healed within 12 months. Medication side effects requiring cessation of one or more agent occurred in 7 patients (17%). One relapse occurred after 8 weeks of antimicrobial therapy.

Conclusion

Our experience demonstrates the efficacy of exclusive medical management of MU infection with healing of lesions within 12 months of therapy initiation in 98% of cases, an acceptable toxicity profile and good cosmetic results. Further research to determine both the optimal and minimum durations of medical therapy is required.
Risk factors for Paradoxical reactions related to antibiotic treatment of Mycobacterium ulcerans.

Presenter: Daniel O'Brien

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Background

Risk factors for paradoxical reactions related to the treatment of Mycobacterium ulcerans infection with antibiotics have not been determined. We describe factors associated with their incidence in a patient cohort from south-eastern Australia.

Methods

Paradoxical reactions were defined on clinical and histological criteria. Data was collected prospectively on all confirmed cases of M. ulcerans infection managed at Barwon Health Services, Australia, from 1/1/1998-31/12/2011. A poisson regression model was used to assess incidence rates and associations of variables with paradoxical reactions.

Results

32/156 patients (21%) developed paradoxical reactions at a rate of 25.0 (95% CI 17.7-35.4) per 100 person-years. On univariate analysis, surgical excision (RR 1.73, 95% CI 0.71-4.20, p=0.20) and the commonly used orally administered antibiotics (rifampicin RR 0.93, 95% CI 0.22-3.88, p=0.92; ciprofloxacin RR 0.77, 95% CI 0.37-1.53, p=0.44; clarithromycin RR 0.71, 95% CI 0.32-1.59, p=0.40) were not associated with the development of paradoxical reactions. On multivariable analysis adjusting for age, sex, lesion type, major excision, diabetes and the use of amikacin in the initial antibiotic regimen, age ≥ 60 years (RR 2.84, 95% CI 1.12-7.17, p=0.03), the presence of an oedematous lesion (RR 3.44, 95% CI 1.11-10.70, p=0.03) and the use of amikacin in the initial antibiotic regimen (RR 6.33, 95% CI 2.09-19.18, p<0.01) were associated with the development of paradoxical reactions.

Conclusions

Paradoxical reactions are common in the antibiotic treatment of M. ulcerans, and may be increased in older adults, oedematous forms of disease, and in those treated with amikacin.
Use of rifapentine and clarithromycin in an all-oral regimen for Buruli Ulcer

Presenter: Deepak Almeida

Deepak V. Almeida\textsuperscript{1,2}, Paul J. Converse\textsuperscript{1}, Si-Yang Lee\textsuperscript{1}, Sandeep Tyagi\textsuperscript{1}, and Jacques H. Grosset\textsuperscript{1,2}.

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Background

The standard WHO recommended treatment for Buruli ulcer is rifampicin (RIF, R) plus streptomycin (STR, S) injections given daily (7 days a week) for 8 weeks. Replacement of streptomycin with an effective oral drug would be ideal, since an all-oral regimen would be easier to implement. Currently clarithromycin (CLR, C) is the only suitable alternative to STR, however it is bacteriostatic against \textit{Mycobacterium ulcerans} and therefore not as potent as STR. In our previous studies we evaluated the oral combinations of RIF-CLR and rifapentine (RPT, P) - CLR with the reasoning that RPT, a rifamycin derivative with a longer half may be as good as RIF-STR. However, we could not evaluate results due to a PK interaction, hence in this experiment we repeated the study while taking care to rule out any PK interaction.

Material and methods

235 BALB/c mice were infected in the right hind footpad with \textit{M. ulcerans} 1615 (Malaysian strain). The day after infection and on initiation of treatment, 5 mice were sacrificed to determine the baseline CFU counts. Treatment was started after 31 days with mice randomized to one of 4 treatment groups which were untreated controls, RS, RC, and PC. R and P were given at a dose of 10 mg/kg by oral gavage, C was given at a dose of 100 mg/kg and S was injected subcutaneously at 150 mg/kg. C was administered 1 hr later than R and P to overcome PK interactions encountered in the previous study. Mice were treated 5 times a week, for each treatment group, the duration of drug administration was 2, 4, 6 or 8 weeks. On treatment completion 5 mice were sacrificed for CFU, all mice treated with P were kept for an additional week before sacrifice to wash out the rifapentine and 10 were kept without treatment for at least 6 months to detect swelling of footpads. Response to treatment was determined by reduction in CFU counts during treatment and potential sterilization was determined by the median time to footpad swelling. Footpads of mice were evaluated weekly for swelling and were considered positive when the average lesion index (ALI) was $\geq 2$, i.e., exhibiting definite swelling and inflammation of the foot.

Results

CFU counts: Mean $\log_{10}$ CFU count/footpad were $4.11\pm0.11 \log_{10}$ the day after infection and increased to $5.55\pm0.47$ at the time of treatment initiation. At the end of 2 weeks treatment, the $\log_{10}$ CFU counts were as follows: untreated controls, $5.91\pm0.34$; RS, $3.10\pm0.92$; RC, $4.09\pm0.45$; and PC, $1.68\pm1.08$. At 4 weeks the $\log_{10}$ CFU counts were, RS, $0.68\pm0.93$; RC, $1.95\pm0.63$; while in the PC group no CFU could be detected. At the end of 6 weeks only those treated with RC had detectable CFU of $0.75\pm0.69$ and at end of 8 weeks, all treated mice were culture negative.
Median time to swelling: The median time to swelling (MTS) for control mice was 5 wks after infection. In RC mice treated for 2 or 4 wks the MTS was 10 wks, but if treated for 6 or 8 wks, the MTS was 34 and 32 wks. For PC mice, only those treated for 2 wks reached 50% swelling – at 18 wks. RS mice never reached 50% swelling regardless of treatment duration.

Conclusion
As seen in previous studies, RS was the most potent regimen; RC was inferior to the current standard as evidenced by the CFU count and time to swelling data. However PC was found to be as good as RS when the mice were treated for 4 weeks or more, indicating the possibility of using rifapentine in all-oral regimens.
Optimizing the chemotherapeutic approach for the treatment of Buruli Ulcer: possible options and research needs

Poster: Martina Casenghi

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*These authors contributed equally to this work

Buruli ulcer disease is a serious necrotising skin infection caused by the environmental pathogen Mycobacterium ulcerans, and represents the third most common mycobacterial infection after tuberculosis and leprosy. This disease, if untreated, can lead to disfiguring and disabling lesions, particularly affecting young populations in resource-poor settings. The antibiotic treatment recommended by the WHO since 2004 has simplified the delivery of care for Buruli ulcer, and provided solid evidence that early and limited lesions can be effectively treated with antibiotics alone. However the current recommendations still present significant disadvantages including the use of aminoglycosides, which are administered by intramuscular injection. These drugs are difficult and costly to administer in resource-poor settings and have significant side effects that represent major obstacles to implementation. Therefore, there is an urgent need to identify alternative oral regimens that can be effective, short-course, have few drug-drug interactions with antiretrovirals (ARVs) and are compatible for use in the paediatric population.

We performed a systematic literature review to identify and evaluate publications pertaining to the use of chemotherapy for the treatment of M. ulcerans infection. We included in vitro, in vivo and clinical studies assessing the activity of single drugs and combination regimens against M. ulcerans and their efficacy for the treatment of Buruli ulcer disease. We excluded all studies that did not report microbiological confirmation of M. ulcerans infection as the causative agent for ulcer development. The objectives of this review were to: (1) evaluate the available literature on existing drugs and drug combinations, as well compounds in the research and development pipeline, that could represent future treatment options for Buruli ulcer disease; and (2) to explore next steps in the research and development agenda towards achieving a more effective and accessible treatment regimen.

Various drugs and drug combinations were identified as having clinical efficacy against Buruli ulcer disease in resource-poor settings, some with potentially more attractive administration and toxicity profiles and the major regimens studied will be presented. However, data are fragmented, limiting the rigorous conclusions that can be drawn. Although recent studies indicate that a fully orally-administered treatment regimen for Buruli ulcer may be as equally effective as regimens containing aminoglycosides, further research is critically needed to identify and evaluate new potential treatment candidates. The anti-tuberculosis drug research and development pipeline represents an important and potentially rich source of novel compounds for Buruli ulcer treatment. Based on these findings, we propose a research and development agenda aimed at delivering new, more efficacious and readily implementable treatments against Buruli ulcer in resource-limited settings.
Surveillance, decentralization and technology
« Decentralization of Buruli ulcer control activities in Songololo region: Results, challenges and prospects »

Presenter: Delphine Phanzu

Authors: Phanzu Mavinga Delphin, Imposo Bofunga Bosongo Désiré, Mputu Kembo Dieu-Merci, Lukanu Ngwala Philippe

Until 2009, the diagnosis and medical/surgical treatment of patients with Buruli ulcer in Songololo region was the preserve of specialized facilities including general referral hospitals and selected referral health centres located in the most endemic areas. The objective of the project was to strengthen community-based early detection and expand the geographical coverage of treatment through the decentralization of control activities.

The two endemic areas in Songololo region, Kimpese and Nsona-Mpangu, which are situated in the Cataractes district of Bas-Congo province, have benefited from an intervention essentially comprising (i) the training and supervision of health professionals, (ii) the training of community intermediaries, (iii) the establishment of a case-confirmation and standardized treatment network, (iv) the provision of routine surgical supplies, medicines and other medical consumables.

We report on the results achieved after three years of implementation, remaining challenges and future prospects.
Analysis of the epidemiological situation of Buruli ulcer in Benin from 2003 to 2011

Presenter: Jean Gabin Houezo

Dr Jean Gabin HOUEZO, Dr Ghislain SOPOH, Dr Ange DOSSOU, Dr Yves BAROGUI, Dr Gilbert AYELO, Julia AGUIAR, Dr Didier AGOSSADOU, Mr Esaï ANAGONOU

Introduction
The system of epidemiological surveillance of Buruli ulcer in Benin is based on a network of health workers and community organizations for the detection and early treatment of cases. Since 2003, the use of the BU02 register recommended by WHO has facilitated the compilation of a documented database making it possible to identify cases by individual patient name and the time and place of occurrence. An analysis of the information in the database indicates a concentrated pattern of distribution that varies from one year to the next.

Method
This is a descriptive, retrospective and analytical study focusing on cases of Buruli ulcer originating in three endemic communes and treated between 2003 and 2011 in the Allada, Lalo and Zagnanado centres.

Results
In all, 7543 cases were treated over this period, or an average of 838 cases a year. The three endemic communes are Lalo (1211 cases, 16%), Zè (734 cases, 10%) and Ouinhi (472 cas). The study shows that, in each commune, the cases are concentrated in certain districts and villages. In Lalo, for instance, the most endemic districts are Adoukandji (328 cases) and Gninzoumè (230 cases), and the cases are concentrated in the villages of Adoukandji and Tandji, respectively. The same situation occurs in Zè and Ouinhi where the cases are concentrated in the districts of Sèdjè-dénou (predominantly in the village of Sèdjè) and Dasso (Agonkon village).

The study also notes a decline in the epidemic curve both at district and at village level, in line with the national trend; the same phenomenon can be observed internationally, in some countries. The factors associated with this decrease should be investigated.

Conclusion
This report analyses BU trends at district and village levels identified over the period 2003-2011; it reveals a concentrated pattern of distribution with a trend towards inversion of the epidemic curve over time.
Alternative approach to using OpenClinica in “Offline” mode: A case of WHO Buruli Study

Presenter: Raymond Omollo

Authors: Raymond Omollo1, Michael Ochieng1
Affiliation: 1Drugs for Neglected Diseases initiative (DNDi)

Background
OpenClinica (see http://www.openclinica.com) is one of the world’s most widely adopted Clinical Trials software which is currently being used to manage Clinical Trials data in over 100 countries across the world. DNDi Data Center is currently using the Community edition of the software to manage a multi-site, multi-country WHO’s Buruli ulcer study in West Africa in an offline mode. This approach was adopted due to weak internet infrastructure across the study sites which makes it difficult to use online version of OpenClinica.

Methodologies/Findings
In operating OpenClinica in offline mode, we setup the final study database which is then replicated in all study site computers, site users then proceed to collect data in their respective computers and then generate database dump and send to Data Center for synchronization with the central database. At the DC, OC Event Scheduler which adds subjects and schedules their event in the central database is executed.

Once this is done, the site data is imported in the central database using a utility which uses Web services. A report showing the status of the imported data is then generated and filed.

Conclusions
OpenClinica remains one of the world’s preferred software of choice in Clinical Data Management for clinical trials; however, lack of an offline version for this software is limiting its acceptance and use in sub-Saharan Africa due to poor and unreliable internet infrastructure. This has prompted us to invent ways that can help in using the free version of OpenClinica in offline mode, a number of processes are involved but most of it is automated making the whole process feasible.

Key words
OpenClinica, Clinical Trials
Monitoring outcomes at the end of antibiotic treatment using BU01, POD and BUFLS forms with 23 new cases in 2012 at Kukuom Health Center Asunafo South District, Brong Ahafo Region of Ghana

Poster: Julien Aké

Authors: Lehman LF, Tabiri OF, Tienaah W, Buabeng ID and Ake J.

Kukuom health center is located in Asunafo South District of Brong Ahafo Region of Ghana. Kukuom has a population of 6,383 people. In 2012 the Asunofo South District detected 45 new cases of Buruli ulcer (BU) of which 23 (51%) had been seen at the Kukuom Health Center. Observation of the Kukuom Health Center documentation in 2011 demonstrated almost no information was found on the BU01 forms or documentation was done much later than on initial patient contact. No documentation was done in 2011 on the prevention of disability (POD) forms that included the Buruli Ulcer Functional Limitations Score (BUFLS). Efforts were made by the district health directorate and Kukuom Health Center to improve documentation and care of BU patients and to know more clearly what care areas were in need of attention and resources during BU antibiotic treatment and after BU antibiotic treatment. They were also interested in knowing if the care during the period of the antibiotic treatment had improved the patients’ outcomes. The forms used by health workers to record patient information were reviewed.

Objectives

- To assess the completeness of documentation on the BU01, POD and BUFLS forms.
- To determine if wounds, pain, limitations of movement and percent of limitation in function are less at the end of the period of antibiotic treatment where other care was provided to prevent disability.
- To identify care areas which require attention during treatment and after completion of BU specific antibiotic treatment

Methods

A monitoring form was utilized to collect, and assess all information on the BU01, POD and BUFLS forms of the 23 persons who had been put on and completed BU antibiotic treatment. All cases had been clinically suspected to have BU by three trained health workers and all had PCR completed. The monitoring tool allowed for a quick “snapshot” overview of all new patients treated for BU in 2012. In most cases the same health worker evaluated the same patient at the beginning and end of antibiotic treatment. To demonstrate the change on pain, LOM and functional limitations at the end of BU antibiotic treatment, a statistical analysis was made using Pearson Test to compare proportions of cases at the beginning and end.

Findings

There was significant improvement in documentation in 2012 compared to 2011 on BU01 forms and the inclusion of documentation of POD and BUFLS forms for all patients. The completeness of information on the POD and BUFLS forms was 18 out of 23 (78%) filled completely versus only 10 of 23 (43%) of the BU01 forms filled completely. The documentation that was most complete on the BU01 form was the registry of daily antibiotic treatment, demographic and BU lesion information. The least completed was the last section on the form and when the wound was healed and if there was LOM. The information most completed on the POD forms were all YES and NO responses to impairment and disability areas. This permitted us to identify
information that was missing on the BU01 form. The percentage of functional limitations (BUFLS) was recorded on all at the beginning and end of antibiotic treatment.

Ninety-one percent (21) had information on whether they saw a traditional healer or not. Of these 21 persons, 12 (57%) sought a traditional healer before reporting to the health center. The majority, 18 (78%) were 15 years or older with half between 15 to 50 years of age and the other half more than 50 years old. A total of 5 (22%) were children ranging from 2.5 to 14 years. The majority, 13 (57%) were female. The assessment demonstrated that all new cases had PCR done of which sixty-one percent (14) were PCR positive and that the majority (83%) completed their antibiotic treatment within seventy days. The majority of lesions were categorized as 1 or 2 (57%). However when looking at those who were PCR positive, only 6 of the 14 had a category 1 or 2 (43%) lesion and all children were PCR positive. Seventy percent (16) of lesions were on the lower leg followed by twenty-two percent (5) on the upper limb. (Table 1)

Table 1: Location of lesions by agegroup among 23 persons of Kukuom Health Center in 2012

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Lower Limb</th>
<th>Upper Limb</th>
<th>Abdomen</th>
<th>Head and Neck</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 14</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15 – 50</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>6</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16 (70%)</td>
<td>5 (22%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

Table 2: Comparison of wound, pain, Limitations of Movement and Buruli Ulcer Functional Limitation Score at the beginning and end of BU antibiotic treatment at Kukuom Health Center in 2012

<table>
<thead>
<tr>
<th></th>
<th>Wound</th>
<th>Pain</th>
<th>LOM</th>
<th>BUFLS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning of BU antibiotics</td>
<td>15 (88%)</td>
<td>16 (94%)</td>
<td>13 (76%)</td>
<td>16(94%) persons with a limitation</td>
</tr>
<tr>
<td>End BU antibiotics</td>
<td>15 (88%)</td>
<td>2 (12%)</td>
<td>4 (24%)</td>
<td>8(47%) persons with a limitation</td>
</tr>
<tr>
<td>Variation p value</td>
<td>0%</td>
<td>-87.2%</td>
<td>-68.4%</td>
<td>-0.5%</td>
</tr>
</tbody>
</table>

This presentation shows the significant (< 0.05) change that could be observed with the POD forms and BUFLS form (See table 2). Although the same number of wounds was present at the end, all decreased in size, one healed and one person, having an oedematous lesion, developed a wound during treatment.

Observation of problems at the end of BU antibiotic treatment demonstrated that wound management is still needed for 88% of this group. In addition 42% still had functional limitations, 24% had LOM and 12% had pain that required attention and possible referral.
Comments
The results of good care could be observed in the 17 patients having complete information documented at the beginning and end of BU antibiotic treatment (Table 2). The intensity of pain and percentages of limitations of movement could easily be seen. The improvement in LOM was limited to a YES/NO response for the whole limb with only a few documenting on the body chart or quantifying the number of movements that were limited. The monitoring form made it clear which patients required continued care after BU antibiotic treatment and the type of care that would be needed. Further study is needed on the PCR results and decision on antibiotic treatment.

Lessons Learned
- The POD forms could realistically be used within BU control programs at all levels of the health system to identify problems needing care during and after the completion of antibiotic treatment. It can also be used to plan for resources needed to provide care.
- The POD forms permitted comparison of impairments and disability at the beginning and end to evaluate quality and outcomes of care. It could be made better if more quantitative data were available with the YES/NO responses and drawings.
- Besides wound management, attention is needed to manage pain, movement and functional limitations (BUFLS).
- It appears that oedema could have been a factor influencing the pain, LOM and BUFLS.
- Documentation consistently and accurately helps to measure quality of care and individual outcomes as well as permit the comparison of outcomes across health facilities.
- The Monitoring Form enabled the district team to easily collect data and summarize findings from the BU01, POD and BULFS forms to determine quality of services and make management decisions.

