



**WHO ANNUAL MEETING
ON BURULI ULCER**

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ABSTRACTS

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Buruli ulcer in Australia, 2009

Presenter: Professor Paul Johnson

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During 2009 there were 35 new human cases of Buruli ulcer (BU) notified to the WHO Collaborating Centre for *Mycobacterium ulcerans* in Melbourne, compared with 40 cases in 2008 (table 1). In 2009, 29/35 cases (83%) occurred in Victoria, the remaining 6 were from northern Queensland (figure 1).

In Victoria, 20/29 (69%) were exposed in the Bellarine Peninsula towns of Point Lonsdale, Barwon Heads, Queenscliff, St Leonards and Indented Head. There were also 5 cases linked to the Mornington Peninsula, a previously endemic area that appears to be becoming active again.

For all Australian cases, ages ranged from 3-89 years, 19 were males and 16 were females (figure 2).

Two recurrent human cases were also recorded for 2009 (defined as a new lesion occurring in a patient recorded in a previous report). Both had positive PCRs for *M. ulcerans* but negative cultures, suggesting both were “paradoxical reactions” rather than true recurrences.

There were also 10 cases of laboratory confirmed BU in animals, mainly from the Bellarine peninsula (7 possums and one dog). Another possum was from Phillip Island, and a koala with a large ulcerative lesion was identified from Raymond Island, near Bairnsdale. Most animal cases were identified through active case finding and it is likely that Buruli ulcer in animals is more common than previously suspected.

Table. Laboratory Confirmed Human Cases of BU in Australia – 2004 to 2009

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

Figure 1. Location of laboratory confirmed human cases of BU, Australia, 2009

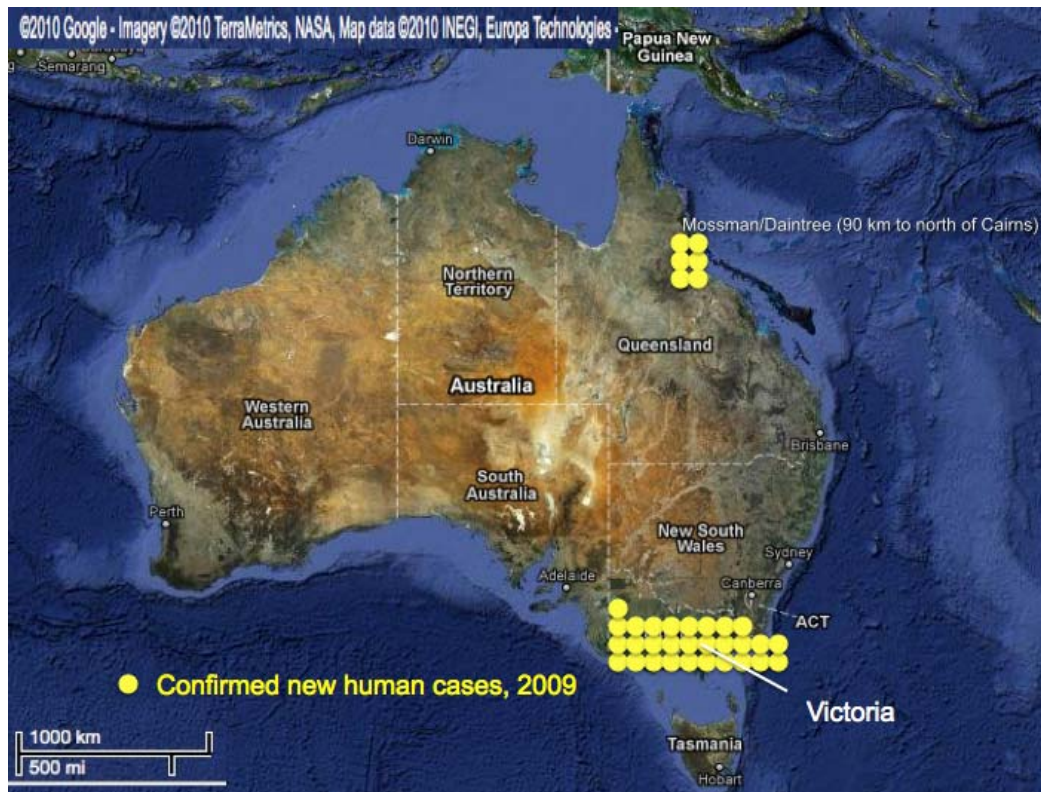
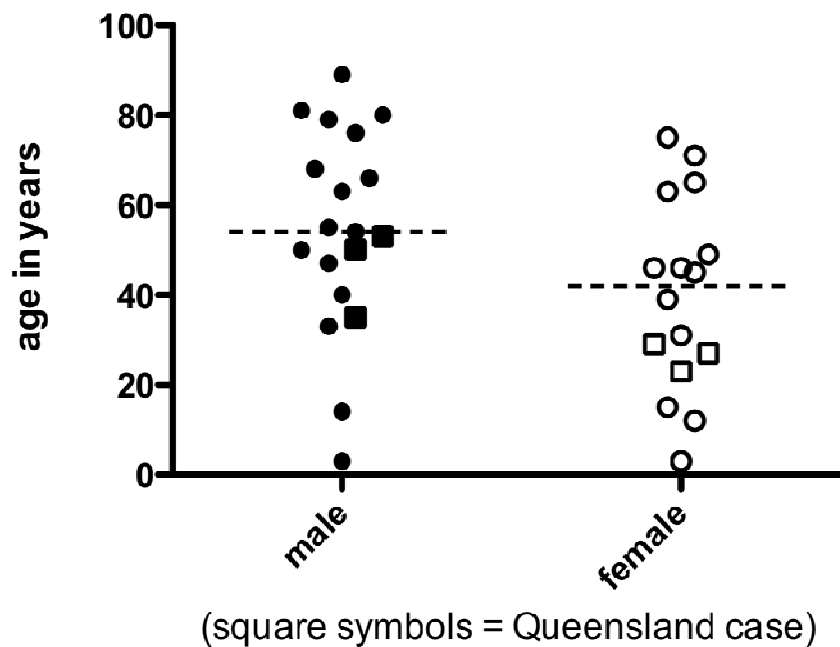