Recommendations
1. The POD and BUFLS forms are used along with the BU01 form to document and monitor the patients condition and quality of care.
2. Use the Monitoring form to evaluate quality of services and make managerial decisions.
3. Include oedema which is in POD forms on future Monitoring forms.
4. Study the feasibility of using the Monitoring form by cell phone technology.
5. The quantification of LOM and oedema could be better using the 2012 WHO BU Drug trial POD protocol.

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Improving care for Buruli ulcers in the Bankim District of Cameroon: A proof of feasibility for a community based intervention for early case detection

*Poster: Paschal Awah*

**Authors:** Paschal Awah, Ferdinand Mou, Joseph Koin, Evaristus Mbah, Alphonse Umboock

**Introduction**
Buruli Ulcers is a neglected tropical disease that affects mainly people living in poverty and in poor rural communities. Its vector and risk areas are various. Cameroon is one of the countries with a high infection rate and where some research and interventions are being set up to tackle the disease. However, the disease seems to be neglected both by affected individuals, communities and governments where BU is common. A feasibility study was conducted to demonstrate, to these groups of stakeholders, that involving community stakeholders may reduce time for referral and improve treatment outcomes and lend credibility to public health interventions aimed at treating BU.

**Material and methods**
The design was prospective. Forty eight confirmed cases of BU were admitted into the treatment centres in the Bankim Health District of Cameroon from April to November 2011. The patients were followed up daily to ensure adherence to treatment. Advanced cases were lodged in halfway homes and in treatment centres where their feeding was subsidized.

**Results**
The ages of research participants ranged from 2-68 years, with a mean of 22 years. Eighty-one percent of the confirmed cases were in their advanced stages (categories two and three) at admission, during the first four of the eight months of the intervention. From the fifth month, the number of categories one started to increase while categories three started to reduce reduced. Mean hospital stay did not significantly change. The involvement of traditional healers, family members, community relay agents and the fact that many of the burdens in terms of transportation and lodging provided to patients contributed to the recorded changes.

**Conclusion**
Delay to seeking care for Buruli ulcers could be reduced through involving motivated community stakeholders in the planning, identification, referral and follow up of suspected cases and patients with BU and if transport and lodging facilities are subsidized.

Keywords: Buruli Ulcers, Delay, Treatment, Community stakeholders, patients
Initial assessment findings; interventions, achievements and challenges, Lofa, Nimba and Bong Counties

Presenter: Tarnue Mulbah

Neglected Tropical and Non Communicable Diseases Program Buruli Ulcer Disease, Ministry Of Health and Social Welfare, Liberia

Liberia is among few if not the only country in the sub region that has just begun reporting on Buruli Ulcer cases. The country is a post-conflict one which has a fragile health system in a resource poor setting recovering from the ravages of war. Liberia has now established an integrated NTDs program under one Program Manager; the diseases include Buruli Ulcer, Lymphatic Filariasis, Onchocerciasis, Schistosomiasis and Soil Transmitted Helminthes.

Cases of Buruli Ulcer have been confirmed in Liberia; with support from MAP International, an assessment on Buruli Ulcer was conducted in three (Bong, Lofa and Nimba) of the fifteen counties with the aim to establish the existence of buruli Ulcer in the country.

The initial assessment collected specimen from 61 suspected cases of Buruli Ulcer of which 21 (17 Males, 4 Females) were confirmed PCR positive. One of the 21 cases expired before the treatment was initiated. Sixteen (16) cases have since then completed 56 days of treatment, 13 have healed completely but 2 cases have developed complications. Two cases have reported relief from discrimination and stigmatization, and have both volunteered to work along with their respective Communities in the areas of creating community awareness on the early Buruli Ulcer case detection.

The project has identified a national BU Coordinator and three NTDs focal persons within the three counties. BU and Leprosy have been included in the counties surveillance diseases listing and supervision checklists. Seventeen (11%) of 149 health facilities in the pilot project counties have treated BU cases,

BU and Leprosy are currently integrated into the national NTDs program. They have been included in the Nursing Board national curriculum, while Leprosy remains part of the curricula of all other health training institutions. Progress is being made to include BU in all other curricula. There are plans in 2013 to conduct Buruli Ulcer assessment in the remaining twelve counties.

In the midst of these successes there are challenges, the 14 year civil war destroyed technical capacity and physical infrastructure including health facilities on a massive scale. Most roads are in very bad conditions, making it difficult to access endemic communities, thus weakening interventions at the community level. Few of the challenges ranges from strong resistance to the incorporation of vertical programmes for other NTDs like soil transmitted helminthes (STH) and Leprosy into a single integrated national NTD control programme, faces logistical challenges for NTDs implementation and limited financial support.
Buruli ulcer and HIV co-infection
BU-HIV co-infections: cases treated at Allada and Lalo Centers for the detection and treatment of Buruli ulcer, Benin, 2005–2012

Presenter: Yves Barogui

Yves Barogui, Ghislain E. Sopoh, Ange D. Dossou, Jean Gabin Houezo, Didier Agossadou Johnson, Roch Christian, Annick Chauty, Julia Aguiar

Introduction

Buruli ulcer (BU) is the most widespread mycobacterium in the world after tuberculosis and leprosy. This emerging disease is present in more than 30 countries. Its prevalence and impact in endemic countries are poorly understood. This is because the documentation currently available for assessing the scale and gravity of BU is very fragmented and the risk factors are poorly understood.

A link between BU and HIV was recently demonstrated. In addition, it was shown that carriers of the human immunodeficiency virus were more likely to present with disseminated forms of BU. Scant data are available on HIV-BU coinfections, and at no time since the initiation of medical treatment for BU have the therapeutic outcomes of HIV-BU coinfections been evaluated.

We report here on the clinical aspects and the course of certain cases of HIV-BU coinfection managed at the Allada and Lalo BU treatment centres in Benin.

Method

This is a retrospective study based on data relating to patients coinfected with HIV and BU who were treated at the centres for the detection and treatment of BU (CDTUB) at Allada and Lalo between 2005 and 2012.

A voluntary HIV test is routinely offered to patients with suspected BU upon admission. If they test positive, they are treated for HIV and BU.

BU patients confirmed by Ziehl-Neelsen staining and PCR (IS2404 strain) with HIV coinfection are recorded. Data on these patients are compiled in Excel and analysed using IMB SPSS Statistics 19.

Result

Epidemiological and clinical

- Cases of BU confirmed by Ziehl-Neelsen and PCR (IS2404 strain) with HIV coinfection have been recorded.
- Most coinfected patients have ulcerative category-III lesions
- Some patients presented with disseminated lesions
Laboratory
- Blood counts revealed cases of severe anaemia and leukocytosis. The white blood cell count was normal in 9 patients; 3 patients had leukocytosis.
- CD4 count less than 200 cells/µL in some patients.

Treatment
- All patients were administered streptomycin and rifampicin and all had surgical treatment for BU.
- Patients were placed on anti-retroviral therapy depending on the clinical course of the disease and their laboratory results.

Outcome
- Most of the patients were cured and experienced no sequelae.

Conclusion
These results in patients coinfected with BU and HIV are very encouraging. It seems to us that, when coinfected patients receive appropriate antiretroviral therapy and streptomycin and rifampicin, the therapeutic outcomes are fairly promising.
Immune protection mechanism in Buruli ulcer disease (BUD) is not fully understood, it appears like other mycobacterial disease, adaptive immune response championed by CD4+ activation of macrophages could be crucial for protection. While it is clearly known that HIV/AIDS which leads to reduced CD4 helper T cell activity is a risk factor for TB, the role of HIV infection in Buruli ulcer has not been extensively studied, and no report is available in Ghana.

In this report (from 2009 to 2012), we screened 195 PCR confirmed cases attending the Amasaman Municipal hospital and the Obom Health Centre for HIV/AIDS infection by following the national protocol. Oralquick was used for initial screening and samples that tested positive were confirmed by the Inno-Lia HIV I/II immunoblot assay.

Of the 195 cases, 57 (29.2%) were ≤ 15 years; age ranged between 5 months and 99 years, males were 95 and females were 100.

HIV prevalence among Buruli ulcer patient was higher (8.6% 17/195) than the national figure of less than 2%. Affects both sexes, 9/17 were females and 8/17 were males. Co-infection occurs mostly in adults (>15 years: N=15) who presented with large and multiple lesions; 12 presented with category III and of these 4 had multiple lesions. Ten of the cases had their CD4 counts determined and the value ranged between 37 and 1296 with a mean value of 594.2 ±439.8 and median value of 692.5. While 13 received SR8, four of them based on the clinician’s judgment were treated with SR for 12 weeks.

Two of the co-infected patients healed without surgery, and duration of treatment was four months, 7 cases healed between 28 and 42 weeks with surgery, though 2 of them have had their wounds re-opened; 2 died during treatment, 2 absconded and four of them who have been treated for between one year and two yeas are still on wound management.

Histopathological analysis of cases that underwent surgery after treatment indicated that there is granuloma formation as occurred in other patients; however we found the granulomas to be mainly formed from macrophages, indication of limitation in CD4 response.

This study is supported by the Stop Buruli Initiative funded by the UBS-Optimus Foundation.
Does HIV have an influence on Buruli Ulcer clinical presentation and incidence?

Presenter: Vanessa Christinet

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Introduction

Buruli Ulcer (BU) is caused by Mycobacterium ulcerans. It is the third most common human mycobacterial disease after tuberculosis and leprosy and is considered a neglected tropical disease. The bacterium is responsible for extensive undermined cutaneous ulcers inducing esthetical and functional deformities. The impact of HIV on BU has not been clarified yet in contrary to other mycobacterial diseases: although tuberculosis does classify an HIV-infected patient as having AIDS, leprosy is not considered an opportunistic disease. Little research has been done on the interaction of HIV and BU. A few studies have been conducted in order to investigate HIV infection as a risk factor for developing BU but without conclusive results. There are a few descriptive case reports showing extensive and overwhelming BU in HIV infected patients (PLWHIV), but the same clinical picture has also been described in HIV negative individuals. The aim of our research is to investigate the impact of HIV on the clinical expression of Mycobacterium ulcerans (MU).

Method

Retrospective analyses were done of the data of the Médecins Sans Frontières Switzerland program in Akonolinga, Cameroon. The project has been running since 2002 and aims at treating patients with BU. The first analysis was done on the HIV prevalence among patients diagnosed with BU enrolled since 2008, as systematic HIV testing of all BU patients was then introduced. Secondly, different BU clinical severity parameters were compared between HIV positive and HIV negative adult patients. These different parameters were then stratified according to various levels of CD4 of the HIV infected patients. Kruskal-Wallis and T-test were used for comparison. A Kaplan-Meier survival analysis was elaborated in order to investigate the time to heal BU according to different CD4 strata. Uni- and multivariate analyses (Cox model) were done to investigate the factors influencing the time to heal BU. Linear regression has been used to investigate the correlation between baseline size of the main BU lesion with the level of immunosuppression induced by HIV infection and measured by baseline CD4 cell count.
Results

Since introduction of systematic HIV testing in 2008, more than 90% of the BU patients have been tested for HIV. Among patients treated for BU, the HIV prevalence, stratified by sex and age, is systematically 4 to 6 times higher than the local estimated prevalence. In the comparative analyses among adults, we observed that PLWHIV tended to have larger and multiple BU lesions. PLWHIV needed more surgical interventions and a longer time to heal. These tendencies were coherent with the analyses according to immunosuppression strata. The survival analyses showed that the most severely immunosuppressed patients have a significant longer time until complete BU lesions healing. In the multivariate analyses low CD4 cell count were independently associated with a prolonged BU lesions healing time. We found a significant association between the severity of immune suppression and the lesion size.

Conclusions

We found a higher prevalence of HIV infection among patients hospitalized for BU, as compared with the expected HIV prevalence in a comparable population.

In addition, our data suggest an effect of HIV on the clinical expression of BU. The higher proportion of patients with severe BU among HIV positive patients and the continuous negative association of severe BU with immunosuppression strengthen the hypothesis of a negative effect of immunosuppression on BU clinical expression.
Between 2005 and 2012, we received 1305 patients with Buruli ulcer, confirmed by a positive PCR.

We proposed an HIV test to every patient. They all accepted the test. 22 patients were HIV positive. We informed them and proposed a confirmation test and a counseling in the HIV service in the general hospital in Pobè. One patient knew that she was HIV positive and she had already received antiretroviral treatment.

One patient is positive with VIH 1 & 2 and is co-infected with hepatitis B.

Those 22 patients: 12 females between 7 and 60 years old, and 10 males between 25 and 60 years old.

8 have a BCG scar.

Delay of consultation is between 3 and 40 weeks.

19 patients received traditional cares before consultation to CDTLUB.

- 2 patients are category 1,
- 8 patients are Category 2
- 12 patients are catégorie 3 (more than 15cm).
- 17 patients have ulcerative lesion and one had a bone lesion.
- 5 patients had non-ulcerative lesions.
- 6 patients healed their lesion without surgery.
- 16 patients had surgery (necrosis ablation under general anesthesia with or without graft).
- 3 patients presents new lesion during the first year of follow-up.

Patients who received ARV treatment before 30 days of streptomycine/rifampicine(SR) or finished their SR treatment first.

Average time of hospitalization was 186 days.
Laboratory confirmation: progress and challenges
Laboratory Examination of Buruli Ulcer in Japan 2010-2012

Presenter: Kazue Nakanaga

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Background
The first case of Buruli ulcer in Japan was reported in 1980 of a 19-year-old Japanese woman who had never been abroad. The causative agent is not fully equal with *Mycobacterium ulcerans* but closely related strain “*Mycobacterium ulcerans* subsp. *shinhshuense*”. Since then, the reported number of cases in Japan were gradually increased, and 36 cases in total by Dec. 2012. Unfortunately, the Buruli ulcer in Japan have a risk of being late for diagnosis because usual Japanese dermatologists and surgeons are unaware Buruli ulcer or recognize Buruli ulcer as tropical disease. For making an earlier diagnosis in the future, we conducted a validation study about laboratory examinations for differential diagnosis.

Materials and Methods
All the subjects are received at Leprosy Research Center from Apr. 2010 to Dec. 2012. 1. Clinical samples of the patient suspected as Buruli ulcer: There were frozen or chilled pus, skin exudate, skin biopsies (39), and thin sections of formalin fixed paraffin embedded skin biopsy tissue (28) included. 2. Cultured bacterial samples (13) from the suspected patients.

Laboratory diagnostic tests were as follows: 1. Bacterial isolation test, 2. PCR test; targeting IS2404, (esxA, esxB). 3. Sequencing test; 16S rRNA gene, hsp65 gene, rpoB gene and the 16S-23S intergenic spacer region (ITS region).

Results and Discussion
The bacterial isolation was successful in 6 cases in 22 cases tested, 3 cases were “*Mycobacterium ulcerans* subsp. *shinhshuense*” and other 3 were non tuberculous mycobacteria (NTM). PCR test targeting IS2404 was positive for the DNA of frozen or chilled skin biopsy tissue or cultured bacteria of 20 samples (20/35, including 19 Buruli ulcer cases, positivity 100%). This PCR test was considered to be most important for the early diagnosis. The equal PCR test was also positive for 15 samples of formalin fixed paraffin embedded skin biopsy tissue (15/25, 8 Buruli ulcer cases). This PCR test with paraffin embedded skin sample is proven to be very useful for retrospective analysis. All isolates from Bruli ulcer patient have the identical 16S rRNA gene sequences in “*M. ulcerans* subsp. *shinhshuense*”. Of the 7 NTM strains, one is identified as *M. peregrinum*, the other one is identified as *M. gordnae*, other 2 and 3 are identified *M. chelonae* and *M. marinum* respectively by sequencing test.

Conclusion
PCR test targeting IS2404 was considered to be the most important test for the early diagnosis, and also for the retrospective analysis.
Molecular diagnosis and confirmation of Buruli ulcer cases in Côte d’Ivoire in 2012.

Presenter: Solange Kacou-Ngazoa

Kakou-Ngazoa ES\textsuperscript{1}, Aka Nguetta\textsuperscript{1}, Coulibaly N D\textsuperscript{1}, Assié N\textsuperscript{2}, Koffi Aboua\textsuperscript{2}, Yavo A\textsuperscript{1}, Sangaré F\textsuperscript{1}, Mambé P\textsuperscript{1}, Kadjo C\textsuperscript{3}, Asse H\textsuperscript{2}, Aoussi S\textsuperscript{1}, Dosso M\textsuperscript{1}

\textsuperscript{1}Molecular biology platform, Pasteur Institute
\textsuperscript{2}National Buruli Ulcer Control Programme
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Buruli ulcer is a skin infection caused by \textit{M. ulcerans}. Since 2009, the number of cases in Côte d’Ivoire has risen in spite of expanded national surveillance. The disease is diagnosed on the basis of clinical and laboratory criteria. Buruli ulcer is a continuing health problem in Côte d’Ivoire despite the establishment of a national control programme and the eradication efforts of the World Health Organization. Since 2010, WHO has recommended that 50% of cases should be confirmed by molecular diagnosis based on the presence of genetic material of \textit{M. ulcerans} by PCR.

In order to achieve the WHO target of confirmation of 50% of Buruli ulcer cases, the Pasteur Institute of Côte d’Ivoire, as the national reference laboratory, has collaborated with a number of partners to test specimens taken from suspected BU patients nationwide.

Equipment and method

A total of 1104 clinical specimens from 530 suspected patients were processed at the molecular biology laboratory in 2012.

The swabs were treated according to the protocol approved by WHO for laboratories performing molecular biology confirmation. Basically, the swabs were hydrated in 2 ml of sterile water and agitated in order to recover the Mycobacteria at room temperature. The residue obtained after centrifuging was re-suspended in 200 µl of sterile water and used for extraction, employing the alkaline-thermal lysis method.

PCR was performed according to the recommended protocol for a reaction of 25 µl containing 5 µl of DNA and 20 µl of Master-mix. Fluorescence curves were generated by an AB 7300 thermocycler. Each sample was tested twice to validate the results. Negative and positive controls (reference DNA) were included in all the tests as a method of quality assurance.

Results and conclusion

The breakdown of patients from all the endemic health districts in Côte d’Ivoire was as follows: Sakassou (31), Sinfra (70), Daoukro (17), Yamoussoukro (56), Katiola (9), Béoumi (14), Bouaké (92), Abidjan, NGO Afrisol (19), Bondoukou (5), Vavoua (20), Daloa (34), Zoukougbeu (56) and Toumoudi (21). A total of 217 patients (40\%) were confirmed as positive by PCR IS204 or PCR KR in real time.

114 patients were children under 15, accounting for 21.5\% of the total. 90\% of the molecular tests were requested long after the date of specimen-taking.
Buruli ulcer is a public-health problem in Côte d’Ivoire. The rate of case confirmation by molecular diagnosis at the Pasteur Institute is 100%, and 40% of patients tested positive in 2012. The remoteness of certain endemic sites can lead to delays in case confirmation and subsequent treatment.

**Keywords**: Buruli ulcer, *M. ulcerans*, PCR, KR, IS2404, Côte d’Ivoire
Prevention of disability
Community-based rehabilitation in the context of the Buruli ulcer control project at the Kimpese Medical Evangelical Institute (IME), Democratic Republic of the Congo

Presenter: Désiré Imposo


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Introduction

Despite progress made by various Buruli ulcer (BU) control programmes in a number of countries in recent years, BU remains a disabling disease. The decentralization of control efforts to the community level, which has facilitated early detection and treatment of the disease, has significantly reduced the number of cases in which BU causes disability. Nevertheless, certain negative factors such as beliefs, use of traditional medicine as a first recourse, remoteness from health facilities, and unwillingness to seek immediate treatment have ensured that the disease continues to thrive.