Figure 2. Buruli ulcer in Australia 2009, by Age and Sex.



Buruli ulcer in Japan: 14 cases of “*Mycobacterium ulcerans* subsp. *shinshuense*” infection in 1980-2009

Presenter: Dr Kazue Nakanaga

Kazue Nakanaga, Yoshihiko Hoshino, Norihisa Ishii

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Background: The first case of Buruli ulcer in Japan was reported in 1980. This was a 19-year-old Japanese woman who had never been abroad, only lived in Japan. This case report was published in 1982 by Mikoshiba and Tukamura, et al. The causative agent was advocated as “*M. ulcerans* subsp. *shinshuense*” because it was closely related to *M. ulcerans*. However, since then, there has been no more case report for over 20 years in Japan. But after a report of a 37-year-old woman with skin ulcer lesion in 2003, the reported number of cases has gradually increased: 1 case in 2004, 1 case in 2005, 3 cases in 2007, 2 cases in 2008, and 5 cases in 2009. Using these clinical samples and/or isolated bacteria, we conducted a comprehensive study on these all 14 cases and we report about clinical manifestations, treatment, etiological speculation, and differential diagnosis.

Results: Of 14 cases, 10 (71.4%) were females and 4 (28.6%) were males. The average age for females was 31.9 years (range 8–63) and the average age for the males was 68.3 years (range 46–81). The lesions occurred on the unprotected sites of the body: arms (6 cases), legs (6 cases), right auricle of ear (1 case), and both arm and leg (1 case). While a skin ulcer lesion occurred in all cases, most of which were smaller than 5 cm in diameter classified into category I. But we experienced one severe case classified as category III. In eight of the cases, the patients presented with painless lesions like a typical *M. ulcerans* infection; however, in 6 cases, the lesions were painful. Ten cases were operated by surgical excision and 7 out of the 10 cases were subsequently treated with skin grafting. All 14 cases were received with various regimen of antibiotics treatment. No relapse case was reported up to now. Geographical distribution of the cases was not focused, but all 14 cases were in Honshu (the largest island of Japan): 6 cases in Chugoku region (western part of Honshu), 5 cases in Chubu region (central part of Honshu), 2 cases in Kinki region (between Chugoku and Chubu region), 1 case in Tohoku region (northern part of Honshu). Although close interview with the patients was done, there were no evidence concerning aquatic environments, the sources of infection therefore remain unclear.

Laboratory diagnostic tests were done as follows, #1. PCR targeting IS2404 which is specific for *M. ulcerans* and #2. Bacterial isolation test. With these isolates, further three tests were performed, #3. 16S rRNA gene sequencing #4. PCR targeting 8 marker of mycolactone producing enzymes on the pMUM001 and #5. DDH test (Commercially available identification test of 19 mycobacteria by DNA-DNA hybridization)

Of the former two laboratory diagnostic tests, PCR to IS2404 was detected from the DNA of affected skin tissue of all 12 cases tested. The bacterial isolation was successful in 10 cases. An isolation period, that was defined as the first day when yellow pigmented bacterial colony was observed, was variable (the shortest took 4 weeks and the longest 11 months). All 10 isolates have the identical 16S rRNA gene sequences which were virtually similar with those of *M. ulcerans*. Only different sites between them were the 492, 1288, 1449-1451 (*E. coli* positions), the nucleotide of those positions were A, C, TTT in *M. ulcerans* (African strain), and were G, G, --- in “*M. ulcerans* subsp. *shinshuense*”. With the second PCR test targeting on pMUM001, all 10 isolates amplified with 7 markers, but lacked the band representing the serine/threonine protein kinase gene MUP011. The results of the DDH tests were *M. marinum* with all 9 isolates tested. Both “*M. ulcerans* subsp. *shinshuense*” and *M. ulcerans* were not included in defined 19 species of DDH test, misdiagnosis have been possibly occurred in clinical assay.