Implementation of community-based rehabilitation

Patients without sequelae have reintegrated into their respective communities without too much difficulty, whereas those with sequelae have mostly been marginalized and in some cases actively excluded owing to their disability. With support from our partner American Leprosy Mission (ALM), and in order to address this problem and restore dignity to these individuals, our BU control project is currently developing a community-based rehabilitation programme for people with BU sequelae and other disabilities, while simultaneously employing the other conventional techniques for controlling BU.

A review was conducted at the outset to compile a database as a prerequisite for rolling out activities. Next, trainings were organized for coordinators, the Kimpese and Nsone-Mpangu rural health district management teams, community health intermediaries, village volunteers, persons living with disabilities caused by BU, leprosy and other diseases, and selected members of the community.

Community-based rehabilitation activities

From the health standpoint, conventional control activities (prevention, health education, curative care) were continued. Some individuals were given crutches and others were offered repair and reconstructive surgery. In the sphere of education, about 15 schoolchildren who had missed school through illness were given school supplies and in some cases their school fees were paid for them. As regards means of subsistence, social integration and empowerment, two mutual assistance associations were established by the persons concerned (one association per health district) to act as a springboard for current and future interventions and various forms of support.

Conclusion

Community-based rehabilitation is a strategy that deserves to be incorporated into comprehensive control strategies to enable persons with disabilities caused by BU, leprosy and other diseases to reclaim their basic rights and restore their dignity. It can also be a tool for fighting poverty.
Prevention of disability in BU in the context of community-based rehabilitation: first lessons learnt in Cameroon

Presenter: Valérie Simonet

Valérie Simonet¹, Alphonse Um Boock², Simon Yonga³

General considerations

The vertical programme system, the institutional response and decentralization in the prevention and management of disabilities associated with BU have revealed their limitations. As a result, since 2010 FAIRMED has encouraged discussion on this topic in a spirit of equity and with a view to rationalizing invested resources.

Intervention

This discussion resulted in the development of a tripartite project involving FAIRMED, the Ministry of Welfare and the Ministry of Health, which was launched at a workshop in September 2010 and promotes a participative approach. The development of a national strategy for community-based rehabilitation gained extensive approval at the workshop, and gave birth to the Integrated Prevention of Disabilities and Rehabilitation Project (PIER).

Mbalmayo, a town in the Central region that is endemic for BU, was chosen as a pilot district for community-based rehabilitation on the model recommended by WHO. Since December 2012, a specially trained community-based rehabilitation technical officer has been delivering the minimum package of community-based rehabilitation activities. Under his supervision, 18 volunteers are monitoring disabled persons living in their immediate neighbourhood. A management committee comprising members of the community supports their action through local resource mobilization, advocacy and other targeted interventions. The premises designated for these activities also act as a forum enabling people with various disabilities to share their experiences. In the longer term, the Ministry of Welfare should coordinate the community-based rehabilitation programme nationally and scale up this pilot project.

Current and forthcoming results

This initiative, which was originally intended to support the BU control programme, has largely exceeded its original remit; it is now an element used in comprehensively strengthening prevention and rehabilitation at Mbalmayo, both in terms of health and at the community level. By addressing a problem that was recognized by the local population and by health professionals and local decision-makers alike, this project has generated enthusiasm and support for the participative approach at the highest level.

Persons affected by BU could benefit from this approach in several ways, including through better early detection and improved coordination of preventive actions, community-based monitoring by specially trained volunteers on a case-by-case basis, under the supervision of a professional conversant with basic BU care, access to an available forum to exchange experiences, counselling, psychosocial support and exercises, reduction of stigma, and better referrals and counter referrals.

Outlook

Activities in this community-based rehabilitation district will be launched and the information required to replicate the pilot model in other areas of Cameroon will become available in the course of 2013.
Participation restrictions, functional limitations and stigma in former Buruli ulcer patients in Ghana

Presenter: Janine de Zeeuw

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Background

Buruli ulcer (BU) disease is a stigmatising health condition that may lead to physical disabilities and may impact on social life, such as employment and relationships in the community. The International Classification of Functioning, Disability and Health, a model to describe functioning and health, defines problems in social life as participation restrictions. These restrictions are an important indicator in rehabilitation interventions. This study aimed to measure the extent of participation restrictions, along with functional limitations and stigma among former BU patients.

Methods

Individuals previously affected with BU disease treated in the Agogo Presbyterian Hospital in Ghana from 2005-2011 were interviewed using the Participation-scale (P-scale), Buruli Ulcer Functional Limitation Score (BUFLS), and Exploratory Model Interview Catalogue (EMIC) to measure stigma. Higher scores indicate more problems. Participants eligible for enrollment finished treatment at least 3 months earlier and were at least 15 years of age. The P-scale was also applied to healthy community controls matched for age, sex and location. Native interviewers who had been trained conducted the interviews.

Results

In total 125 participants (75 former BU patients and 50 community controls) were included in the study. Former BU patients had significantly higher (p<0.001) scores on the participation scale (median 13; IQR 5-29) compared to healthy controls (median 2.0; IQR 0.75-7.25). Frequently reported problems among former BU patients related to employment, relationship with life partner and meeting new people. Spearman’s Rho correlation between participation restrictions and functional limitations among former BU patients was r=0.62; p<0.001. Among former BU patients, Spearman’s Rho correlation (r) between participation restrictions and stigma was 0.64; p<0.001.

Conclusion

Former BU patients in Ghana experience substantial participation restrictions in social life compared to healthy controls. Especially, participation problems were experienced related to employment, long-term relationships and meeting new people. Strong relations were found with functional limitations and stigma.
Togolese physiotherapists training program in cooperation with the German Leprosy and Tuberculosis Relief Association.

Presenter: Yuki Shimomura

Project SCOBU, Kobe International University
Hideki Koeda, Tomoki Niiyama, Kazuyuki Fukunishi, Tetsuya Fujikura, Yuki Shimomura

Project SCOBU, Kobe International University has started a pilot training program focused on BU treatment for Togolese physiotherapists and health workers in co-operation with German Leprosy and Tuberculosis Relief Association since March 2012. The first session of the program was carried out in September 2012 and provided up-to-date knowledge of rehabilitation medicine.

Togo has a national PT's training program in l'École Nationale des Auxiliaires Médicaux de Lomé. The school has graduates most PTs in Togo however, they have had almost no opportunity to improve their rehabilitation engineering or skills because of lack of funds and human resources.

The project continues to study difficulties to manage PTs activity in Togo and will plan better training workshops for the program.
Evaluation of functional mobility in Buruli ulcer: limits and utility of an existing tool

Poster: Valérie Simonet

General considerations:
Evaluation of functional mobility in persons affected by BU is key for preventing disabilities because it:

1. Facilitates objective assessment of progress and decision-making (whether to continue, modify or stop rehabilitation)
2. Facilitates objective assessment of what is considered a functional limitation in order to standardize completion of BU01 forms
3. Provides information about the quality of treatment
4. Sets treatment objectives at the individual or the health-facility level and facilitates assessment of how far these have been met.

There is no simple tool for rapid measurement of functional mobility, either at referral centres or at peripheral health facilities.

Intervention
To rectify this shortcoming, a functional mobility score, albeit not standardized, has been included in the WHO field guide entitled « Buruli ulcer. Prevention of disability. Basic rehabilitation », and treatment centres in Cameroon have been invited to make use of it.

Results
The situation has been reviewed in certain treatment centres in Cameroon. The functional mobility score has its limitations but is very helpful for interpreting the course and results of treatment and also for identifying limitations. There is a correlation between use of this tool and the number of patients reported as having a functional limitation in the BU01 form.

Discussion
The Buruli Ulcer Functional Limitation Score (BUFLS) provides information about the overall quality of prevention of disability, but it cannot provide information about the development of functional mobility, nor enable professionals to take decisions about interventions requiring implementation or to set objectives. A tool such as the functional mobility score could greatly facilitate the work of supervising prevention of disability activities and it would be useful to adapt and standardize it from this perspective.
Wound management and surgery
Wound management in the treatment of Buruli ulcer at two Buruli ulcer screening and treatment centres in Benin

Presenter: Ghislain Sopoh

Dr Ghislain Sopoh ; Dr Ange Dossou ; Dr Yves Barogui, Dr Gilbert Ayelo ; Dr Jean Gabin Houezo ; Dr Didier Agossadou ; Dr Alphonse Umboock ; Dr Roch Christian Johnson

Wound management occupies an important place in the treatment of Buruli ulcer. The quality of dressings influences the duration of hospitalization and the quality of scar formation. Although the management of wounds and especially chronic ulcers have been described in detail, there is currently no standard protocol applicable to Buruli ulcer used in screening and treatment centres in Benin. We present here:

1. A review of the existing modern dressing methods and techniques
2. A description of the dressing methods and techniques currently employed in two Buruli ulcer screening and treatment centres in Benin
3. An analysis of the strengths, weaknesses, advantages and disadvantages of the various dressing methods and techniques described
4. The prospects for improving the quality of the dressings in the two Buruli ulcer screening and treatment centres assessed.
Management of Buruli ulcer: Prevalence and risk of nosocomial infections at Bankim screening and treatment centre.

Poster: Alphonse Um Boock

Alphonse Um Boock, Stéphanie Melie, Fidèle Watong, Bladine Noumen, Christian Minyem, Catherine Bilong

Introduction

Buruli ulcer is an infectious disease caused by *Mycobacterium ulcerans*, a mycobacterium present in the environment.

The infection causes extensive destruction of the skin and soft tissues and the formation of large ulcers, usually on the arm or leg. Bankim health district is a major focus of the disease.

However, in Cameroon and elsewhere, inpatient treatment for the disease often takes an inordinately long time, sometimes up to two years.

According to the literature, nosocomial infections are to blame for these long periods of hospitalization. Despite the positive results achieved by the Bankim screening and treatment centre, no study has ever been carried out on this topic at this health facility.

Method

This will be a prospective cohort study over 24 months designed to ascertain the risks of contracting a nosocomial infection, and to gather information about the prevalence of nosocomial infections.

A sample of 85 patients will be recruited at the Bankim screening and treatment centre for Buruli ulcer. The study will focus solely on category II and III patients.

A bacterial assessment will be carried out at the start of the study and periodically thereafter, even if there are no signs of infection.

Results

As at 31 January 2013, 7 patients have already been recruited and their assessments are now being conducted at the Pasteur Centre in Yaoundé.

The results for the first quarter of 2013 will be known by the end of March.

Conclusion

The study of nosocomial infections could be a tracer for the quality of dressings. The resulting recommendations will form a basis for reducing the duration of inpatient treatment.

Keywords: Buruli ulcer, nosocomial infection, category II and III patients.
Discovering the Current Wound Management Practices of Rural Africans: A Pilot Study

Poster: Linda Benskin

Unrelenting heat, poor sanitation, lack of knowledge, and poverty contribute to disabling wound prevalence that often exceeds 20% in rural areas of tropical developing countries. Wounds in this environment are usually poorly managed at very high cost. Traditional health practitioners and village health workers, rather than health professionals, provide health care in most villages. Wound management education for these nonprofessional health providers should include only sustainable practices which prove to be safe and effective in tropical villages. However, usual practice data, needed for comparison studies, is absent from the published literature.

This pilot study introduced an innovative data collection method to overcome cultural obstacles which have prevented researchers from obtaining meaningful quantitative data in this challenging setting. Between August and October of 2012, seventy-five participants from 25 diverse villages in Ghana provided detailed descriptions of their current usual topical wound management methods by completing patient stories representing seven wound types typically found in this setting. Responses were tabulated and categorized as congruent or not congruent with modern topical wound management principles within three domains and six subcategories (two for each domain).

Four research questions organized the data analysis. Several significant differences in the wound management of three provider groups were found. The wound management practices of nonprofessional health care providers were identified and described in detail for the first time. These results are foundational to the process of developing culturally and environmentally appropriate wound management protocols for indigenous wound care providers in rural areas of tropical developing countries.

The unique data collection method introduced in this study can easily be adapted to rural areas of other tropical developing countries. When sufficient data have been accumulated, the information can be utilized to design comparison studies so that the ecological validity of the wound management protocols in planned educational programs can be ensured.
Importance of scar management in the treatment of Buruli ulcer

*Poster: Ange Dossou*

AD DOSSOU, GE SOPOH, JG HOUEZO, GA AYELO, YT BAROGUI, RC JOHNSON, DC AGOSSADOU,

The introduction of bi-antibiotic therapy and the development of decentralized treatment have made a significant contribution to early screening and better management of patients; they have also made treatment less onerous. Many patients are now cured without recourse to surgery, or by surgery limited to debridements subsequent to immediate grafts.

Following a decade of treatment and regular monitoring of patients, it now appears that scars of wounds that heal without surgery often reopen and in the long term are prone to cancerization. Scars in this category are situated for the most part at the major joints (elbow and knee). Histological lesions are often epidermal carcinomas and life expectancy following diagnosis does not exceed one year.

It therefore seems advisable to establish a system of rigorous monitoring of patients following discharge from hospital and to introduce proper management of hypochromic and retractile scars.
The author describes the case of a large Buruli ulcer (PCR positive, ZN positive) situated on the right arm and complicated by high radial palsy. Treatment was a combination of Rifampicin and Streptomycin for 8 weeks as recommended by WHO for *Mycobacterium ulcerans* infection and covering of the large ulcer with a *latissimus dorsi* strip after debridement of the necrotic tissue. The high radial palsy was treated by means of a triple tendon transfer with a view to restoring extension function in the wrist, fingers and thumb: *pronator teres* was transferred to *extensor carpi radialis brevis*, *flexor carpi ulnaris* to *extensor digitorum communis*, and *palmaris longus* to *extensor pollicis longus*. The surgery was complemented by immobilization and rehabilitation. Operative follow-up was simple, involving gradual recovery of the restored functions.

**Illustrations**

Large Buruli ulcer (before treatment)  |  Result (following treatment)
Radial palsy (before treatment)  |  Result (following treatment)

**Author and correspondent**

Professor KIBADI KAPAY Anatole, MD, PhD, plastic surgeon

Chief, Reconstructive and Aesthetic Plastic Surgery Unit, Hand and Burns Surgery (Kinshasa University Hospital), University of Kinshasa, Democratic Republic of the Congo
«Multiple-strip reconstruction in the case of a significant loss of tegumental tissue and facial deformity due to Buruli ulcer (*Mycobacterium ulcerans* infection)».

**Abstract**: Anatole Kibadi Kapay

**KIBADI K., IMPOSO B., DILU J.**

*Mycobacterium ulcerans* infection, commonly called Buruli ulcer, causes necrotic ulcerating skin lesions. Large ulcers, disabling deformities and serious sequelae necessitate plastic surgery. Serious cases such as this one require reparative surgery and sometimes several stages of reconstruction using multiple strips.

The multiple-strip reconstruction of a significant area of tegumental tissue and facial deformity was realized in several phases: (Phase 1) Covering with pediculated strip of *latissimus dorsi*; (Phase 2): Weaning of the *latissimus dorsi* strip; (Phase 3): Harvesting of forehead strip to cover nasal tissue loss; (4ème temps): Weaning of forehead strip; (5ème temps): Repair of residual losses: realization of a nasolabial strip to replace lost residual nasal tissue.

It is therefore possible, in our facilities and working conditions, to repair large areas of facial tissue using the techniques of reconstructive surgery. For this patient, the interim result is satisfactory and encouraging; she is now able to smile again. Other elective procedures are envisaged (left commissuroplasty).

**Author and correspondent**

Professor KIBADI KAPAY Anatole, MD, PhD, plastic surgeon

Chief, Reconstructive and Aesthetic Plastic Surgery Unit, Hand and Burns Surgery (Kinshasa University Hospital), University of Kinshasa, Democratic Republic of the Congo
Towards rational use of antibiotics for suspected secondary infections in Buruli ulcer patients.

Poster: Yves Barogui

Background
The emerging disease Buruli ulcer is treated with streptomycin and rifampicin and surgery if necessary. Frequently other antibiotics are used during treatment.

Methods/Principal findings
Information on prescribing behavior of antibiotics for suspected secondary infections and for prophylactic use was collected retrospectively. Of 185 patients that started treatment for Buruli ulcer in different centers in Ghana and Bénin 51 were admitted. Forty of these 51 admitted patients (78%) received at least one course of antibiotics other than streptomycin and rifampicin during their hospital stay. The median number (IQR) of antibiotic courses for admitted patients was 2 (1, 5). Only twelve patients received antibiotics for a suspected secondary infection, all other courses were prescribed as prophylaxis of secondary infections extended till 10 days on average after excision, debridement or skin grafting. Antibiotic regimens varied considerably per indication. In another group of BU patients in two centers in Bénin, superficial wound cultures were performed. These cultures from superficial swabs represented bacteria to be expected from a chronic wound, but 13 of the 34 (38%) S. aureus were MRSA.

Conclusions/significance
A guide for rational antibiotic treatment for suspected secondary infections or prophylaxis is needed. Adherence to the guideline proposed in this article may reduce and tailor antibiotic use other than streptomycin and rifampicin in Buruli ulcer patients. It may save costs, reduce toxicity and limit development of further antimicrobial resistance. This topic should be included in general protocols on the management of Buruli ulcer.
Training
Impact of the Microbiology *Mycobacterium ulcerans* course on the diagnosis of Buruli ulcer in Sub-Saharan Africa

*Presenter: Sara Eyangoh*

What has been the impact of the Microbiology of *Mycobacterium ulcerans* (M2U) course organised in Centre Pasteur since 2006 on the diagnosis of Buruli ulcer? Actually, we took into account the relevance of this training, the degree of its effectiveness in terms of educational achievements of participants and ensuring transfer of learning was actually achieved in the professional field. These three levels of assessment were judged on the basis of tests and questionnaires.

Indeed, four training sessions have been organised (January 2006, September 2007, November 2009, November 2011) with full cover of the participation fee of participants. The main objective of the M2U course was to improve the technical capacity of participants, in order to facilitate the implementation in their respective laboratories for confirmation of cases of Buruli ulcer in endemic areas, and indispensably to improve the quality of clinical diagnosis. Thus, with funding from the International Network of Pasteur Institutes, supported by WHO and Sanofi Espoir Foundation, 50 health personnel, biologists and technicians from 11 countries (Benin, Congo, Côte d'Ivoire, Gabon, Ghana, Guinea Conakry, Nigeria, Uganda, CAR, DRC and Togo) in addition to Cameroon have been trained, one technician from the Supra Reference Laboratory ITM Antwerp and 4 students from Cameroon and French nationalities were also trained.

At the end of each training, examinations showed that participants had acquired over 85% of the skills that were covered by the training objectives. Moreover, the evaluation of the relevance has an evolution and adaptation in line with each training session contexts needs. (1) we moved from 7 days of full-time training in 2006 to 10 days in 2007 to increase the duration of practical work, including environmental collection and analysis and extend the training to English-speaking countries, (2) the 2009 course integrated real-time PCR and (3) the 2011 supported by the WHO network of buruli ulcer laboratories incorporated the notion of external quality control for the improvement of laboratory capacity.