Conclusion: All 14 reported cases of Buruli ulcer in Japan were confirmed to be the “*M. ulcerans* subsp. *shinshuense*” infections. Those were comparable with *M. ulcerans* infection in view of clinical manifestation, however, different points were found as follows, #1. No focalization of cases in a certain area, #2. No supposable relation with aquatic environment, #3. A few, but certain conserved genetic differences were seen between both isolates.

“Efforts to unravel the mystery surrounding transmission of Buruli ulcer in Africa and Australia: What do we know today, what are the gaps and how do we proceed?”

Presenters: Professors Richard W. Merritt¹ and Paul D.R. Johnson²

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Part A. Africa

Buruli ulcer disease is often referred to as the “mysterious disease” because the mode of transmission has remained unclear despite the growing body of investigators working in this area. In Africa, nearly all epidemiological studies have associated disease outbreaks with villages in close proximity to human-disturbed freshwater habitats, including both standing and moving water bodies. In a recent review of *M. ulcerans* infection throughout the world, it was determined that poor wound care, failure to wear protective clothing, and living or working near water bodies were identified risk factors in most studies. Field studies in Africa have identified *M. ulcerans* DNA in many aquatic taxa. However, the organism has only been isolated once from an environmental sample. Research has demonstrated that aquatic and semi-aquatic insects, as well as some other non-insect aquatic invertebrates and plant biofilms, may serve as environmental reservoirs or intermediate hosts for this bacterium in nature, and may play an important role in maintaining bacteria within food webs in the aquatic environment. Laboratory experiments have either confirmed this or demonstrated that *M. ulcerans* could survive in secondary and tertiary consumers for up to 21 to 35 days, respectively, suggesting that some of these more mobile consumers would be capable of introducing *M. ulcerans* into new habitats through dispersion. Despite these findings, there are stringent criteria that exist in biomedical research and criteria that have been recently developed in the field of disease ecology for evaluating the roles of living agents as biologically significant reservoirs and/or vectors of pathogens, including *M. ulcerans*. While the above research reveals that various routes of transmission may occur, it is clear that the exact mode of transmission, if indeed there is a single mode, remains unknown in Africa at the present time.

Part B. Australia

Transmission of *M. ulcerans* in Australia occurs in two distinct climactic zones—a northern tropical zone with a similar climate to the African Buruli endemic regions, and a southern temperate zone. Since 2002, an intensely localised outbreak of BU has occurred on the Bellarine Peninsula, about 80 km south of Melbourne. Our investigation of this outbreak has provided evidence linking mosquitoes to the transmission of *M. ulcerans*. The decision to investigate mosquitoes was based on reports from patients that their BU developed at a site of a previous mosquito bite, the observation that BU often occurs at sites targeted by mosquitoes (ankle, elbow and in one case the tip of the ear), and very short exposure times (eg just one 4 hour visit to an endemic area in one case). Between 2004-2006 we trapped and tested by PCR more than 12, 000 mosquitoes from the Bellarine peninsula and identified *M. ulcerans* DNA in 4.3/1,000 of them. We also noted a correlation between the proportion of PCR-positive mosquitoes and the number of BU cases in a given location. A case-control study found that the use of insect repellent reduced the odds of being diagnosed with BU while frequent mosquito bites increased the odds. Finally, we identified a positive year-to-year correlation between our state-wide incidence of Ross River and Barmah Forest virus (both transmitted by mosquitoes) and the incidence of BU, suggesting that years in which mosquito disease transmission is high are also likely to have increased BU notifications. Although we are the first to implicate mosquitoes, our findings support laboratory and field work performed by others suggesting that insects play some role as either vectors or reservoirs of *M. ulcerans*. The possibility of mosquito transmission does not exclude other modes such as direct contact with contaminated soil, vegetation or water, and the evidence implicating mosquitoes is not yet conclusive. However, it is sufficiently strong to justify further detailed laboratory vector-host transmission experiments, and the systematic screening of biting insects in other Buruli endemic areas.

Development of rapid diagnostic tests for Buruli ulcer: where are we today and where do we go next?

Presenters and authors: Professors Timothy P. Stinear¹ and Gerd Pluschke²

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Developing a rapid and cost-effective point-of-care diagnostic test for Buruli ulcer is a major priority and several research strategies are currently being pursued. These include immunodiagnostic approaches such as antigen capture (detecting bacterial proteins in samples taken from an ulcer or nodule), serodiagnosis (detecting antibody responses to the bacteria in samples of patient blood) and human biomarker approaches (detecting characteristic immune system signatures such as changes in chemokines in blood). There are also other approaches focused on detecting the *M. ulcerans* macrolide toxin mycolactone and research is underway to rapidly detect *M. ulcerans* DNA in clinical specimens using a PCR-like technique amenable to resource-limited settings called loop mediated isothermal amplification (LAMP). Whilst these studies are all in various stages of discovery phase, sufficient progress has been made to suggest that antigen capture and human biomarker analysis hold considerable promise as candidate diagnostic platforms for further development. With ongoing financial support it is possible that one or more point-of-care tests for Buruli will become available for validation studies within the next three years.