In addition, the evaluation of transfer of learning showed that participants, once back at their workstations, implemented the skills acquired during the training. This concerns particularly six countries: Benin, Côte d'Ivoire, Ghana, CAR, DRC and Togo. For 2 countries (Gabon and Congo) the transfer was not effected in 3 of the countries due to lack of resources. We received no feedback from 3 countries (Nigeria, Uganda and Guinea Conakry).

Finally, the third assessment concerns the impact of the training. Although it is difficult to give the actual impact link with the training, it is clear that countries endemic for BU in sub-Saharan Africa are getting acquainted with the use of these methods in their diagnostic activities and samples are no longer sent to laboratories in the North for diagnosis as was the case in 2006. Several countries now participate in external quality control, and trained staff is involved in research programs credit to a better understanding of this disease. It also appears that this training has been a real source of motivation especially for technicians and a determining factor for maintaining technicians at their workstations. This course also allowed interaction between people working in different countries on Buruli ulcer and thus could eventually lead to an inter-country Buruli ulcer network.
The Buruli ulcer treatment center of Allada: A possible WHO collaborating center for Buruli ulcer training

Presenters: Ghislain Sopoh/Emile China

Ghislain Sopoh¹, Ange Dossou¹, Gilbert Ayelo¹, Emile China², Jean Gabin Houezo¹&³, Didier Agossadou¹, Raoul Saïzonou¹, Kingsley Asiedu²

1. BU treatment center of Allada
2. Follereau Foundation of Luxembourg
3. BU and Leprosy control program, Benin
4. WHO, Bénin
5. WHO, HQ

The diagnosis, management and control of Buruli ulcer are evolving and new health workers are also coming on board. There is therefore the need for theoretical and practical training on regular basis for health workers from BU endemic countries in Africa so that they are properly equipped to deal with the disease. This training may cover different areas such as surveillance, diagnosis, treatment management (including antibiotics, wound and scar management, prevention of disability, rehabilitation).

From 2002 to 2010, the CDTUB of Allada underwent several improvements of its infrastructure to meet the increasing demand for hospitalization resulting from continued growth in the number of inpatients and also to make the facilities available for training and research on BU. The infrastructure currently comprises technical structures (95-bed wards, operating theater, well-equipped laboratory, physiotherapy room and orthotic workshop), a training unit with several meeting facilities and a guest house. The hospital also has a number of vehicles for field work. Currently there is 48 staff (5 doctors, 18 nurses, 7 laboratory technician, 1 physiotherapist and 17 administrative and support staff).

The center offers the following services: emergency care, routine consultations, hospitalizations, surgery, wound dressings, diagnostic services (laboratory, radiography, ultrasonography), physiotherapy, coordination and supervision of ten decentralized health services, epidemiological surveillance, monitoring, evaluation and data management, staff training and management of research projects, mass communication and outreach, screening and secondary prevention activities, socio-educational and social reintegration activities.

With all these resources available, we believe that all conditions are met to facilitate quality training, integrating theory and practice, for health workers from all endemic countries, knowing that the major advantage of such an international training is the exchange of experiences. The training facilities may also be of benefit to other disease control programs which can easily be integrated into Buruli ulcer activities and vice-versa such as leprosy and yaws.

The Ministry of Health of Benin and the National BU and Leprosy program support this proposal. We therefore call on the commitment and support of all partners so that we can make another progress in Buruli ulcer control and research.
Differential diagnosis
Histoplasmosis caused by Histoplasma duboisi: a disease not to be confused with Buruli ulcer

Poster: Désiré Imposo


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Introduction

African histoplasmosis caused by Histoplasma duboisi or Histoplasma capsulatum var. duboisi is a relatively rare mycosis. In the interval between its discovery in 1952 by Vanbreusegen and 1992, a total of 237 cases were reported. According to the data available to us, annual frequency does not exceed 4 cases. However, since 2011 our region has seen an upsurge in cases that has never been observed elsewhere. This could completely change our understanding of the epidemiology of this disease. This mycosis is particularly remarkable owing to its resemblance to Buruli ulcer.

The causal agent

The causal agent of histoplasmosis is a dimorphic encapsulated fungus, Histoplasma capsulatum. This fungus exists in its filamentous form in the soil and can produce highly resistant spores that are dispersed in the air. It is these yeast forms that are responsible for the clinical forms of histoplasmosis. Two varieties are distinguishable, var. capsulatum and var. duboisi, which are quite distinct in terms of their geographical distribution and their clinical and anatomical spectrum. The capsulatum variety (also known as small-form histoplasmosis, American histoplasmosis or Darling’s disease) has essentially been described in the Americas, but also in Africa, Oceania/New Caledonia and Asia. All European cases have been imported. The duboisi variety (syn. Large-form histoplasmosis, African histoplasmosis) is less frequent and has only been described in tropical Africa, from the Sahara to South Africa and in Madagascar.

Clinical aspects

The first case observed in our hospital was that of an 11-year-old boy from a small urbanized rural community situated 15km from the hospital. He was admitted to the orthopaedics unit on 2 May 2011 with apparently chronic disseminated ulcers complicated by osteomyelitis. A diagnosis of Buruli ulcer was suggested, but subsequent investigation of secretion specimens ruled out this disease. Surgery was indicated, in the course of which the product of curettage, a cerebroid material, was sent for pathology analysis. In the interval, nonspecific antibiotic treatment proved ineffective. Other nodular or gummatous lesions, occasionally abscessed, appeared during hospitalization and the patient’s overall condition continued to deteriorate. The result of the pathology investigation subsequently revealed histoplasmosis due to Histoplasma duboisi. The patient was given a course of ketoconazole and the lesions completely disappeared six months later.
One month after the admission of the initial patient, a second, female patient was admitted with a similar clinical picture; two months later, two other female patients were admitted almost simultaneously, with less serious lesions. The surgical unit gradually began to diagnose other, simpler cases with non-complicated nodular lesions. Four patients were referred to peripheral health centres by two physicians who had been trained in techniques for diagnosing Buruli ulcer.

To date, more than 20 patients have been admitted to and treated at our hospital.

When the patients were questioned to ascertain the reservoir of the pathogen, almost all of them stated that they had been in contact with certain domestic animals, specifically cats or poultry. However, it was noted that the disease did not occur in patients living on the same plot of land, on neighbouring plots, or in patients from the same family or living under the same roof. Some of the patients were tested for HIV; the results were negative.

**Conclusion**

Although African histoplasmosis was described in the Democratic Republic of the Congo in the 1970s, it is a new phenomenon in our region. Its high frequency (which does not reflect the actual situation) merits a higher level of surveillance and more in-depth epidemiological study. Signs of onset: papilla, painless slow-growing nodule; in complicated forms: dissemination, osteomyelitis resembling category I, II or III Buruli ulcer, requiring differential diagnosis. In doubtful cases, histoplasmosis is confirmed by direct examination and/or histopathology.
Differential diagnoses for Buruli ulcer: clinical cases at Akonolinga, Cameroon

Poster: Laurence Toutous Trellu

L Toutous Trellu¹, Yolanda Mueller², Elizabeth Tschanz¹, Patrick Nkemenang³, Bitoungui Mboua³, Geneviève Ehounou³, Eric Comte⁴, Jean-François Etard²

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Introduction

Owing to clinical presentations that vary over time and for one and the same individual, diagnosis of Buruli ulcer (BU) can be difficult even in endemic areas. Ulcerative forms of BU can be confused with other kinds of ulcers found in tropical settings. The most frequent are phagedenic ulcers, ulcers of vascular origin, diabetic ulcers or neurogenic ulcers secondary to leprosy, sickle-cell anaemia, yaws, chronic herpes, ecthymas, or any other ulcer of infectious origin, in addition to skin cancers. The differential diagnosis of non-ulcerative lesions mainly includes infectious bacterial lesions (dermo-panniculitis, abscess), tuberculosis, parasitosis or deep mycosis, but also other nodule-forming conditions such as benign lipomas and, exceptionally but most serious of all, malignant tumours (sarcoma, lymphoma). The diagnosis will differ according to the patient’s context and age. In addition, atypical presentations of BU have been described in the case of HIV infection.

Hypothesis

A sound knowledge of the typical presentation of BU and its complications facilitates highly accurate clinical diagnosis. However, in the absence of confirmation through paraclinical examination, errors in diagnosis are very probably under-estimated. Confirmation of the diagnosis by laboratory testing makes it possible to ascertain that the proper treatment has been proposed and can avoid the use of unsuitable antibiotics.

Intervention

Médecins sans Frontières is supporting a BU programme at Akonolinga, a marshy endemic area in the centre of Cameroon, and expanding its recruitment in order to enhance treatment for wounds and skin diseases in hospital settings. A study over the past 12 months has been focusing on improving diagnosis with a view to better targeting of specific antibiotic therapy. 166 patients presenting with 191 suspected BU lesions were assessed photographically, cytological and laboratory specimens were taken for PCR and culture and skin biopsies were performed for histology. The median age of the patients was 32 and 22.3% were under 15. Most of the patients attending the treatment centre (62%) were men. The median duration of the course of the disease was 20 weeks and 58% received conventional treatment. In 34.9% of cases there was a history of initial injury.

Recruitment is continuing and the cases presented are clinical situations described in detail as being non-BU confirmed cases, but which could have benefited from empirical treatment for BU.
A case-report of Elephantiasis and Buruli ulcer co-infection

Poster: Lydia Mosi

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The Afrique One consortium “Ecosystem and population health: bridging the frontiers in health” is an African led initiative with a strategic vision for developing coordinated and integrated capacity building in health research at the human-animal-wildlife interface in a given ecosystem. There are 7 Universities and 4 research institutes from 6 countries in sub-Saharan in partnership with 3 northern institutions. The role of the consortium is to enhance research capacity and build formal operational links among African regional institutions specifically in the area of zoonotic diseases. In this frame, Post Doctoral research is the key issue which acts as a starting block for a competitive and independent research.

The fellow of the Swiss centre for Scientific Research (CSRS) in Côte d’Ivoire is working on a project titled “Zoonotic risks of non-tuberculous mycobacteria between humans and small mammals (Potential transmission of Buruli ulcer) in Côte d’Ivoire and Ghana”. The hypothesis suggests that overlapping environmental habitats of the pathogen, animals and humans directly influences the transmission of M. ulcerans and other mycolactone producing mycobacteria (MPM). We aim to address the distribution and the transmission with respect to (i) active case surveillance of human and small animal disease burden; (ii) zoonotic risk analysis between animals and humans living in close proximity; (iii) and molecular characterization of NTMs in described endemic areas (Côte d’Ivoire and Ghana).

The study is being conducted in Côte d’Ivoire, in 6 communities in the districts of Taabo and Daloa, and in Ghana, in 4 communities located in the Amansie central district. During active-case-surveillance for the project in Côte d’Ivoire, an interesting case of co-infection of Elephantiasis and Buruli ulcer was observed and documented. We present here the results of this case-study along with clinical and laboratory confirmation of both infections that could contribute to the transmission model. We are grateful to the Wellcome Trust for supporting the Afrique One consortium.
Social studies
The influence of social and economic capital on health seeking decision-making among Buruli ulcer patients in Southern Ghana

Poster: Collins Ahorlu

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Background

Buruli ulcer is considered as a re-emerging disease particularly in West Africa where it has suffered neglect over the years until recently. Though not fatal, due to delayed health seeking it is associated with grave disabilities, which may lead to amputation and loss of vital organs like the eye. The disease affects children under 16 most and therefore leads to school dropout and other social and economic consequences for the affected family. Early treatment with antibiotics for 56 days is effective, but though, treatment is generally free, most cases report at the clinic late. Treatment decisions in terms of choice and timing may affect early case detection and access to early effective treatment, which is the flagship of BU control.

Method

This was a descriptive study designed to determine the how social and economic capital in the communities influences health seeking behaviour of BU patients, in terms of where and when to seek help. Data was collected using a semi-structured questionnaire to interview BU patients in the selected communities. Data from forty patients was analysed for this paper.

Results

Forty patients were interviewed constituting female to male ratio of 2:3. About 87.5 said they have someone to turn to for financial support (Economic capital) when they need it. They received financial supports for; clinic attendance (95%) self medication using biomedicine (45%) home prepared concoctions (37.5%), Faith healing (15%) and consulting traditional healers (7.5%). Also, 92% of respondents said they have someone to turn to for advice (social capital) regarding decision on when and where to seek BU treatment.

Conclusion

The study shows that social and economic capitals are important in treatment decision making among BU patients. These capitals eventually determine where and when treatments are sought for BU rather than the cost of treatment.
Psychosocial and school support for children affected by Buruli ulcer at the Ayos subsidiary regional hospital.

Poster: Jean-Paul Amougou Amombo

Dr Amougou Amombo¹, Um Boock Alphonse², et al.
1: Director, Ayos Hospital
2: FAIRMED representative

Cameroon is an endemic country for Buruli ulcer.

The Nyong basin is a major focus of this disease; it was here that national BU control efforts started, specifically in the Akonolinga and Ayos health districts.

Accordingly, Ayos Hospital was designated the national referral centre for the treatment of BU.

The conventional treatment services offered by the hospital include an educational component, which has proved to be a significant innovation. A school for sick children was built at the hospital, because more than half of BU patients are children and hospital stays involving antibiotic treatment can last up to three years.

The objective of the school is to enable the children to continue their education during their stay in hospital.

A total of 164 under 15 have attended this school, despite the significant stigma they had experienced. Thanks to this school, most of them now have a brighter future.

The aim of this presentation is to show the impact of the social and educational centre at a Buruli Ulcer Screening and Treatment Centre and its role in the social reintegration of child patients.
Socio-cultural beliefs and wound care at the Obom sub-district of the Ga South Municipality of Ghana

Poster: Eric Koka

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4 National Buruli ulcer control programme, Ghana Health Service, Accra

Background
Most often, we are faced with cultural issues in wound care and seeking treatment at the biomedical health facilities. Based on clinical suspicion, a microbiological study was conducted that showed that secondary infection of Buruli ulcer wounds could be a reason for delay in wound healing in the Ga-South and West districts of Ghana. A study was conducted with the aim to understand some of the cultural belief systems in the management of wounds and patients practices that could contaminate wounds at the Obom sub-district of the Ga South Municipality of Ghana.

Method
This was an ethnographic study using in-depth interviews, Focus Group Discussions and participant observation techniques for data collection. Interviews and Focus Group Discussions were conducted with opinion leaders and Buruli ulcer patients in selected communities. In all, ten in-depth interviews and two Focus Group Discussions were conducted. Also, observations were done on Buruli ulcer patients to document how they integrate local and modern wound management practices in their day to day handling of their wounds. Content analysis was done after the data were subjected to thematic coding.

Results
A number of cultural practices and beliefs came up in the data, most of which significantly affected the patients’ wound care and treatment seeking behavior. These included cultural beliefs that prohibit certain category of people such as pregnant women, lactating mothers and women who menstruate from dressing wounds. It was usually believed that wounds were caused by charms or spirits and, therefore, required the attention of a native healer. In instances where some patients’ wounds were dressed in the hospital by clinicians whose condition/age/sex contradict their belief, the affected often dress the wounds later at home. Some of the materials often used for such wound dressing include urine and concoctions made of charcoal and gun powder with the belief of driving out evil spirits from the wounds; practices which may cause secondary infection of wounds.

Conclusions
Clinicians must therefore be aware of these cultural beliefs and take them into consideration when managing BU wounds as this would help to reduce the secondary infections that have been observed among BU patients. Though, there is a need for education and counseling, these must not be seen as a magic bullet that would change these practice overnight, hence the need for clinicians to respond to the needs of the patients by seeing them as partners in the management of their wounds to reduce secondary infections and improve healing.
Research
Environment and transmission
Through a house-by-house survey in the Mapé Dam basin of Cameroon, the prevalence of Buruli ulcer (BU), leprosy and yaws in the local population was determined. Following the survey, BU monitoring continued and a total of 88 qPCR positive BU cases were identified. Analysis of the geographical pattern of the homes, water contact sites and sites of agricultural activities of BU patients indicated that they have closer contact to local rivers as opposed to the artificial lake that has developed by damming the Mapé River since 1989. The population adjusted age prevalence of BU was low in young individuals and increased after the age of 40. It further showed, that very young children (<5) are underrepresented among cases indicating that they may be less exposed to risk factors of transmission. Analyzing BU lesion distribution, we found that lesions cluster around the ankles and at the back of the elbows. This lesion pattern does not match published mosquito biting site patterns which are usually focused on the head or feet and ankles. On the other hand, the distribution of the BU lesions is more similar to the published pattern of small skin injuries. The option of multiple modes of transmission can however, based on these data, not be excluded.
Studies on the environmental distribution of *Mycobacterium ulcerans* in Ghana and Australia reveal some similarities and differences.

*Presenter: Janet Fyfe*

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The highly focal distribution of Buruli Ulcer (BU) cases is a common feature in all geographic locations where outbreaks of this disease are encountered, though the reason for this remains unclear. Despite the fact that *Mycobacterium ulcerans* DNA has been detected in various aquatic samples in west Africa, and *M. ulcerans* has been successfully cultured on rare occasions from water bugs, the primary ecological niche(s) and mode(s) of transmission remain controversial.

Advances in DNA extraction procedures and molecular detection and identification of *M. ulcerans* in environmental samples have led to the discovery that in BU-endemic regions in southeastern Australia, the local *M. ulcerans* strain infects both humans and arboreal native marsupials, in particular, common ringtail possums. Furthermore, the detection of high levels of *M. ulcerans* DNA in the faeces of these animals, both with and without associated ulcerative disease led us to suspect that the organism is able to persist in the coastal vegetation which is the primary food source of the ringtail possum and potentially replicate within the marsupial gastrointestinal tract.

In September 2011, we took a similar approach to environmental sampling and testing in the Eastern Region of Ghana. The aim was to collect a variety of environmental samples from both aquatic and non-aquatic sources from a BU-endemic village and two non-endemic villages in the Densu River valley. In particular, animal faecal samples were collected from all three villages.