Collaboration between HUG and MSF: the HIV-Buruli project at Akonolinga

Presenter: Dr Vanessa Christinet

Introduction

Akonolinga district is located in Centre province in Cameroon, 125 km east of Yaoundé, the capital. The main causes of morbidity in Cameroon are malaria, diarrhoeal and respiratory diseases (1). Prevalence of HIV is relatively low, and is estimated to be 5% (2). A survey of Buruli ulcer prevalence in Akonolinga district found overall prevalence to be 47 per 10 000 inhabitants and prevalence of active forms to be 25 per 10 000 inhabitants (3).

In 2002, MSF set up a project at Akonolinga with the initial primary objective of treating Buruli ulcer patients. Since June 2007, HIV screening has been provided. Roch C. Johnson et al (4) have shown that in Benin, HIV prevalence is higher among patients hospitalized with Buruli ulcer than among the control population. In addition, other data (5) suggest that HIV is a risk factor for the development of bone complications in patients infected with Buruli. Accordingly, we have set the following objectives:

- Evaluation of the impact of HIV infection on expression of *Mycobacterium ulcerans* (Buruli ulcer)
- Evaluation of the impact of antiretroviral treatment on expression of *Mycobacterium ulcerans*
- Measurement of the frequency of HIV infection in patients hospitalized for Buruli ulcer

Achievements

Since 2008, MSF has been systematically collecting complete data on patients admitted to hospital for Buruli ulcer and has built up a data base that includes data on HIV, Buruli ulcer and its evolution and socio-demographic data. Data going back to 2004 is nevertheless available.

Results

Our survey began after the introduction in 2008 of systematic HIV screening of all newly-hospitalized patients. It shows increased HIV prevalence among adults; out of 60 adults admitted to the programme, 20 were HIV positive (33%). As regards the cohort of co-infected patients including patients screened since 2004:

- The average CD4 count is 383 cell/mm³ (DS : 267 cell/mm³)
- 41/53 (77%) of patients were women
- 45/53 (85%) of patients presented the ulcerative form of Buruli ulcer
- 37/53 (70%) presented Buruli ulcer with no apparent complication

We still have scant paediatric data as it is more difficult to organize systematic screening on account of the need for parental consent for HIV screening.

The major challenges

- Obliging health-care workers systematically to carry out screening outside the health facilities in which it is usually carried out
- Developing collaboration between HIV outpatient treatment facilities and those specialized in Buruli ulcer (Akonolinga hospital)
- Difficulty of recruiting patients infected with Buruli ulcer at an early stage in the disease; HIV tests are carried out on patients hospitalized with major lesions ascribed to Buruli ulcer, whose diagnosis has not always been confirmed. These two factors certainly introduce elements of bias into our analysis.

The solutions proposed

- Training staff in comprehensive case management of the infectious diseases present in the region. This comprises:
 1. screening for Buruli by carers treating HIV
 2. HIV screening of all patients presenting early or late Buruli ulcer lesions, not only in hospitals, but also in health centres or more remote health facilities. .
- Tools for early diagnosis need to be provided and approved to prevent the development of large lesions requiring invasive treatment. Early detection of Buruli ulcer and determination of prevalence of HIV seropositivity in patients detected at an early stage would be important in understanding the link between these two diseases.
- It is essential to exclude active tuberculosis before administering antibiotic treatment with streptomycin and rifampicin. This is all the more important for HIV patients, who are at greater risk of developing tuberculosis.
- The possibility of IRIS (immune reconstitution syndrome) must be considered in the case of HIV patients beginning antiretroviral treatment.

Conclusions

These results seem to confirm the link between HIV infection and infection with *M. ulcerans*, simply because prevalence of HIV is far higher among adults with Buruli ulcer than among the general population (33% vs 5%). This encourages us to continue with our investigation and to improve methods of diagnosing Buruli ulcer and screening for HIV among Buruli ulcer patients in order to shed light on the link between HIV and Buruli ulcer.

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- (3) Klaudia Porten et al. (2009) Prevalence of Buruli Ulcer in Akonolinga Health District, Cameroon: Results of a Cross Sectional Survey. PLoS Negl Trop Dis. www.plosntds.org/article/info:doi%2F10.1371%2Fjournal.pntd.0000466
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What is the role of Civil Society in Buruli Ulcer Control? An account of Health Foundation of Ghana and Fontilles Programme in two districts of Ghana.

Presenter: Mrs Lynda Arthur

Country Director, Health Foundation of Ghana

Introduction

Early case detection and standardized case management are two of the seven global control strategies for Buruli ulcer (BU) disease in endemic countries. In line with these strategies, Fontilles Lucha contra la Lepra of Spain supported Health Foundation of Ghana (HFG) and its partners, notably, the Ghana Health Service (GHS), to initiate early case detection and management of Buruli ulcer cases in two highly endemic districts of Ghana; Amansie Central District in Ashanti Region and Asunafo South District in the Brong Ahafo Region. The period for project implementation was in 2008/09. The project focused on active community sensitization, case screening and strengthening of health system (training and the provision of medical equipment and consumables).

Activities undertaken

Activities undertaken included:

- Mass sensitization of communities on BU – use of audio visual equipment and BU documentary with commentary in local dialect.
- Active case screening by health workers and project staff – to identify all skin conditions including Buruli ulcer, take specimen and refer for treatment where necessary.
- Training of Community-based surveillance volunteers – to educate community members, to recognize cases, to refer and follow-up cases in endemic communities.
- Training of basic school teachers – to work together with trained CBSVs to undertake sensitization especially in school children who are the most affected group.
- Training of health staff at the district level in early case detection, specimen collection, antibiotic treatment, wound care and basic prevention of disability procedures.
- Provision of medical/surgical consumables and equipment (hospital beds and mattresses, gauze, bandages, other theatre supplies, bed sheets, etc) to support treatment of cases.
- Advocacy meetings with stakeholders at the district level (District Assemblies, Traditional and Opinion Leaders, Key Focal Persons and District Health Management Teams)

Outcomes

Results from key interventions in the two districts have been summarized below

Indicator	Results		Total
	Amansie Central	Asunafo South	
No. of communities sensitized on BU	5	4	9
No. of people sensitized on BU	1,850	1752	3,602
No. of people screened for BU and other skin conditions	1,009	445	1,454
No. of suspected BU cases identified	12	11	33
No. of other skin conditions identified	84	11	95
No. of BU specimen taken	8	0	8
No. of CBSVs trained	80	80	160
No. of teachers trained	80	80	160
No. of Health workers trained	80	80	160
No. of facilities supported with medical equipment and consumables	2	1	3
No. of advocacy meetings held	2	1	3
No. of monitoring activities undertaken	2	2	4

Challenges

- Absence of a network of trained community based organization to assist in the control of Buruli ulcer.
- Myths and stigma associated with Buruli ulcer and its treatment.
- Poor access roads making it difficult to get to hard-to-reach endemic communities.
- Inability for health workers to follow up cases identified from the sensitization and screening programmes.
- Staff apathy to patient care as a result of inadequate motivation for health workers in endemic communities.
- Low human and logistical capacity at the peripheral level health facilities hindering treatment of BU cases especially category I & II cases.
- Patients' accessing treatment due to long distance between endemic communities and treatment centres.
- Lack of political will at the district and sub-district level hampering the control of BU

Solutions to challenges/Recommendations

- There is the need to co-opt and train community based organization to partner the Ghana Health Service in the control of Buruli ulcer at the community level.
- There is an urgent need for resource mobilization/funding to support community based organization/faith based organizations in BU control.
- Intensification of community sensitization and active case screening through the use of video shows and community discussions to detect more early cases and dispel myths and stigma.
- There is the need to “enable” patients to comply with antibiotics treatment at the community level.
- Establishment/strengthening of more BU treatment centres at periphery of health system.
- Special motivational packages/incentive for health workers in the endemic treatment facilities.
- Intensification of international, national and district-level advocacy programmes to increase awareness and understanding and disseminate current development in BU control.

Stop Buruli Consortium – A research initiative of the UBS Optimus Foundation

Presenter: Dr. Susanna Hausmann-Muela

Head Global Health Research, UBS Optimus Foundation, Zurich, Switzerland

The UBS Optimus Foundation is a grant-making organization which supports projects for the benefit of children all over the world. In its focus on Neglected Tropical Diseases, the Foundation aims to catalyze research on relevant, but particularly under-financed infectious diseases. In 2007, the Foundation identified *Buruli ulcer* as a disease where a relatively small investment has the potential to make a big difference.

With the goal to bundle Buruli ulcer research and to grant a coordinated effort in investigation, the Foundation convened researchers from different disciplines to a workshop. The Stop Buruli consortium developed a proposal with individual projects axes which are coordinated according to an overall research agenda. From the beginning, the Foundation encouraged the researchers to think along the line of research which is useful for public health and application. Furthermore, the consortium was being built up with a prominent role of endemic countries and good links to National Buruli Programs.

Today, the consortium consists of four members from endemic countries, three from Africa and one from Australia, two from Europe and two from the USA (see list below).

Research is carried out along four main axes: new tools for diagnosis, transmission studies, antibiotic treatment, and socio-economic aspects. The transdisciplinary approach, together with the strong involvement of the African partners, makes this consortium promising for developing and validating innovative tools and interventions to public health application.

Important first results have been achieved in relation to diagnosis, with the production and evaluation of a panel of *Mycobacterium ulcerans* specific protein antigens and of prototype antigen capture assays. Furthermore the Fine Needle Aspiration (FNA) technique for detection, cultivation, and fine typing of *M. ulcerans* has been validated. Comparative genome sequencing has facilitated the generation of the first genetic fine-typing system for *M. ulcerans*.

The role of the Stop Buruli consortium goes beyond research. The consortium has carried out training for health staff in correctly performing FNA, and it has produced a technical video and training material which will become available to health staff in all endemic countries. Moreover, the consortium will increasingly take on a role of advocacy for Buruli ulcer, both in the scientific community and the general public. A first press release was issued last autumn in all member countries. The website www.stopburuli.org reports regularly on the progress of the Buruli ulcer research consortium and its work.