As for the Australian samples, DNA was extracted using the FastDNA® SPIN Kit for Soil with the FastPrep® Instrument. Extracts were initially screened for the presence of IS2404 via gel-based and real-time PCR, and positive samples further tested for the presence of IS2606 and KR. Only those samples testing strongly positive for all three targets and with a $\Delta C_t$ (IS2606 – IS2404) of <2 were considered strong candidates for the presence of significant numbers of *M. ulcerans*. On this basis, a variety of sample types from all three villages were found to have estimated bacterial loads of $10^5$ - $10^6$ *M. ulcerans*/gram. These included faecal samples from lizards, chickens and goats, snails from a farm, a palm husk with an insect and vegetation collected beside a path. These preliminary results confirm that in this region of Ghana, as in south eastern Australia, *M. ulcerans* is not confined to aquatic habitats. Furthermore, particularly in Ghana, *M. ulcerans* may be present in the environment within villages with no prior history of BU. Further studies are required to identify the primary ecological niche(s) and to determine whether the modes of environmental distribution and transmission are similar in these two distinct geographic and climatic regions.
Potential wildlife sentinels for monitoring the endemic spread of human Buruli Ulcer in south-east Australia

Presenter: Connor Carson

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The last 20 years has seen a significant series of outbreaks of Buruli/Bairnsdale Ulcer (BU) in temperate south-eastern Australia (State of Victoria). Here, the prevailing view of Mycobacterium ulcerans as an aquatic pathogen has been questioned by recent research identifying native wildlife as potential terrestrial reservoirs of infection; specifically, tree-dwelling common ringtail and brushtail possums. In that previous work, sampling of environmental possum faeces, and swabs from ulcerative skin lesions in live captured animals, detected a high prevalence of M. ulcerans DNA in established endemic areas for human BU, compared with non-endemic areas. Here, we report research from new BU endemic foci recently identified in Victoria, confirming spatial associations between human BU cases and the presence of M. ulcerans DNA in possum faecal samples detected by real-time qPCR (IS2404, IS2606 and KR). Sampling of ground-collected possum faeces in survey grids showed focal distribution of high numbers of positive faecal samples corresponding with BU human case clusters; corresponding grids in closely adjacent BU-free areas were predominantly negative. Moreover, time series environmental sampling showed that the detection of M. ulcerans DNA in possum faecal samples predated a surge in human BU cases in a previously non-endemic area. Possums may be useful sentinel animals to predict endemic spread of human BU in Victoria, for public health planning. However, further research is needed to establish whether spatial and temporal associations represent evidence of direct or indirect transmission between possums and humans, and the mechanism by which this may occur.
**Mycobacterium ulcerans** isolated from a Belostomatid in the Asante Akim North district of Ghana

**Presenter: Anthony Ablordey**

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**Background**

Cultivating *M. ulcerans* from environmental sources has been very difficult to achieve. Low bacilli loads in the environment, deleterious effects of decontamination procedures, slow growth and resultant overgrowth in culture by fast growing organisms hinder successful cultivation of the bacterium (1, 2). Currently only one *M. ulcerans* isolate has been obtained in pure culture from an environmental specimen (aquatic insect from the *Gerridae*) (1). The cultivation involved growth in Bactec 12B broth of decontaminated sample suspension and subsequent inoculation of mouse footpad (MFP). *M. ulcerans* was isolated on LJ medium after MFP passage. The aim of our study in the three most endemic communities of the Asante Akim North district of Ghana was to cultivate *M. ulcerans* from macro-invertebrates (both aquatic and terrestrial).

**Methods and results**

A total of 9012 samples, pooled to 421, were investigated. The samples were identified by an entomologist. Of these 367 were IS2404 (-) and 54 were IS2404 (+). PCR positive homogenates were inoculated in Bactec broth and onto LJ medium. The 15 cultures that subsequently grew were tested for IS2404 and only two (from Notonectidae and Belostomatidae insect families) became positive. Colonies were then examined by IS2404 PCR, 16S rDNA sequence analysis, multi locus VNTR typing and whole genome sequencing. Isolate M3 from Belostomatid sp of insect had 16S rDNA, VNTR (Loci 1,4,6,8,9,14,15,19) sequences all consistent with *M. ulcerans*. Whole genome sequencing confirmed the identity of this isolate as *M. ulcerans*. Interestingly, while high-resolution molecular epidemiology based on SNP comparisons showed isolate M3 was related to other *M. ulcerans* isolates from Ghana, it was also distinct to clinical isolates from the same region.

**Conclusions**

More effort needs to be given to obtaining environmental *M. ulcerans* isolates to help understand the questions of reservoir and transmission. Here we have shown that *M. ulcerans* obtained from an insect in Ghana is genetically distinct to isolates obtained from humans in the same region, raising the possibility that several strains of *M. ulcerans* may circulate in the environment, without necessarily causing disease.

**References**


Spatial variation of *Mycobacterium ulcerans* abundance in aquatic habitats: implications for disease prevention and transmission

**Presenter: Eric Benbow**

M. Eric Benbow, Heather R. Williamson, Mollie D. McIntosh, Richard W. Merritt, Daniel Boakye, Charles Quaye, Pamela LC Small

Understanding the transmission dynamics of *Mycobacterium ulcerans* to humans has eluded investigators for many years. Recently there have been important developments in effective molecular tools to identify and quantify *M. ulcerans* from both patients and environmental sources. Even with the use of these tools in excellent studies to better understand *M. ulcerans* in the environment, there still remains a central question regarding the spatial and temporal variability of this mycobacteria, used in this study to represent all other mycolactone producing mycobacteria (MPMs), in nature. Understanding this variability will allow researchers to more cost effectively target sampling intensity from environmental sources for specific habitats and certain times of the year, while also providing critical information for better describing the environmental niche and any hosts important to transmission.

In this study, we evaluated the spatial distribution of MPMs (most presumably *M. ulcerans*) at several scales of resolution to better understand how positivity rates vary with matched quantitative estimates depending on the scale of inquiry. We did this using qPCR of the ER domain to provide quantitative estimates of genome forming units (GFU) in relation to associated positivity rates at three spatial scales of inquiry from sampling that took place in West Africa aquatic habitats: 1) among village/hamlets; 2) among three habitat types (plants, water filtrand, artificial substrates) in one waterbody; and 3) among different microhabitats of the artificial substrates. Using the relationships at the village scale, we also estimated country-wide scale variability in *M. ulcerans* distribution.

We found that the spatial scale of inquiry was the most important factor determining the abundance of *M. ulcerans* in the environment. At larger scales, as positivity rates increased, the variability (patchiness) in *M. ulcerans* abundance decreased, but at the microhabitat scale, this relationship was the opposite, demonstrating increased patchiness of *M. ulcerans* when it was most abundant among standardized artificial substrates. These results suggest for studies evaluating *M. ulcerans* abundance among villages or waterbodies, that standardized sampling from multiple habitats is important to estimate the risk of *M. ulcerans* transmission in those locations. This also holds true for studies evaluating the distribution of *M. ulcerans* within one habitat such as one waterbody. However, in studies where the microhabitat (and thus potential environmental reservoirs) is being evaluated, it is important to identify specific substrates and increase the sampling intensity both in number of samples and frequency of collections. These results suggest that careful consideration should be taken when developing experiments or surveys of *M. ulcerans* from environmental samples, as the scale of inquiry plays an important role in the ability to make strong inference and interpretation of the environmental distribution of this human pathogen. This information is needed for making local scale (within a village) estimates of transmission risk important to understanding Buruli ulcer disease in West Africa.
Demography of Buruli Ulcer in Lalo Commune

Presenter: Pamela Small

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The epidemiology of Buruli ulcer has been studied intensively in the past 15 years both in African countries and in Australia. Results from these studies show that over 50% of the cases occur in children <15 years of age and that the distribution of cases occurs equally in males and females. However data from these studies have not been normalized against the population age pyramid of the communities surveyed. This has made it impossible to determine whether Buruli ulcer is a "children's disease" with a specific association with children such as measles or group A pharyngitis, or whether cases occur predominantly in children <15 because over 50% of the total population fall into this age group.

To address this issue we have gathered detailed demographic information in the villages and hamlets of Tandji, and Tchi-Ahomadegbe which lie within 10km of each other in the Couffo drainage. Both areas are characterized by high Buruli ulcer prevalence. Population data was gathered using local language (Fon and Adja) by a team including local community volunteers, Buruli Ulcer Control Program personnel, and a field team from University of Tennessee. Basic demographic data was gathered from all residences in Tandji (pop. 1388) and three communities in Tchi-Ahomadegbe (total pop. 1423). Information collected includes age and gender of each person, case information, monthly BU detection rate, livelihood information, and months resident in the village (out-migration rate).

Construction of age pyramids for the total population compared with similar data for cases shows that age-specific associations do occur but they are not the same for all communities. We also show significant differences in the monthly detection rate for Tandji and Tchi-Ahomadegbe, both of which are served by local health clinics. Population data was gathered using local language (Fon and Adja) by a team including local community volunteers, Buruli Ulcer Control Program personnel, and a field team from University of Tennessee. Basic demographic data was gathered from all residences in Tandji (pop. 1388) and three communities in Tchi-Ahomadegbe (total pop. 1423). Information collected includes age and gender of each person, case information, monthly BU detection rate, livelihood information, and months resident in the village (out-migration rate).

Construction of age pyramids for the total population compared with similar data for cases shows that age-specific associations do occur but they are not the same for all communities. We also show significant differences in the monthly detection rate for Tandji and Tchi-Ahomadegbe, both of which are served by local health clinics. These differences may be explained by differences in livelihood. Livelihood in Tandji involves cultivation of maize as a subsistence crop, but significant populations are involved in rice cultivation, wild-crafting the aquatic palm, Calamus deerattus and furniture making. In contrast, along with subsistence maize cultivation, rice is a dominant crop throughout the Tchi-Ahomadegbe area. Rice cultivation was introduced to Tchi-Ahomadegbe by the Chinese and artesian wells were constructed. These wells perpetually spew forth volumes of water creating permanent swamps and allow vast areas to come under rice cultivation. Participation in rice cultivation both as a land owner and day laborer is a dominant activity in Tchi-Ahomadegbe. Case detection per month in Tchi-Ahomadegbe follows a predictable course with peaks as high as 11 cases in October-November over a 5 yr period. In other months only 1-3 cases are detected. In contrast, cases are spread out more evenly throughout the year in Tandji. In terms of detection rate the only similarity between Tchi-Ahomadegbe and Tandji is that the month when cases are least likely to be reported is at the end of the dry season in March.

A key finding from these studies is that even within a small geographic area, major differences are found in ethnicity, agricultural practices, crop calendar and environment, extent of out-migration, and monthly case detection rate.
Preventing Buruli ulcer in the wet agro-ecosystems of West and Central Africa: Preliminary data on the location of *M. ulcerans* in the Buruli ulcer endemic area of Ze in the southern Benin.

*Presenter: Akindayo A. Sovunmi*

for the BU-AgroEcoHealth Platform, IITA- West and Central Africa

**Background**

The wet agro-ecosystem is believed to hold the key to reduction of poverty in West and Central Africa through increased land use for improved agricultural productivity and food security. This high agricultural pressure on wet lands is becoming increasingly associated with elevated incidence of Buruli Ulcer (BU) in the communities living or working in wet ecological areas and therefore, inhibiting efforts to sustainable food security in West and Central Africa. In our 5 years strategic plan we have targeted the BU endemic locality of Ze as the pilot site for research and intervention to prevent the transmission of *Mycobacterium ulcerans* (MU) from the environment to humans and reducing the incidence of BU in the communities. Ze is a rural locality in the southern Benin Republic where economic activities are heavily dependent on crop production, fishing and their related activities. This is the first environmental survey conducted at Ze which mobilizes a consistent amount of human resources and has adopted a robust and integrated ecological sampling methodology coupled with the use of molecular detection techniques for investigating and identifying MU niches in the environment.

**Methodology and Results**

As a starting point for this research, we have set up at the International Institute of Tropical Agriculture (IITA ) an AgroEcohealth research and knowledge exchange platform for fighting BU in West and Central Africa. We have brought together through the platform a multi-disciplinary team of scientists from different West and Central African countries to address the modes of transmission of MU from the environment to humans. One hundred and thirty-two samples from surface waters (including cultivated fields), ground waters, sediment , soil, fish, amphibians, biofilms, aquatic insect, terrestrial vegetation, aquatic vegetation were collected at Ze. Samples were macroscopically described, the physico-chemical properties of collected surface and ground water were determined in situ and, 2 PCR analysis were conducted on the various specimens for screening the presence of the IS2404 locus (PCR-IS2404) and detecting the presence of the enoyl reductase ( ER) domain (PCR-ERD) for the *Mycobacterium ulcerans* (MU). Preliminary results from this research conducted at the end of the rainy season revealed that. Available moisture in Ze was acidic: (surface water =5.12±0.42; groundwater pH=5.81±0.38 and biofilm = 6.13±0.25) and very low in dissolved oxygen (surface water =1.10±0.57ppm; groundwater =2.47±0.42ppm and biofilm = 0.88±0.28ppm).

Surface water, aquatic insect ground water and aquatic vegetation tested positive for PCR IS2404 (6 positive samples out of 150 tested so far) .When these 6 samples were subjected to PCR-ERD, 2 revealed the presence of the enoyl reductase ( ER) domain for *Mycobacterium ulcerans*.
Conclusion

This study provides evidences of the presence of *MU* in the environment of *Ze*. Data generated from this first field survey serves as a strong starting point for the planned systematic and longitudinal environmental sampling to be conducted in Ze. These preliminary results will be useful in the detection of MU niches, the mapping of the distribution of MU in the BU endemic locality of Ze and the analysis of the modes of transmission of MU to humans.
Using satellite imagery and GIS approaches to explore geospatial patterns of Buruli Ulcer (BU) in Central Cameroon

*Poster presenter: Konstanze Lechner*

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In 2012, a pilot study has been started to explore geospatial patterns of Buruli Ulcer (BU) in Central Cameroon. In this collaboration between MSF and the Center for Satellite Based Crisis Information (ZKI) of the German Aerospace Centre (DLR) BU case data and a large variety of geospatial data and satellite imagery are jointly analysed mainly for the district of Akonolinga, Cameroon. Main focus of the work comprised the regionalization of BU case data in order to visualise the distribution of recorded BU cases in the study area, and furthermore, to identify spatial clusters and patterns which may indicate areas with an increased infection risk. Moreover, satellite imagery and geographic data were used to derive environmental factors, e.g. land cover, elevation, population distribution, in order to model and spatially predict potential infection risk areas of BU.

A great potential to contribute to a better understanding of spatial characteristics and possible causal mechanism of BU was identified. Future investigations should combine satellite imagery and geographic data with BU systematically collected case data to allow better correlation and more significant geostatistical analysis.
Application of *M. ulcerans* whole genome sequencing to understand Buruli ulcer transmission

*Poster presenter: Tim Stinear*

Anthony Ablordey¹, Nana Ama Amissah¹, Jessica Porter², Caroline Lavender³, Janet Fyfe³, Tim Stinear²

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Is it possible to tell if the *M. ulcerans* isolate obtained from a patient living near a certain river is the same as the *M. ulcerans* isolate obtained from an insect in that river, or the same as an isolate from another patient in a nearby village? If we can say with a high degree of certainty that these isolates are identical then we can start to build testable hypotheses about chains of transmission. Equally, transmission hypotheses can be modified if we can also say that isolates within the same area are slightly different. Whole genome sequencing (determining and then comparing the six-million-letter long string of As, Gs, Cs & Ts that make up the DNA of the *M. ulcerans* genome) is the ultimate tool that we can use to say with a high degree of certainty if any pair of *M. ulcerans* isolates are the same or different. We have begun applying this approach to Buruli ulcer endemic areas in Ghana and Australia. Sequencing and comparing the genomes of more than 30 *M. ulcerans* isolates from human and environmental sources in these regions - where the provenance of the isolates is well documented - has highlighted both promise and problems. There are technical issues surrounding optimal analysis of the data. Knowing how much genome variation between isolates should be considered significant is also an issue. Our foray into whole genome sequencing to support Buruli ulcer epidemiological investigations is just beginning, but our initial findings indicate significantly more isolate variation even at the village scale than we’ve previously considered, encouraging us to think about multiple reservoirs of *M. ulcerans*. As we collect more sequences from carefully collected isolates, with well-documented histories, the power of this approach will significantly escalate, and will lead us to a deeper understanding of Buruli ulcer transmission.
The incubation period for Buruli ulcer (*Mycobacterium ulcerans* infection)

**Poster presenter:** Paul Johnson

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**Objective**

Buruli Ulcer (BU), is a necrotizing cutaneous and subcutaneous infection caused by the environmental pathogen *Mycobacterium ulcerans*. Although the mode of transmission remains unclear, residence in or contact with a BU endemic region is a known risk factor. In Victoria, Australia, where endemic areas have been carefully mapped, an estimation of the incubation period (IP) for BU can be determined using information from patients who have limited or single exposures to these areas. We aimed to estimate the IP for BU.

**Method**

A retrospective review was undertaken of 408 notifications of BU in Victoria from 2002 to 2012. Telephone interviews using a structured questionnaire and medical and notification record review were performed for all notified cases. Patients with a single visit exposure to a defined endemic area were included and the period from exposure to disease onset determined (IP). We also recorded information about timeliness of diagnosis of BU following first presentation to a doctor.

**Results**

We identified 111 of 408 patients (27%) who had a residential address outside known endemic areas, 23 (6%) of whom reported a single exposure within the previous 24 months. The median age of the 23 included patients was 30 years (range: 6 to 73) and 65% were male. The median time from symptom onset to diagnosis was 71 days (range: 34-204 days). The first medical professional consulted was a general practitioner (GP) on 87% of occasions, with BU suspected by the GP in 17% of cases. The midpoint of the reported IP range was utilized to represent a point estimate of the IP for each individual case (Figure 1). Subsequently, the mean IP for the cohort was calculated at 135 days (IQR: 109-160; CI 95%: 113.9-156), or 4.5 months. The shortest IP recorded was 32 days and longest 264 days (Figure 1). IP did not vary by sex, age, endemic area exposed, duration of exposure or location of lesion.
**Figure 1:** The IP range (X-axis) for each patient (Y-axis) is demonstrated, from shortest to longest. The midpoint (IP) for each patient is represented by the middle line of each bar.

**Conclusions**

The estimated mean IP for Buruli ulcer in southeastern Victoria is 135 days (IQR: 109-160 days). Diagnostic delay with the potential to increase morbidity and cost of treatment is common when patients present to doctors outside endemic areas.
Understanding transmission of *Mycobacterium ulcerans* in mammals and mosquitos

*Poster presenter: Renee Marcsisin*

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It is not known how *M. ulcerans* is transmitted from the environment to humans. An epidemic of Buruli ulcer affecting Point Lonsdale and other small towns on the Bellarine Peninsula near Melbourne has allowed our team to make some significant breakthroughs regarding transmission. We have shown that possums and rodents are reservoirs of the bacterium, harboring high concentrations of *M. ulcerans* specifically in their intestines and shedding the bacteria in their feces. We have also shown that a significant proportion of mosquitoes in Point Lonsdale harbor *M. ulcerans*. These findings raise the possibility that *M. ulcerans* is a zoonosis that is spread from animal reservoirs to humans via biting insects such as mosquitoes. We have designed a laboratory-based experimental program to formally test this new model of Buruli ulcer transmission. This research will fulfill the Barnett Criteria required to demonstrate insect vectoring of a human disease and so provide the definitive evidence needed to control the spread of Buruli ulcer.
Arsenic in water and soil: A possible contributory factor to *M. ulcerans* infections in Buruli ulcer endemic communities in the Amansie West District of Ghana

*Abstract: Samuel Fosu Gyasi*

Samuel Fosu Gyasi, Esi Awuah, John Asiedu Larbi and George Asumeng Koffuor

Buruli Ulcer (BU) is assuming public health importance in Ghana, prompting research into possible ways by which the disease can be managed. A longitudinal study was conducted to determine the level of arsenic, pH, total dissolved solids as well as the conductivity of some selected communities in the Buruli Ulcer endemic communities in the Amansie West District of Ashanti Region, Ghana over a period of 24 months. Results from the study showed revealed that, mean arsenic concentration for the streams in the entire sampling community was (0.6325 mgL\(^{-1}\); Range [0.01-1.458]). This figure exceeded both the World Health Organization (WHO) and Ghana Environmental Protection Agency (GEPA) recommendations for arsenic in drinking water (0.01mgL\(^{-1}\)). When the levels of arsenic were analysed based on endemicity, the study showed that arsenic concentration of streams in BU endemic communities were non significantly higher (0.6774 ± 0.1293 mgL\(^{-1}\)) compared to their non-endemic counterparts (0.5877 ± 0.1183 mgL\(^{-1}\)). Both however exceeded the WHO recommendation for drinking water (0.01mgL\(^{-1}\)). The results further showed the influence of seasonal variation with arsenic levels in all the streams. Mean arsenic levels in the streams during the dry sampling periods over the period of study were generally high in all the communities compared to the wet sampling and these were all significant \((p< 0.05)\). Results of the study revealed that, during periods of dry sampling, streams in all the three endemic communities had mean arsenic levels greater than 1.0 mgL\(^{-1}\) (1.072, 1.119 and 1.322 mgL\(^{-1}\) ) far exceeding the WHO recommendations for drinking water of 0.01 mgL\(^{-1}\) by over 100 \(^\circ\) folds. Results from this study has confirmed that, Amansie West District is generally polluted with arsenic and these levels were high during the dry periods.
Diagnostics
**Insertion Element - SNP typing provides insights into the population structure and evolution of the genetically monomorphic pathogen *Mycobacterium ulcerans* in Africa.**

**Presenter: Koen Vandelannoote**

Koen Vandelannoote\(^1,2\), Kurt Jordaens\(^2,3\), Pieter Bomans\(^1\), Herwig Leirs\(^2\), Lies Durnez\(^1\), Dissou Affolabi\(^4\), Ghislain Sopoh\(^5\), Julia Aguiar\(^6\), Delphin Phanza\(^7\), Kibadi Kapay\(^8\), Sara Eyangoh\(^9\), Louis Bayonne Manou\(^10\), Richard Odame Phillips\(^11\), Anthony Ablordey\(^12\), Leen Rigouts\(^1\), Françoise Portaels\(^1\), Bouke de Jong\(^1\), and Miriam Eddyani\(^1\)

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*Mycobacterium ulcerans* is an unusual bacterial pathogen with elusive origins. The highly clonal nature of *M. ulcerans* has until today complicated molecular analyses on the population structure and the evolutionary history of the pathogen, as typing methods with sufficient resolution are lacking. More recent typing methods for *M. ulcerans* and other genetically monomorphic pathogens therefore focus on single nucleotide polymorphisms (SNPs).

In an effort to gain fundamental insights in the population structure and evolutionary history of African *M. ulcerans* we redesigned a SNP typing technique (Käser et al. 2009) to investigate a comprehensive panel of *M. ulcerans* isolates originating from most known African disease foci. These isolates were selected based on diversity in space and time from our reference collection of *M. ulcerans* isolates. As our collection of African *M. ulcerans* isolates did not represent all endemic countries and their different regions to the same extent, we also fine-tuned the technique for application directly on clinical specimens.

Within our panel consisting of 158 *M. ulcerans* isolates and 14 clinical samples, a total of 75 variable nucleotide positions were found that clustered the panel into 23 different African ISE-SNP types, the largest number of distinct *M. ulcerans* genotypes identified to date, save for genotyping methods relying on whole genome sequencing. After associating these ISE-SNP types to available geo-epidemiological data, we were able to track the epidemic spread of particular strains on a continental scale. The various ISE-SNP types were unevenly distributed over the different greater African hydrological drainage basins. It appears that geographic barriers (formed up by elevated regions and salt water) bordering these hydrological basins, separated an ancestral genotype into discontinuous parts by the formation of a physical barrier to gene flow.

Our data suggests this resulted in differentiation by the slow accumulation of point mutational changes of the original founder clone into different closely related variants distributed over the various basins. BU infections in these areas thus appear to result from locally confined transmission of a single circulating clone, with only occasional transfer of clones between basins.
Phylogenetic analysis furthermore differentiated two well supported clades within the African ISE-SNP types: a “pan-African” and a Gabonese/Cameroonian clade. The ISE-SNP types from the pan-African clade are found widespread throughout Africa while the ISE-SNP types of the Gabonese/Cameroonian clade are much rarer and found in a more restricted area, which suggests that the latter clade evolved more recently.
Development of an antigen capture assay for *Mycobacterium ulcerans*

**Presenter: Katharina Röltgen**

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A specific and sensitive diagnostic test for Buruli ulcer, which can be performed without specialized equipment and experienced personnel, could greatly support the diagnosis of *Mycobacterium ulcerans* infection in the field. However, the development of point-of-care assays for mycobacterial diseases has turned out to be a major challenge. Direct *M. ulcerans* antigen detection within BU lesions may represent a suitable approach, when developed into an appropriate test format.

For this purpose, we generated high affinity antibodies against a set of highly abundant *M. ulcerans* protein antigens not present in *M. tuberculosis* and evaluated their suitability as key reagents in ELISA-based antigen capture assays. Preliminary analyses of clinical swab specimens revealed for one of the tests a sensitivity comparable to microscopic detection of acid fast bacilli (AFB) in smears from clinical specimens. In order to increase this initial sensitivity rate, we are currently testing and optimizing the performance of the developed immunological reagents in other assay formats.

This study is supported by the Stop Buruli Initiative funded by the UBS-Optimus Foundation.
Fluorescent thin layer chromatography revisited: Absolute ethanol preserves mycolactone in mouse tissue

Presenter: Paul Converse

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Background

Laboratory confirmation of the diagnosis of Buruli ulcer can be made by acid-fast (AFB) smear microscopy, specimen culture on mycobacterial growth media, polymerase chain reaction (PCR), and/or histopathology. All have their drawbacks, including: non-specificity, prolonged culture at 32°C, relatively sophisticated laboratory facilities, and expertise, respectively. Mycobacterium ulcerans produces a unique toxin, mycolactone (ML) that can be detected by thin layer chromatography (TLC) or mass spectrometric analysis. While the latter requires sophisticated equipment and facilities, the former is relatively simple but can be complicated by the presence of other lipids in the specimen. A method using a boronate-assisted fluorogenic chemosensor in TLC can overcome this challenge by selectively detecting ML when visualized with UV light. However, the method has had, at best, limited success in experimental and clinical tissue samples. We describe here simple changes to the procedure that could make the detection of ML consistently possible under field and central lab conditions.

Methods

Mice were infected with M. ulcerans in the right hind footpad. Footpads were harvested weekly into screw cap containers and shipped from Baltimore to Cambridge for fluorescent TLC (F-TLC) analysis. In a first set of convenience samples, the mouse tissues were shipped at ambient temperature “dry” but subsequent specimens were shipped in absolute ethanol to inhibit potential tissue esterases that could destroy mycolactone A/B. In a second experiment, all samples (N=3 per time point) were sent in absolute ethanol. In this experiment, the presence of ML was also evaluated at different times after the initiation of antibiotic treatment of mice with either the combination of rifampin (RIF, 10 mg/kg) and streptomycin (STR, 150 mg/kg) or RIF and clarithromycin (CLR, 100 mg/kg). Additional mice (N=5 per time point) were evaluated for M. ulcerans CFU.

Results

In the first set of mouse footpads, ML was undetectable in samples sent for analysis “dry”. However, when swollen footpads (N=2 per time point) were immediately placed in absolute EtOH, ML was detectable when subjected to F-TLC analysis and increased with greater swelling. A footpad with advanced swelling sent “dry” had no detectable ML. In the second set of experiments, ML was undetectable until 4 weeks after infection and just before swelling was apparent. ML concentrations increased with increased swelling. Treatment with RIF-STR, and also with RIF-CLR, for one week, but not after one or three days, resulted in a reduction of ML concentration. Results of CFU analysis and for ML after longer treatment will be presented. In addition, swollen footpads kept in absolute EtOH for up to one month showed no loss of detectable ML by F-TLC. All contralateral uninfected footpads were negative for ML.
Discussion

The results of the current experiments indicate that F-TLC for ML has high potential as a confirmatory diagnostic procedure for *M. ulcerans* infection. Indeed, detectability in “sub-clinical” mouse footpads suggests that the procedure may permit diagnosis and treatment of patients with lesions of doubtful etiology before the onset of large nodules or plaques and frank ulcers. Mouse footpad lesions are relatively circumscribed. It will be important to determine which parts of large human lesions (e.g., center, undermined edges, etc.) have the greatest likelihood of containing detectable ML. The preservation of ML in absolute EtOH indicates that the technique could initially be performed in central laboratories in Buruli ulcer endemic countries and then, with increasing familiarity and expertise, be carried out closer to the clinics themselves.
Assessment of PCR inhibitors and comparison of Real time versus conventional PCR in samples for Buruli ulcer diagnosis in Benin

Poster presenter: Dissou Affolabi

Dissou Affolabi, Koen Vandelannoote, Nadege Adè, Frank Faïhun, Aroga Elegbe, Ghislain Sopoh, Yves Barogui, Didier Agossadou, Miriam Eddyani, Bouke de Jong, Séverin Anagonou

Background

Among confirmatory tests available for Buruli ulcer (BU) diagnosis, PCR methods show excellent sensitivity and specificity. Conventional PCR (C-PCR) has been widely used; however compared to real time quantitative PCR (qPCR), C-PCR lacks sensitivity. So far, implementation of qPCR in low resource countries has been limited by the high cost of the equipment and reagents used. Recently, this equipment has been miniaturized, leading to the decrease of the cost of the test and allowing its implementation in the National Reference Laboratory for Mycobacteria in Cotonou, Benin. In addition, false negative PCR results can occur due to various inhibiting substances present in the DNA extract with either C-PCR or qPCR, but the actual prevalence of this phenomenon is not known in our setting.

Objectives

1. To compare the performances of qPCR versus C-PCR in BU diagnosis in Benin. 2. To assess the probability of false negative PCR results due to PCR inhibitors in DNA extracts from skin samples of BU suspects in Benin

Methods

Swabs, tissues, liquids from fine needle aspiration received from BU suspected cases in Allada, Lalo and Zagnanado, were subjected to C-PCR and qPCR after DNA extraction using the Maxwell® 16 Tissue DNA purification kit (Promega®). The presence of PCR inhibitors in each sample was also systematically assessed using the qPCR.

Results

In total, 367 samples were analyzed. Among them, 310 gave concordant and 57 discordant results: 54 samples were found positive with qPCR alone while only 3 samples were positive with C-PCR alone; the difference was statistically significant (p < 0.01). Samples found positive with only qPCR contained approximately 1000 copies or less of the insertion sequence IS2404. Of the 367 samples analyzed, PCR inhibition was detected in only one sample. By adding different amounts of Mycobacterium ulcerans DNA to this sample, no DNA was detected even after up to 1000 copies of IS2404 were added. However, this sample was not able to inhibit 10 000 copies of IS2404 or more. Thus, the probability of false negative PCR results due to inhibitors in skin samples in Benin was determined to be around 0.12%.

Conclusion

In Benin, qPCR improves BU diagnosis and the probability of false negative PCR due to inhibitory substances is low using Maxwell® 16 Tissue DNA purification kit.
Rapid Diagnostic of childhood tuberculosis using blood and urine by Single tube Nested-PCR

Poster presenter: Juliana Costa-Lima

COSTA-LIMA, JFC1,2; CARVALHO, MSZMG2; SANTOS, FCF2; GUEDES, GMR3; LIRA, LAS2; LIMA, AS2; MONTENEGRO, LML2; SALAZAR, MP2; DUARTE, RS1; MELLO, FCQ1; SCHINDLER, HC2.

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Introduction

On childhood’s TB, the diagnosis is peculiar because it’s difficult to confirm bacteriologically the disease. The child is frequently paucibacillary and doesn’t have sputum in most of time. Mycobacterium tuberculosis complex is very infectious and the control of this is mainly through early diagnosis and an appropriate treatment. Gold standard have low sensitivity (Se), is nonspecific (in acid fast bacilli - AFB) and it takes at least 4 weeks for an accurate result. Molecular techniques are being proposed as an auxiliary tool in the detection of Koch’s bacillus direct from clinical specimens. The PCR have a high Se and specificity (Sp), in addition to rapidity in obtaining the results.

Methods

We collect peripheral blood (4mL) with EDTA (an specific anticoagulant) and a pool of 3 samples of urine (10mL/day) from each patient. To extract the DNA from all samples, we used the commercial kit Qiagen Midi kit. Then, we performed the single tube Nested-PCR (SNTPCR) in house, which is composed for 15 (1st PCR) + 45 cycles (2nd PCR), using 2 sets of primers, an inner (TJ5 and TJ3) and another outer (OL15 and STAN3). The amplification products were visualized under UV light after the electrophoresis in 1.5% agarose gel. The used gold standard was a set of clinical symptoms, epidemiology, laboratory findings and specific therapeutic response. Statistical analyses were done at SPSS 13.3, and the conclusions were taken for 5% of significance.

Results

We used 53 children (54.2% of female sex), with the mean age 7.78 ± 4.65 (0-15 years old). Patients came from infirmary (59.3%) and outpatients of public hospitals of Recife-PE (Northeast of Brazil). The performance of STNPCR in blood was: the Se = 50.0% (CI = 31.1% – 68.9%), and the Sp = 92.0% (72.5% – 98.6%), the positive predict value (PPV) = 87.5% (60.4% – 97.8%) and negative predictive value (NPV) = 62.2% (44.8% – 77.1%). In urine, the performance of the PCR system was: Se = 34.6% (17.9% - 55.6%), Sp = 96.2% (78.4% - 99.8%), PPV = 90.0% (54.1% - 99.5%) and NPV = 59.5% (43.3% - 74.0%). When we calculate the performance of STNPCR putting together the blood and the urine, the values were: Se = 62.1% (42.4% – 78.7%), Sp = 88.5% (68.7% – 97.0%), PPV = 85.7% (62.6% – 96.2%) and NPV = 67.6% (49.4% – 82.0%).
Conclusions
The performance of STNPCR for diagnosis of childhood TB proved to be efficient and faster than the culture, more specific than bacilloscopy and can use samples minimally invasive. Mainly in children, the use of urine and blood is very useful because it has an outpatient collects (without need for hospitalization). The PCR technique could indicate a recent infection or the disease in development, for both, the clinic is still sovereign. The STNPCR presents a high PPV and Se, this system of PCR consists in a powerful tool to early confirm the M. tuberculosis, using blood or urine. Based on the literature and in our results, we can conclude that the use of more than one clinical specimen of the same patient, it increases and improves the accuracy and performance of STNPCR.

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Molecular typing of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer in Côte d’Ivoire

*Poster presenter: Solange Kakou-Ngazoa*

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**Rationale**

*Mycobacterium ulcerans* is an environmental mycobacterium that causes Buruli ulcer (BU), a skin disease with a major socioeconomic impact on the populations it affects. Côte d’Ivoire is the hardest-hit country in Africa, where BU is a significant public-health problem.

The mode of transmission and the environmental reservoir of the disease are still poorly understood; exposure to certain aquatic ecosystems is thought to increase the risk of transmission of the disease to humans. The geographical diversity of the strains of *M. ulcerans* has been demonstrated by the use of the gene 16S rRNA to classify Mycobacteria. VNTR (Variable Number of Tandem Repeats) genetic markers have recently been investigated as a method of molecular typing of human and environmental strains of *Mycobacterium*.

**Objective**

The aim of this study is to identify the genetic profile through molecular typing of human strains of *M. ulcerans* in Côte d’Ivoire.

**Equipment and method**

21 clinical specimens from endemic areas provided by the National Buruli Ulcer Control Programme were investigated. Genomic DNA was extracted according to the phenol/chloroform protocol. Using the PCR technique, the *MIRU1, ST1, Locus 6* and *VNTR19* markers were selected as the most commonly utilized. The DNA of 8 reference strains was used for validation purposes and as positive controls.

The real-time PCR method was used to detect the target IS2404, whereas the *MIRU1, ST1, Locus 6 and Locus 19* targets were detected by conventional PCR in order to identify the genetic profiles.

**Results**

The *IS2404* target was detected by real-time PCR in all the specimens. A total of 10 specimens (47%) simultaneously amplified all the markers, presenting the profile common to African strains (3,1,2,2) and (1,1,2,2) and the marker *ST1* was the most amplified at 71% (15/21). Isolated strains from patients presented the profile *VNTR C and D* of *M. ulcerans* from West Africa.

**Keywords:** ulcer, Buruli, *Mycobacterium*, Côte d’Ivoire, locus, typing
Loop Mediated Isothermal Amplification (LAMP) test; towards development of field diagnostic test for Buruli ulcer disease.

Poster presenter: Anthony Ablordey

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Background

Buruli ulcer (BU) caused by Mycobacterium ulcerans (M. ulcerans) is as an important public health problem in several rural communities in sub-Saharan Africa (1). Currently the preferred strategy for the control of the BU is to detect the disease at the early stages and initiate treatment promptly. This reduces or prevents the disfiguring complications that often result when the disease is left to progress to advanced stages (2). One of the primary obstacles to the realization of this strategy is the absence of a diagnostic test that is sensitive, rapid and simple to perform, yet appropriate for use in the low resource settings where the disease is most prevalent. In order to develop such a test, we explored the use of isothermal DNA amplification on crude and purified sample preparations and a colorimetric detection assay for the detection of M. ulcerans in fine needle aspirates and swabs taken from early BU lesions.

Method

We developed a set of six primers to isothermally amplify the multi copy M. ulcerans target IS2404. A disposable pocket warmer was used to generate a constant temperature for amplification. We described this method as Pocket warmer Loop Mediated Isothermal Amplification (pw LAMP) (3). We compared the pwLAMP to the conventional LAMP (using a heat block as heat source) and IS2404 PCR a reference assay for the detection of M. ulcerans. The effect of purified and crude DNA preparations on the detection rate of the LAMP assays were also investigated and compared with that of IS2404 PCR. Thirty clinical specimens from suspected BU cases were examined by LAMP and IS2404 PCR.

Results

The lower detection limit of both LAMP methods at 60°C was 300 copies of IS2404 and 30 copies of IS2404 for the conventional LAMP at 65°C. (The performance of pwLAMP at 65°C was not investigated). When purified DNA extracts were used, both the conventional LAMP and IS2404 PCR concordantly detected 21 positive cases, while the pw LAMP detected 19 cases. Nine of 30 samples were positive by both the LAMP assays as well as IS2404 PCR when crude extracts of clinical specimens were used.
Conclusion

The LAMP method performed on purified extracts can be used as a simple and rapid test for the detection of *M. ulcerans* in clinical specimens and has potential to be developed as a point of care test for Buruli ulcer. However obtaining purified DNA extracts as well as maintaining optimal isothermal conditions under field conditions are two important challenges to be addressed in order to further develop the pwLAMP assay for use as point of care test.

References


Evaluation by ultrasonography of the real extension of the necrosis in Buruli ulcer lesions.

*Poster presenter: Elisa Zavattaro*

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Buruli ulcer (BU) is a chronic necrotizing and highly destroying skin disease, usually with painless onset. It affects primarily subcutaneous adipose tissue and is caused by *Mycobacterium ulcerans*, which produces its pathologic effects through mycolactone.

The standard treatment is represented by a combination of 2 active antibiotics that must be administered for long periods. In severe cases and in those not treated precociously, surgical debridement might be required.

The efficacy of therapy is sometimes difficult to evaluate, since that the apparent worsening of lesions observed in some cases during the treatment does not always mean the persistence of active infection. In such cases, even in presence of an enlargement of the ulcer, probably due to immunologic reaction, laboratory tests (Ziehl-Neelsen, culture and PCR) might result negative.