An international research consortium Further information and contact

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FP7 Project BuruliVac: Identification and development of vaccine candidates for Buruli Ulcer Disease (Duration: 36 months, Starting date: 01/03/2010)

Presenter: Professor Bernhard Fleischer

The general objective of BuruliVac is to identify and develop novel vaccine candidates suitable for translation into clinical application. This objective will be achieved by a multidisciplinary approach involving among others basic and applied research in immunology, bioinformatics, molecular genetics, tropical medicine, microbiology and clinical bacteriology. Extensive capacity building is an essential part of BuruliVac.

As currently no existing vaccine lead candidate is available, the consortium will identify and develop new vaccine candidates of different types, will evaluate them using bioinformatics, applied genomics and proteomics and will subject them to consecutive test systems. For evaluation of vaccine candidates regarding their application in humans, the consortium will also study the immune response and disease immunopathology to define correlates of protection. Essential pre-clinical testing *in vitro* and *in vivo* will select a small number of candidates that is amenable to be introduced into clinical studies. A vaccine against *M. ulcerans* could be used to protect persons at risk in highly endemic areas or as a therapeutic vaccine to shorten duration of treatment and to prevent relapses. The scientific principles underlying this vaccine can be applied to other mycobacterial infections.

Projectweb-site:

http://ec.europa.eu/research/health/infectious-diseases/neglected-diseases/projects/012_en.html

Coordinator:

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DIAGNOSIS

Report on laboratory confirmation of BUD cases in Togo, 2009

Presenter: Dr Gisela Bretzel

Authors: Herbinger KH¹, Nitschke J², Helfrich K¹, Fleischmann E¹, Beissner M¹, Ebekalisai P², Wiedemann F², Diefenhardt A², Bretzel G¹.

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In 2009 142 diagnostic samples from 52 BUD suspects from Togo were subjected to IS2404 PCR at the Department for Infectious Diseases and Tropical Medicine, University of Munich, Germany, 28 BUD cases were confirmed by PCR.

BUD suspects

Out of the 52 BUD suspects 26 (50%) were female.

The range of age was 2–65 years; the arithmetic mean of age was 23.5 years, and the medium 16 years.

Among the 26 female BUD suspects, the range of age was 4–65 years; the arithmetic mean of age was 23.5 years, and the medium 16.5 years.

Among the 26 male BUD suspects, the range of age was 2–60 years; the arithmetic mean of age was 23.5 years, and the medium 15 years.

The BUD suspects were living in the following regions: 40 (76.9%) in Région Maritime, 8 (15.4%) in Région Centrale, and 1 (1.9%) each in Région Kara and Region des plateaux. From 2 (3.8%) of the BUD suspects, the region was not known.

All 52 BUD suspects were new cases; 44 (84.6%) attended the hospital in Tsévié, and 8 (15.4%) attended the hospital in Sotouboua.

Out of them, 41 (78.8%) presented with ulcers, 7 (13.5%) with nodules, and 2 (3.8%) each with oedema and plaque.

Confirmed BUD cases

Out of the 52 BUD suspects, 28 (53.8%) were PCR confirmed as **BUD cases**.

Out of the 28 confirmed cases, 16 (57.1%) were female.

The range of age was 4–60 years; the arithmetic mean of age was 14.2 years, and the medium 10.5 years.

Among the 16 female BUD cases, the range of age was 4–60 years; the arithmetic mean of age was 15.7 years, and the medium 10.5 years.

Among the 12 male BUD cases, the range of age was 6–33 years; the arithmetic mean of age was 12.2 years, and the medium 10 years.

All 28 BUD cases were living in Région Maritime.

All 28 BUD cases were new cases and attended the hospital in Tsévié.

Out of them, 20 (71.4%) presented with ulcers, 4 (14.3%) with nodules, and 2 (7.1%) each with oedema and plaque.

The duration of disease among the 28 BUD cases was as follows: 9 (32.1%) less than 1 month, 8 (28.6%) 1–2 months, 8 (28.6%) 3–5 months, 2 (7.1%) 6–11 months, and 1 (3.6%) 14 months.

The lesions were located as follows: 6 (21.4%) on the left arm/shoulder, 9 (32.1%) on right arm/shoulder, 5 (17.9%) on the left leg/buttock, 5 (17.9%) on right leg/buttock, and 3 (10.7%) on abdomen/back.

The lesions were classified according to WHO categories as follows: 4 (14.3%) category I, 8 (28.6%) category II, and 9 (32.1%) in category III. From 7 (25.0%) BUD cases, the exact category of lesion was not known.

Samples

60 sets of specimens from 52 BUD suspects were subjected to IS2404 PCR analysis at DITM.

Out of altogether 142 diagnostic samples 57 (40.1%) were PCR positive.

Out of 55 swab samples 20 (36.4%) were PCR positive.