We have previously reported the ultrasonographic patterns observed in different lesions provoked by *M. ulcerans* infection, corresponding to the presence of oedema, adiponecrosis and fibrotic changes.

In the present work, we investigated by Ultrasonography (US) the features of Buruli ulcer lesions, mainly in their periphery, and compared them with histological examination of the same area. Our aim was to better define the exact size of the surgical debridment to be done, in order to eliminate the necrotic tissues, shortening healing time and ensuring the success of grafting.

We report the results of our preliminary study.
Pathogenesis and host response
Mycolactone activation of Wiskott-Aldrich syndrome proteins underpins Buruli ulcer formation

Presenter: Laure Guenin-Macé

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Mycolactone is a diffusible lipid secreted by the human pathogen Mycobacterium ulcerans, which induces the formation of open skin lesions referred to as Buruli ulcers. Here, we show that mycolactone operates by hijacking the Wiskott-Aldrich syndrome protein (WASp) family of actin-nucleating factors. By disrupting WASp auto-inhibition, mycolactone leads to uncontrolled activation of Arp2/3-mediated assembly of actin in the cytoplasm. In epithelial cells, mycolactone-induced stimulation of Arp2/3 concentrated in the perinuclear region, resulting in defective cell adhesion and directional migration. Consistently in vivo, injection of mycolactone into mouse ear altered the junctional organization and stratification of keratinocytes, leading to thinning then rupture of the epidermis. This degradation process was efficiently suppressed by co-administration of the N-WASp inhibitor wiskostatin. Our study therefore elucidates the molecular basis of mycolactone activity, and provides a mechanism for Buruli ulcer pathogenesis. As it identifies the mycolactone binding domain in WASp, it opens avenues for inhibitor design.
Further insights into the mechanism of innate immune response suppression by mycolactone

Presenter: Rachel Simmonds

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Infection with Mycobacterium ulcerans is characterised by a profound inhibition of both innate and acquired immune responses. This is attributable to the production of the cytotoxic polyketide macrolide, mycolactone. In previous studies we showed that, in monocytes, sub-toxic levels of mycolactone inhibited the LPS-dependent production of proinflammatory mediators including TNFα, IL6 and COX2 and that this occurred by a post-transcriptional mechanism. We have now shown that these mediators are similarly inhibited in primary human macrophages and in the murine macrophage cell line, RAW264.7. In all cases, the data supported a hypothesis that that mycolactone inhibits the translation of these mRNAs. In order to investigate this, we have now carried out detailed translation analysis using a technique called polysome profiling, which separates out actively- vs. non-translating mRNA fractions. We compared the profiles between cells treated or not with mycolactone prior to LPS stimulation of the cells. Unexpectedly, northern blotting for TNFα, IL6 and COX-2 mRNA revealed that, despite a complete block in protein production, all three transcripts were primarily located in polysomal fractions from mycolactone exposed cells. This association was sensitive to translational disruptors (homoharringtonine and puromycin), suggesting that the mRNA is being actively translated. We present evidence supporting a new model for selective post-transcriptional regulation by mycolactone that may help explain this molecule's pleiotrophic effects.
Schwann cells are damaged by mycolactone

Presenter: Masamichi Goto

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Introduction
Painless nature of the lesion is one major character of Buruli ulcer. We have studied the pathological mechanism of this phenomenon, and revealed that local nerves are damaged by the inoculation of M. ulcerans or by the injection of mycolactone in mouse models. In both models, intraneural Schwann cells showed vacuolar degeneration.

We also tested the cytotoxic effect of mycolactone on the cultured Schwann cells in comparison with cultured fibroblasts. Schwann cells were more susceptible to mycolactone than fibroblasts, confirmed by cell counting and TUNEL studies. In order to further elucidate the mechanism of the nerve damage, we examined the expression of apoptosis-related substances in cultured Schwann cells.

Materials and Methods

Detection of apoptosis by Western blotting: SW10 mouse Schwann cells (ATCC CRL 2766) and L929 mouse fibroblast cells (ATCC CCL1) were cultured for 24 hours. Synthesized mycolactone A/B (supplied from Prof. Yoshito Kishi, Harvard University, U.S.A.) diluted to final concentration of 3 ng/ml, 30 ng/ml, 300 ng/ml was added and incubated for 12, 24, 48 and 72 hours. Cleaved caspase-3, poly ADP ribose polymerase (PARP) and phospho-histone H2A.X (H2A.X) were detected by Western blotting. For the internal control, tubulin was used.

Detection of apoptosis by fluorescence microscopy: SW10 and L929 were cultured in the slide chambers. After fixation and Triton-X treatment, by fluorescent dyes, cleaved caspase-3 was stained in red, nuclear DNA in blue, intracellular actin in green and the cells were examined under a confocal fluorescent microscope.

Results

Detection of apoptosis by Western blotting: In L929 fibroblasts, cleaved caspase-3, PARP and H2A.X were not observed. Tubulin was expressed normally. In SW10 Schwann cells, cleaved caspase-3 was expressed after 12 and 24 hours. PARP and H2A.X were not observed. After 48 and 72 hours of 30 ng/ml mycolactone treatment, tubulin expression was not observed. Detection of apoptosis by fluorescence microscopy: We compared the expression of cleaved caspase-3 after 12 and 24 hours after administration of mycolactone. Only in 12 hours after mycolactone 30 ng/ml, expression was observed in the cytoplasm of some SW10 Schwann cells, but not in L929 fibroblasts.
Discussion

In this study, we have evaluated the apoptosis-inducing property of mycolactone on Schwann cells and fibroblasts by examining apoptosis-related substances. Western blotting and immunofluorescence demonstrated that only SW10 Schwann cells at the distinct time point showed the expression of cleaved caspase-3. Caspase-3 is a key agent in apoptosis that triggers the cleavage of many important proteins such as PARP. PARP and H2A.X are independently involved in the apoptotic process, but they were not detected in both Schwann cells and fibroblasts in this study. Interestingly, down-regulation of tubulin expression was observed in Schwann cells. It is known that anticancer agent paclitaxel stabilizes tubulin and evoke apoptosis to cancer cells, thus mycolactone might have similar effects. Further studies are necessary in order to elucidate how mycolactone triggers apoptosis.
Histopathological features of Buruli ulcer lesions

Presenter: Therese Ruf

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Histopathological analysis of diseased tissue is a valuable tool to diagnose and understand human diseases. In several illnesses histopathology is part of the standard diagnostic protocol and results may impact treatment. Leprosy is one example, where the classification based on histopathological appearance supports the choice of an adjusted treatment regimen.

The histopathological knowledge of the diversity of Buruli ulcer lesions is limited, which is mainly due to the occurrence of the disease in remote rural areas with only minor health infrastructure, limited case numbers and the lack of a sufficient number of systematic analyses.

Within the framework of a number of clinical studies we have analyzed tissue samples from Buruli ulcer patients by histopathology as well as immunohistochemistry. Samples were taken either before, during or after treatment and comprised either 4 mm punch biopsies or surgical excisions. Analyses underlined the clustering and focal distribution of the mycobacteria inside the tissue, with bacteria being mostly present in the deep subcutaneous fat layers up to a depth of 1 cm and only rarely in the dermal layer of the skin. Immunohistochemical studies for sets of markers demonstrated a remarkable diversity in both the presentation of untreated lesions and in the response to treatment. In combination with clinical features, histopathology may therefore help to develop a more differentiated classification of Buruli ulcer lesions and to identify markers most suitable to monitor wound healing after successful antimycobacterial chemotherapy.
Dysregulated inflammation and lipid metabolism in Buruli ulcer patients

Presenter: Fatoumata Niang

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Here, we performed a multi-analyte profiling of serum proteins in Buruli ulcer disease (BUD) patients at various stages of the antibiotic treatment and healing process. Expanding the scope of previous findings, we found that BUD patients displayed a broad yet selective profile of immune suppression, marked by the down-modulation of multiple chemokines, cytokines and immunoglobulins. Interestingly, profound perturbations in transporters of blood lipids, mediators of coagulation and tissue repair were also observed. Comparison of serum harvested at the time of BUD diagnosis, or 4 weeks after completion of antibiotic therapy suggested that BUD correlates positively with pro-inflammatory protein EN-RAGE, inhibitor of coagulation Alpha-2-macroglobulin and Matrix metalloproteinase-2; and negatively with Interleukin (IL)-7, IL-13 and Tumor necrosis factor beta (TNFβ). Furthermore, a preliminary analysis of patients with differential healing responses to treatment highlighted differences in the initial levels of TNFβ, Monocyte chemotactic protein-1, Insulin and Factor VII. These results identify candidate biomarkers for BUD and reveal an association between the physiology of Buruli ulcers and the host lipid metabolism and coagulation.
Spontaneous healing in a mouse model of *M. ulcerans* infection

*Presenter: Estelle Marion*

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**Background**

Buruli ulcer, caused by *Mycobacterium ulcerans*, occurs primarily in West African countries and its incidence is steadily increasing. Without treatment, massive ulcers occur and a lengthy healing process may be observed, but resulting in deep scarring and severe functional disabilities. Administration of Rifampicin and Streptomycin for 8 weeks sterilises the lesions, but the complete healing of the wounds requires additional time and for severe ulcers surgery is often needed. Mouse models are already available to study establishment of *M. ulcerans* lesions or evaluation of therapy but a lack of a suitable animal model, mimicking all clinical stages in particular the healing process, remains an obstacle to understand the pathophysiology of *M. ulcerans* infection.

**Methodology/principal finding.**

*M. ulcerans* was subcutaneously inoculated in three consanguine mouse strains, i.e. BALB/c and C57BL/6, classically used to study mycobacterial infection, and FVB/N. Strikingly, FVB/N mice, although as sensitive as all other mouse strains with respect to *M. ulcerans* infection, presented a spontaneous healing after the ulcerative phase despite stable bacterial load in the healed tissues. The immunological and histological investigations realized at a local, regional and systemic scale showed that spontaneous healing process was accompanied by an activation of the innate immune system. Moreover, the adaptive response initiated by FVB/N mice was not involved in healing process and did not confer a protection against *M. ulcerans*.

**Conclusion/significance**

Our work highlights the importance of host innate immune responses to control *M. ulcerans* infection and a bacterial adaptation in a new environment. This *in vivo* model of *M. ulcerans* infection will be helpful to better understand critical stages of this disease, such as the characterization of the regulation of mycolactone production, a better understanding of the pathophysiology of *M. ulcerans* infection, and the development of new therapeutic strategies to improve healing process.
Early Infection of Mycobacterium ulcerans in a Guinea Pig Model

Presenter: Heather Jordan

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Despite decades of research, the transmission of M. ulcerans from the environment to humans remains an enigma. The two major hypotheses for transmission are 1) that M. ulcerans is acquired through the bite of an insect vector or 2) that bacteria enter open wounds through exposure to a contaminated environment. In previous work we reported development of a model using hairless Hartley guinea pigs to study these alternative routes of transmission. Using this model we were able to produce an infection 100% of the time when the inoculum was injected intradermally, whereas we were unable to establish an infection through application of M. ulcerans to an open abrasion. In the study reported here, we have looked at early time points in infection to see how long organisms can be detected in abrasion sites, and most importantly have included Staphylococcus aureus as a positive control for a bacterium known to infect through open wounds.

Methodology

Hairless Hartley guinea pigs were infected with M. ulcerans by injection, or by application to an open abrasion and sacrificed at 1h, 24h, 48h, 7 days and 14 days. Staphylococcus aureus was applied to abrasion sites of two guinea pigs as a positive control for inflammation and topical infection. For analysis we compared the efficiency of the transmission routes using PCR, histopathology, and culture methods. We also generated cDNA from M. ulcerans RNA isolated from guinea pig tissue from both transmission models for RT-qPCR targeting and comparing mycolactone gene expression.

Results

All abrasion sites healed within 7 days. M. ulcerans was isolated from abrasion sites at 1 hour and 24 hours post infection, but cultures from later time points were uniformly negative. M. ulcerans genome units from qPCR were detected from abrasion sites at every time-point except at the final, 14 day time-point. In contrast, Lesions were apparent at the injection site within 7 days, and all injection sites were positive when assayed via qPCR with high copy numbers of genome units. M. ulcerans were recovered from all injection sites upon culture.

Due to concerns regarding pain produced by S. aureus infection, S. aureus infections were only followed for 48 hr. Microscopic evaluation of abrasion sites infected with S. aureus revealed extensive colonization of the upper dermis where huge numbers of gram positive cocci could be seen decorating cells and the lamina propria. Gross pathology showed extensive vascularization and other signs of inflammation. In samples where M. ulcerans was introduced to an abrasion, acid-fast bacteria were rarely detected by microscopy in abrasion sites. In the single abrasion where acid-fast bacteria were encountered, they occurred as a large cluster of organisms, which did not appear to be cell associated. Early studies have shown that M. ulcerans, unlike other mycobacterial pathogens, is incapable of entering non-phagocytic cells, and does not appear to adhere to cells either in culture or during infection. These results suggest that M. ulcerans can only establish infection through injection because only through direct introduction into the skin is it possible for the bacteria to establish sufficient numbers of organisms to cause disease.
A review of *Mycobacterium ulcerans* infections in animals domiciled in south-eastern Australia

*Presenter: Carolyn O’Brien*

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Despite being recorded in people from over 30 countries world-wide, *Mycobacterium ulcerans* infection in animals has only been reported in areas of south-eastern Victoria, Australia. This is despite efforts to locate animal cases in other endemic areas of Australia and Africa. The reasons for this epidemiological discrepancy are currently unknown, but may be due to specific host or environmental factors (for example, the presence of particular insect vectors), or genetic differences in virulence or host specificity of the particular strain of *M. ulcerans* causing disease in this geographical region.

The first definitive cases of Buruli ulcer were diagnosed in people from Bairnsdale, Victoria, Australia, in the late 1930s. In the 1980s, koalas domiciled on Raymond Island (approximately 16 km southeast of Bairnsdale) were the first non-human animals to be reported with the disease, and it has since been diagnosed in a variety of wild and domestic species, including possums, dogs, alpacas, horses, a long-footed potoroo and a cat.

The study of comparative infectious diseases epidemiology can provide valuable insights into the potential environmental reservoir and routes of transmission in both people and animals. The clinical aspects of the known animal cases are presented, as well as a discussion of future avenues of research that may be able to shed more light on this enigmatic mycobacterium.
The kinetics of mycolactone in a murine model of *Mycobacterium ulcerans* infection

*Presenter: Stephen Sarfo*


**Background**

Mycolactone (ML) is a key virulent factor in the pathogenesis of *Mycobacterium ulcerans* (Mu) disease. Understanding the influence of ML on the pathogenesis of Mu infection could unravel its potential as a biomarker for diagnosis, viability of Mu and monitoring of antibiotic efficacy. We evaluated the evolution of *M. ulcerans* (Mu1615, Malaysian strain) infection in the mouse footpad model by assessing the lesion appearance and CFU number in relation to mycolactone production at different times after infection and after the onset of antibiotic therapy.

**Methods**

BALB/c mice were inoculated in the right hind footpad with ≈ $3 \times 10^5$ Mu1615 in 0.03 ml PBS. Footpads were harvested from mice for Mu culture and for ML detection at different time points after infection up to ≥ grade 3 swelling. After the onset of swelling (~ day 21-23), treatment with rifampin (R, 10 mg/kg by gavage) and streptomycin (S, 150 mg/kg by subcutaneous injection) began on day 24, 5 days/week for 8 weeks. For CFU analysis footpad tissue was harvested, minced, suspended in 2.0 ml PBS, serially diluted, and plated on Middlebrook selective 7H11 plates. Lipids from mouse footpads were extracted using organic solvents and the presence of ML detected and quantified by mass spectrometry MS and a cytotoxicity assay.

**Results**

*Footpad swelling and CFU counts before and after treatment*—Three days after infection, mice harbored $3.29 \pm 0.33 \log_{10}$ CFU in the infected footpads increasing to $5.05 \pm 0.19$ at day 20 and $6.37 \pm 0.32$ by day 55 in untreated mice. Footpad swelling first appeared in some mice 3 weeks after infection and continued to increase over the following 3 weeks (Figure 1) for an average of grade $3.42 \pm 0.43$ in untreated mice whereas CFU plateaued. In contrast, both swelling and CFU declined markedly in the RS-treated mice. Swelling after 3 weeks of treatment averaged grade $0.14 \pm 0.18$ and CFU in footpads was reduced to $0.52 \pm 0.45$. By day 63 (i.e., 39 days of treatment), the RS-treated mice were culture negative and swelling was undetectable.

*Kinetics of mycolactone before and after treatment*—In the infected right footpads, mycolactone A/B concentration measured by MS increased from 1.6 ng/ml on day 13 to a peak of 195.6 ng/ml on day 62. In antibiotic treated mice mycolactone concentration in the right footpads decreased from a peak of 23.7 ng/ml on day 35 to 1.6 ng/ml on day 62 (figure 2). The kinetics of ML measured by cytotoxicity paralleled that measured by MS but significant biological activity was detected also in the left uninfected footpads even though intact ML was not detected by MS.

**Discussion and conclusion:** We propose that RS treatment promotes healing in the host by killing *M. ulcerans* bacilli and thus blocking mycolactone production. In contrast, mycolactone concentrations, footpad swelling and cfu increases in untreated mice. These findings closely link mycolactone kinetics with the clinical and bacteriological responses to Mu infection.
Figure 1. The kinetics of right hind footpad swelling, colony forming units and mycolactone A/B concentration (measured by MS) in untreated mice infected with Mu 1615.

Figure 2. Kinetics of mycolactone A/B in right hind footpads with and without antibiotic therapy.
Mycobacterium ulcerans pathogenesis: a cell wall phenomenon

Poster presenter: Nick Tobias

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The polyketide-derived toxin, mycolactone, is important for virulence in Mycobacterium ulcerans infections. Biosynthesis of mycolactone requires three large plasmid-borne genes (\textit{mlsA1}: 51kb, \textit{mlsB}: 42kb and \textit{mlsA2}: 7kb) that encode type I polyketide synthases (PKS) and two predicted accessory genes, \textit{mup038} and \textit{mup045}. We demonstrate that the Mls PKS multienzymes and Mup045 are associated exclusively in the cell wall of \textit{M. ulcerans}. Another feature of \textit{M. ulcerans} is expression of the small heat shock protein, Hsp18. The function of Hsp18 is unknown but it also associates with the mycobacterial cell wall. We show that Hsp18 forms large multimeric complexes of ~630 kDa and is an active chaperone capable of protecting proteins from stress induced aggregation. To test the hypothesis that Hsp18 is involved in specifically stabilizing the mycolactone polyketide synthase multienzymes, Hsp18 expression in \textit{Mycobacterium ulcerans} was blocked by expression of its repressor HspR_2. Knock down of Hsp18 expression had no effect on mycolactone levels compared to wild type under these conditions. Genome sequencing of 30 \textit{M. ulcerans} isolates revealed evidence of adaptive selection acting on cell wall associated genes. Thus, the cell wall of \textit{M. ulcerans} is a focus for some of the unusual traits of this pathogen. We infer from these observations that the predicted \textit{M. ulcerans} niche environment likely involves close contact with a host organism.
Mycolactone induced cytotoxicity in the mouse footpad

Poster presenter: Yaw Amoako


Background
Mycolactone (ML) produced by Mycobacterium ulcerans (Mu) is central to the pathogenesis of Buruli ulcer and we have shown previously that it can be measured in human and mouse tissue during active infection. In the present studies we have used an assay of cytotoxicity against human embryonic fibroblasts (HELF) to compare mycolactone induced cytotoxicity in the mouse footpad after infection with Mu with that following injection with synthetic mycolactone (kindly supplied by Prof Kishi).