Out of 44 FNA samples 16 (36.4%) were PCR positive.

Out of 43 3 mm punch biopsies 21 (48.8%) were PCR positive.

Issues/Problems/Challenges relating to the confirmation of cases (lab perspective)

In our setting data analysis was conducted at the external reference laboratory (DITM) by means of a database (designed in the context of the BURULICO project). Besides BU01 forms, specific laboratory data forms were used to obtain information on patients and samples. The current format of the database requires to assign separate data forms to each set of specimens.

The most frequent problems encountered:

1. Incomplete information on patients (BU01) and specimens (e.g. epidemiological data, treatment history, origin and type of samples)
2. Incorrect assignment of data forms and lab numbers to the respective sets of specimens (e.g. several sets of specimens collected from one patient at different time points were incorrectly recorded on one data form)
3. Incorrect labelling of samples
4. Storage of PCR specimens in expired buffer solutions or in tubes without buffer (absence of local quality control measures)
5. Occasionally samples have been stored for several months before shipment

Possible solutions:

1. Regular and regularly repeated training in sample and data collection
2. Implementation of local quality control measures regarding completion of data forms, storage and shipment of samples

Issues/Problems/Challenges relating to the confirmation of cases (field perspective)

Training measures in sample and data collection have been regularly conducted by an experienced BU-surgeon (Dr. Jörg Nitschke, external advisor for GLRA). It was noted that during the external advisor's visits the quality of sample and data collection was good, however deteriorated in his absence. Likewise, almost no active case finding has been carried out in the absence of the external advisor.

Possible solutions (Dr.Jörg Nitschke):

It is very well known that until summer 2009 any effective collaboration and cooperation between the DAHW and PLNUB had been missing completely because of lacking engagement as well as professional interest of the coordinators before. The new coordinator, Dr. Basile Kobara has already given evidence being very engaged of sorting out problems of the BU project and motivating collaborators of the hospital and the field. So it is to be expected he will now encourage and endorse responsible people to collect patient's data carefully as a fundamental challenge to epidemiological requirements and statistics on BU incidence and prevalence of the country. He may also initiate more intensive active case finding under the leadership of the main health worker, M. Hegnon. Moreover, he should be charged with controlling and supervision of "Dispensaires" and "Centres de Santé" within the epidemiological area to ensure good compliance and treatment procedures on BU out-patients collectives

Laboratory Confirmation of BU cases at the NMIMR, Ghana: Combining microscopy and PCR to reduce cost and reporting time.

Presenter: Dr Dorothy Yeboah-Manu

Authors: D. Yeboah-Manu¹, A. Asante-Poku¹, E. Danso¹, K. Asan-Ampah¹, Z. Nakubo¹, I. Ibrahim¹, W. Opare² and E. Ampadu².

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Introduction

Due to problems associated with cultivation of *Mycobacterium ulcerans*, the causative agent of Buruli ulcer and the poorly recorded sensitivity of smear microscopy, *IS2404* PCR is the gold standard diagnostic tool. However PCR is expensive and requires elaborate infrastructure. Therefore laboratory confirmation is done in research laboratories where usually samples are analyzed in bulk making confirmation as a quality control tool. We improved microscopy by first concentrating samples before smearing and combined with PCR to reduce cost and reporting time.

BU Suspects

We analyzed Samples from 106 patients from 8 districts and three regions in Ghana. Majority of the samples (71; 67%) were from the Ga-West and South districts in the Greater-Accra region. 33 (31.1%) were from the Akwapim North and South, Atiwa, West-Akim and Manya Kobo districts all in the Eastern region and 2 were from Nkoranza North district in the Brong-Ahafo regions respectively.

53 of the cases (50%) were females; age range between 3 and 82 years; arithmetic mean age of 25.4 years, median 14 years with 10 years as the mode. The remaining 53 cases were men, between 4 months and 82 years; with arithmetic mean age of 26.8, median 15.5 and mode of 10 years.

Among the 106 cases, 104 were new cases and two were recorded as recurrent cases.

Majority of the suspects had ulcers (91; 85.8%): 79 had only ulcers, 9 had edema with ulcers, 1 had ulcers with osteomyelitis, and 2 had nodules and ulcers.

Only 15 (14.2%) cases were pre-ulcerative: 12 nodules, 1 plaque and 2 edemas. The lesion category was recorded for 84 cases and of these, 31 (36.9%), 24 (28.6%) and 29 (34.5%) were categories I, II and III respectively.

Microscopy was performed on samples received from 100 of the suspects and of these, 46 (46%) were positive for AFB and 54 were negative. *IS2404* PCR was performed on all samples and of these 81 were positive (76.4%) and 25 (23.6%) were negative. Combining the two methods, 82 (77.4%) of the cases were confirmed and of these 80 were new cases and the remaining 2 were recurrent cases.

Confirmed Cases

43 (52.4%) and 39 (47.6%) were females and males respectively. Age ranges between 3 and 82 years with an arithmetic mean, median and mode ages of 24.9, 14.5 and 10 years respectively.