Methods
BALB/c mice were inoculated in the right hind footpad with ≈ 3x10^5 Mu1615 (a Malaysian strain) in 0.03 ml PBS. Footpads were harvested from mice for Mu culture and for ML detection at different time points after infection up to ≥ grade 3 swelling. After the onset of swelling (~ day 21-23), treatment with rifampin (R) 10mg/kg by gavage and streptomycin (S) 150 mg/kg by subcutaneous injection began on day 24, 5 days/week for 8 weeks. For CFU analysis footpad tissue was harvested, minced, suspended in 2.0 ml PBS, serially diluted, and plated on Middlebrook selective 7H11 plates. Lipids from mouse footpads were extracted using organic solvents and the presence of ML was quantified by a comparison with that induced by synthetic mycolactone in the HELF cell cytotoxicity assay.

Separately, mouse footpads were injected with synthetic mycolactone 5, 50 or 100 micrograms and sacrificed at 6, 24 or 96 hours. Mycolactone extracted in the acetone soluble lipid fraction was assayed in the cytotoxicity assay and by mass spectrometry (MS).

Results
Mouse footpad infection model:
In the murine infection model, there was detectable cytotoxicity in both infected (right) and uninfected (left) footpads. The cytotoxicity decreased following treatment with RS but remained persistently high in both right and left footpads of untreated mice. In spite of the observed cytotoxicity in the uninfected contralateral footpads, no intact ML was detectable by mass spectrometry.

Mouse footpads spiked with synthetic mycolactone:
There was detectable cytotoxicity in both the spiked (right) and non-spiked (left) footpads of the mice at all time points, which was significantly higher than that in ethanol injected footpads although ethanol injected footpads showed cytotoxicity that was above that in normal footpads. After 6 hours, the level of cytotoxicity was related to the dose of ML injected and there was little change at 24 and 96 hours. No intact mycolactone was detectable by mass spectrometry at any of the time points.

Conclusion
The discrepancy between the results of MS and cytotoxicity could be due to cytotoxic molecules formed during the breakdown of ML.
Biochemistry of *Mycobacterium ulcerans* in Côte d’Ivoire: Variability and Mycolactone

**Poster presenter N’Guetta Aka**

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**Context**

Buruli ulcer is a cutaneous mycobacteriosis that occurs in countries with a wet tropical climate. The pathogen is *Mycobacterium ulcerans*, a slow-growing mycobacterium (8-10 weeks on Lowenstein-Jensen medium). Skin ulcerations in the course of the disease are thought to be caused by an exotoxin secreted by *M. ulcerans*, namely mycolactone. The discovery of specific insertion sequences and genes coding for the synthesis of this toxin was the catalyst for the search for this microorganism in humans and the environment. Purified for the first time in the form of a mixture of cis and trans isomers designating the Mycolactones A and B, five distinct structural molecules have now been identified (A/B–F). The different forms would appear to be associated with the geographical origin of the producing strains of *M. ulcerans*. The A/B form was extracted from strains of African and Malaysian origin, the C form from Australian strains and the D form from strains in Asia. Côte d’Ivoire records the highest number of cases of Buruli ulcer every year (about 2000 new cases), and has approximately 30 endemic health districts. The clinical form of the disease varies between endemic regions and individual patients. Operating on the hypothesis that skin necrosis is associated with the secretion of the toxin by *M. ulcerans*, we have tried to understand the causes of this clinical and geographical variability in Côte d’Ivoire. Do all the strains circulating in Côte d’Ivoire secrete Mycolactone? If so, in what form?

The aim of this study is to describe Mycolactone on the basis of the body fluids of BU patients and the molecular typing of strains isolated from these patients.

**Patients and methods**

Treatment-naïve BU patients (IS2404+) and control patients from endemic zones were recruited between 2005 and 2012. After obtaining written consent, specimens of exudates and serum were collected from each category of subject.

The exudate specimens were cultured on Löwenstein-Jensen medium to isolate *M. ulcerans*. Molecular typing of the strains was performed, targeting three insertion sequences (IS6110, IS2606 and IS2404) and the genes involved in synthesizing Mycolactone (*kētoréductase*, *mlsA* and *mlsB*). The exudate and serum specimens were analysed by High Performance Liquid Chromatography (HPLC) and mass spectrometry to characterize the Mycolactone.
Results and conclusion.

75 patients and 5 controls were recruited in 10 regions of Côte d’Ivoire. Chromatographic analysis detected the presence of Mycolactone in 97% of the patients and its absence in the controls; most of the patients had the A/B form. 61 strains of *M. ulcerans* were isolated and stored for between one and 7 years on sterile semi-skimmed milk. Molecular typing revealed that these strains contained, respectively, IS6110 in 90% of cases, IS2404 in 80% of cases, and IS2606 in 100% of cases. However, no strain appeared to contain ketoreductase.

Buruli ulcer is a public-health problem in Côte d’Ivoire, exhibiting considerable clinical and geographical variability. The body fluids of almost all the patients surveyed revealed the presence of Mycolactone, specifically its A/B form. Molecular typing of the strains thus isolated indicated genetic variability and the total absence of ketoreductase.

**Keywords**: *M. ulcerans*; Mycolactone; Insertion sequences; Ketoreductase (KR).
The Susceptibility of Arsenic-Exposed ICR Mice to Buruli Ulcer Development

*Poster presenter: Samuel Fosu Gyasi*

Samuel Fosu Gyasi, Esi Awuah, John Asiedu Larbi, George Asumeng Koffuor, Alex Yaw Debrah, Nana Yaw Awua-Boateng & Owusu-Afriyie Osei

**Background**

Buruli Ulcer is assuming public health importance in Ghana, prompting research into possible ways by which the disease can be managed. The study aimed at investigating the susceptibility of arsenic-exposed ICR mice to the development of Buruli Ulcer.

**Methodology**

Upon continuous exposure of mice to variable concentrations of arsenic via drinking water, they were inoculated intraplantarly with approximately $15 \times 10^8$ CFU/ml (5 McFarland standard) *Mycobacterium ulcerans*. Cage-side and clinical observations were carried out daily for post-exposure inoculation clinical manifestations. Hematological and histopathological studies were also performed and observations compared with controls. Tissue from developed lesion obtained was confirmed for the presence of *Mycobacterium ulcerans* by Polymerase Chain Reaction test.

**Results**

Inoculated arsenic exposed mice developed erythema on day 25 which progressed to swelling of the footpad, foot oedema, thigh oedema and ulcer within 112 days. The onset and progression was directly related to the arsenic exposure dose. Within this period, there were no developments in the MU-only treated and the normal mice. *Mycobacterium ulcerans* positive lesions however started developing on the hind feet of this treatment group on day 122 (50 days after this manifestation had been observed in *Mycobacterium ulcerans* inoculated arsenic exposed mice). White blood cell numbers decreased significantly ($P \leq 0.01$) and dose-dependently in MU inoculated arsenic exposed mice as well as the arsenic-only treatment group. Histopathological reports revealed that inoculated arsenic exposed mice had dose-dependent liver and spleen damage after 112 days of the study similar to the *Mycobacterium ulcerans* only treatment.

**Conclusion**

Results from the study revealed that, arsenic has an immunosuppressive potential that can hastens a possible MU infection in mice.
Vaccines
Analysis of the vaccine potential of plasmid DNA encoding nine polyketide synthase domains in *M. ulcerans* infected mice.

**Presenter: Krys Huygen**

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As of now, there is no effective vaccine against Buruli ulcer. In experimental foot pad infection of C57BL/6 mice with *M. ulcerans*, a prime-boost vaccination protocol using plasmid DNA encoding mycolyltransferase Ag85A of *M. ulcerans* (MUL4987) and a homologous protein boost has shown significant protection (2). Mycolactone is an obvious candidate for a vaccine, but by virtue of its chemical structure, the polyketide is not in itself immunogenic. However, antibodies against some of the polyketide synthase domains could be detected in Buruli ulcer patients and healthy controls from the same endemic region, suggesting that these domains are indeed immunogenic (1). Here we have analyzed the vaccine potential of nine of the polyketide synthase domains using the DNA prime/protein boost strategy. C57BL/6 mice were vaccinated against ACP1, ACP2, ACP3, ATac1, ATac2, ATp, ER, KRA and KS-ext. As a control mice were vaccinated with pDNA encoding MUL4987 or *M. bovis* BCG. Strong antigen specific antibodies could be detected in response to ATp and Atac2. Strong antigen-specific Th1 type cytokine responses (IL-2 and IFN-γ) were observed in mice vaccinated against ATac2, ER, KRA, KS-ext and particularly against ATp (acyltransferase with propionate specificity). Finally, mice vaccinated with ATp were protected to some extent against challenge with virulent *M. ulcerans* 1615, administered 6 weeks after the protein boost. Protection was however not as strong as conferred by vaccination with MUL4987 or *M. bovis* BCG.

These results show that targeting the polyketide synthase domains may be a new approach in the development of a Buruli ulcer vaccine.


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Subunit – vaccine development for Buruli Ulcer

Presenter: Miriam Bolz

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Until now no effective vaccine is available for Buruli ulcer although it could play an important role in prevention and control of M. ulcerans infections. Within the framework of a collaborative project funded by the European Commission (BuruliVac), our goal was to assess the potential to develop a protein based subunit vaccine. First steps included a multi-dimensional antigen selection process that led to the selection of a panel of potential protective target antigens. In a next step, antigens were incorporated into different vaccine formulations potentially stimulating different arms of the immune system. Mice were immunized and robust immune responses were observed in ELISA and Western blotting analyses done with the recombinant target proteins and M. ulcerans lysates. The most promising candidates are currently tested for their protective potential in a murine M. ulcerans infection model, where mice are immunized and subsequently infected in the left hind foot pad. The course of the infection is followed by measuring foot pad swelling with a calliper and the M.ulcerans load in the foot pads is determined by quantitative PCR and cfu plating. Local immune responses and histopathology are analysed by microscopic examination of histological sections. First results indicate that partial protection is achieved with some of the formulations tested.
Treatments
Effect of hydrated, antibacterial clay minerals on *Mycobacterium ulcerans* *in vitro* and *in vivo* growth

**Presenter: Sarojini Adusumilli**

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Buruli ulcer, caused by *Mycobacterium ulcerans*, begins as a localized skin lesion that can progress to extensive ulceration and necrosis if left untreated. Documented use of two clay minerals as a therapeutic treatment for Buruli ulcer suggests that specific clay mineral products have significant beneficial effects on wound healing. We have previously demonstrated that one of two mineral products used to heal Buruli ulcer infections has antibacterial activity against a broad-spectrum of pathogenic bacteria, suggesting the potential for therapeutic applications of minerals against topical infections. To determine if different hydrated clay minerals exhibit antimycobacterial activity, we tested different clay samples for their effect on *M. ulcerans* growth *in vitro*. *M. ulcerans* 1615 was incubated with 10% suspensions of CB07, CB08, CB09, CB10, and BY07 clay minerals, and survival was determined after 7, 14, 21, and 28 days of exposure. While all clay minerals demonstrated bactericidal activity against *M. ulcerans*, the CB07, CB08, and CB09 clays exhibited complete bactericidal activity at all time points. Filtered suspensions (leachates) of the clay minerals negatively affected *M. ulcerans* growth *in vitro*, but did not exhibit bactericidal activity. To determine if mycolactone-mediated cytotoxicity is affected by clay leachate, we exposed WM115 human epithelial cells to mycolactone in the presence or absence of clay mineral leachate. Preliminary results indicated that mycolactone-mediated cytotoxicity is decreased in the presence of clay leachate. To examine the effect of topical, hydrated clay therapy on ulcer progression *in vivo*, tails of Balb/c (4-6 week old) mice were subcutaneously infected with *M. ulcerans* 1615. After 36 days post infection, mice infected with $10^2$ and $10^3$ *M. ulcerans* CFU developed tail ulcers. Ulcerated areas on mice tails are currently being treated with hydrated clay poultices to assess wound healing and therapeutic benefits. Our *in vitro* data indicates that hydrated clay minerals are bactericidal to *M. ulcerans* and suggests that topical application of hydrated clay poultices may be effective in treating Buruli ulcer *in vivo*.
Dexamethasone-induced immunosuppression does not compromise the efficacy of rifampicin and streptomycin treatment against *Mycobacterium ulcerans* mouse infection

**Presenter: Teresa Martins**

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# Deceased

Buruli ulcer (BU) is a necrotizing disease of the skin, subcutaneous tissue and bone, caused by *Mycobacterium ulcerans*. The pathogenesis of BU is associated with mycolactone, a lipidic toxin with immunosuppressive/cytotoxic properties produced by *M. ulcerans*. Treatment of BU consists of an antibiotic combination of rifampicin and streptomycin (RS). Recent findings describe a waning of the immunosuppressive state in the lesions during the RS regimen, with an increase of inflammatory infiltrations and phagocytosis of bacilli. Although these results point to a synergism between the host immune response and the RS antimicrobial activity, there are also clinical reports on the worsening of lesions during or after RS treatment, probably due to immunopathological mechanisms, the so-called paradoxical reactions.

According to these data on the literature, we were interested in studying the participation of the host immunity on the efficacy of the RS treatment against *M. ulcerans* infection and its implications for the possible management of paradoxical reactions through immunomodulation. For that, we used the mouse model of footpad infection and treatment with RS and followed the dynamics of bacterial burdens and immune response at the site of infection and draining lymph node (DLN). Moreover, we also induced immunosuppression on RS treated mice by the administration of dexamethasone (DEX). During RS antibiotherapy, we observed a rapid elimination of viable *M. ulcerans* organisms, associated with a shift in the cellular infiltrates, from a predominantly neutrophilic/macrophagic to a lymphocytic/macrophagic profile, with macrophage activation. We additionally showed that RS prevents the DLN destruction and depletion of lymphocytes. On the other hand, the inflammatory response in the footpad persisted for a long time after the end of the RS regimen, associated with the presence of non-culturable bacteria. When mice subjected to antibiotherapy were administered with DEX, they still cleared the infection despite a downregulation of the local inflammatory response. In addition, the *M. ulcerans* infection did not reactivate after an additional 3 months of DEX administration following the end of RS.

Overall, our results suggest that although the RS treatment is accompanied by an upregulation of the cellular immune response to *M. ulcerans*, immunosuppression induced by corticosteroids does not ultimately compromise the RS efficacy. These observations support the clinical investigation on the use of corticosteroids or other immunosuppressive/anti-inflammatory drugs for the management of paradoxical reactions in BU patients.
Clinical, hematological and histopathological responses to arsenic toxicity in ICR mice using arsenic levels synonymous to Buruli ulcer endemic communities in the Amansie West District of Ghana

Presenter: Samuel Fosu Gyasi

Samuel Fosu Gyasi, Esi Awuah, John A. Larbi, George Asumeng Koffuor and Owusu-Afriyie Osei

Buruli Ulcer, an ulcerative disease of the skin, subcutaneous tissue and sometimes with the involvement of the bone is caused by *Mycobacterium ulcerans*. Exposure to arsenic in an endemic area has been predicted in recent times as a possible risk factor for the disease. Arsenic poisoning has become one of the major environmental concerns in the world as millions of people have been exposed to excessive arsenic through contaminated drinking water. In this study, ICR mice were introduced to 0.8-4.8 mgL\(^{-1}\) arsenic, synonymous to arsenic detected from streams and soils in Buruli Ulcer endemic communities of the Amansie West District of the Ashanti Region of Ghana, via their drinking water for 52 days. Mice with arsenic exposure of 4.0-4.8 mgL\(^{-1}\) developed inflammation, erythema and open ulcers on skin (with scab formation). The scabs upon examination were negative for acid fast bacilli. Histopathological studies revealed liver and spleen damage. There was hepatic cell swelling with the loosening of cell wall and degenerative change with cells showing cytoplasmic vacoulation as well as nuclear blebbing (i.e. Gradual process of cell loss). The spleen developed a lymphoid background with multinucleate cells formation. Hematological examination revealed significant dose-dependent decrements in white blood cells indicating a detrimental effect on the body’s immune system, a situation which makes the body susceptible to infections including *M. ulcerans*. The mean corpuscular volume of red blood cells also decreased significantly indicating microcytosis. Results from the study has shown that, high levels of arsenic in tissue (possibly from accumulation) cause inflammation, erythema and open ulcers on the skin, and has the potential to cause liver and spleen damage, reduced immune system function, and red blood cell microcytosis in ICR mice.
Herbal Remedies used by BU Patients in the Eastern Region of Ghana

Poster presenter: Linda Seefeld

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Aim of the Study

Buruli ulcer (BU), caused by an infection with Mycobacterium ulcerans, is a debilitating disease of the skin and underlying tissue, which occurs in tropical countries. BU affected patients frequently use locally prepared herbal preparations to treat (pre-ulcerative stages (nodules, plaques or oedemas) of the disease and report to specialized treatment centres only at advanced stages (ulcer) to receive the WHO recommended antibiotic treatment regimen (streptomycin and Rifampicin). The objectives of this study were to collect and document information on the above-mentioned herbal remedies that are traditionally used to treat BU in the Eastern Region of Ghana.

Methods

A mixed methods study was done within one BU endemic sub district of the Eastern Region. The research started with 33 expert interviews (patients, health personnel, community based volunteers, herbalists, teachers and scientists) to explore patients’ behaviour. After that an active case search was done in 48 rural communities and a quantitative Knowledge Attitude Practice (KAP) questionnaire was administered to 244 study participants (122 (formerly) BU affected people as well as 122 unaffected and matched community members). Plants, which were reported and presented by study participants, were documented (photographical documentation and collection of herbarium samples) and then identified and classified by a botanist of the Centre for Scientific Research into Plant Medicine, Mampong-Akuapem.

Results

According to the data of the KAP survey 43.4% of the study participants used herbal treatment for BU suspected nodules. It was reported that a mixture of different herbs was applied with the main aim to burst the nodule or to remove the necrotic tissue ("cotton wool").

28.7% of the survey participants explained that they used herbs for the treatment of their ulcers. Four different techniques were reported: (1) Herbs were ground and applied on the ulcer, (2) herbs were applied on the sides of the ulcer, (3) specific leaves were boiled and spread over the ulcer or (4) herbs were cooked as an infusion to clean and heal the lesion.

In addition to the in-situ-therapy a herbalist explained an oral therapy in the form of bitters mixed with different herbs to supplement the above-mentioned regimens.

Altogether the interviewees presented 28 different plants/ preparations from the immediate vicinity of their homes or the local market, which they used to treat BU symptoms. One plant was reported to be used to differentiate between BU nodules and ordinary "boils".
Discussion and conclusions

The people used an array of different herbs to treat BU within their natural environment and there seems to be no consensus about their effectiveness across the communities. Further research into herbal treatment (specific techniques and plants) and its effectiveness is needed.