The age range of the confirmed males is between 3 and 82; arithmetic mean of 26.8, mode 10 and median 15.5 years.

The females had an age range of between 3-82, arithmetic mean 25.5, median 14 and mode 10 years.

12 (14.6%) of the cases had pre-ulcerative lesions. 9 had nodules, 1 had plaque, and 2 edema and 70 (85.4%) had ulcers.

80 of the cases were classified as new and two were recurrent cases.

The category of 17 was not indicated. Out of the 65 indicated cases, 23 (35.4%) were category I, 20 (30.8%) category II, 22(33.9%) category III.

Samples

15 FNA samples were collected from 15 pre-ulcerative suspects and of these 12 (80%) and 3 (20%) were positive and negative respectively by PCR. Microscopy was done on 14 and 6 (43%) and 8 (57%) were positive and negative respectively

91 swab samples were collected from the ulcerative suspects, and of these 69 (75.8%) and 22 (24.2%) were positive and negative respectively

Microscopy was done on 86 of the samples; out of these 40 (46.5%) and 46 (53.5%) were positive and negative respectively.

Challenges

One of our challenges was to report results early to health facilities. We have now gone about it by improving microscopy so that preliminary results can be available within 24 hours of receipt by using GSM text messaging results to requesting facilities. Out of the 82 confirmed cases 46 (56.1%) were positive by microscopy. Based on this we now do microscopy first and report the result using mobile phones, and do PCR only when microscopy is negative. The other challenge was quality of specimen collection and together with the NBUCP, we have conducted training on specimen collection which has greatly improved quality of specimen collection.

Molecular diagnosis of *Mycobacterium ulcerans* using clinical samples in Côte d'Ivoire from 2000 to 2009

Presenters: Dr Euloge Ekaza, Dr Solange Ngazoa-Kakou

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Buruli ulcer is a cutaneous mycobacterial disease. It is widespread globally, with Africa being the most affected continent. Infections caused by *Mycobacterium ulcerans*, the pathogen responsible for Buruli ulcer, are essentially found in warm humid tropical and sub-tropical rural areas. Côte d'Ivoire has the highest number of cases (2000 new cases each year, with an aggregate total of 25 000 cases in 2006). All 19 health regions in the country are affected by the disease.

Diagnosis of the infection is hampered by the difficulty and slow growth of the bacterium in culture (6 to 8 weeks at 29-32°C). Before 2000, in Côte d'Ivoire, diagnosis of an ulceration most often relied solely on clinical criteria without biological or histological confirmation. This posed a problem for the etiology of skin infections. The presence of acid-fast bacilli (AFB) on biological skin samples after Ziehl-Neelsen staining points to the presence of a mycobacterium in the sample and could be a satisfactory diagnosis for presumed Buruli ulcer. However, other AFB bacilli besides *M. ulcerans* may also be responsible for skin ulcers. Laboratory diagnosis of Buruli ulcer by detection of *M. ulcerans* plays an important role in control of the disease. There are several methods of performing this diagnosis, one of which is gene amplification of a specific sequence of the bacterial genome (IS2404) by polymerase chain reaction (PCR). This technique is sensitive, specific and takes very little time. It makes it possible rapidly to provide patients with treatment, at the same time averting the sequelae associated with the disease. Genetic techniques offer speed and specificity which are without comparison with traditional techniques. The sensitivity of the PCR method is remarkable and makes it possible to use a direct and rapid diagnostic method on clinical samples. We provide below an assessment of 9 years of molecular diagnosis to confirm the presence of the bacterium on skin lesions that are clinically or biologically evocative of Buruli ulcer.

Between 2000 and 2009, a total of 3988 samples were analysed using different PCR techniques targeting specific sequences of the *M. ulcerans* genome. The number of samples analysed varied each year. From 2000 to 2005 the samples were obtained thanks to studies carried out at one or two centres providing case management. We started to receive samples from all sites in the country after 2006, after the national Buruli ulcer control programme had realized that we are capable of carrying out molecular diagnosis. The overall detection rate is 66.92 % (2669/3988) and varies from 31.84 % to 83.90 %. The targets are IS2604 and, IS2404. The conclusion drawn from the samples taken until 2004 was that the low positive rates were attributable to the low levels of presence in the exudates used (which made up 90 % of the samples analysed on account of the ease with which they could be collected). This feature may be explained by the fact that the samples were taken from old ulcers which had been treated several times with pharmaceutical and/or plant antiseptics. Such treatment reduces the bacterial load in the swabs. In order to improve the positive rates, changes were made in 2003 involving the introduction of a sample transport medium (Middelbrook 7H9 +CPC) and validation, after 2005, of « Nested PCR » targeting the IS2404 sequence and mainly involving all samples negative under simple PCR targeting the same insertion sequence. In years in which the detection rate was below 50 %, this was because samples were taken by staff who had not been trained to take the samples. During the nine years, the molecular detection rates were always higher than those of culture and microscopy.

Confirmation of the diagnosis of Buruli ulcer relies essentially on detection and culture of bacteria from samples taken from the lesion. Given the slow rate of growth of *M. ulcerans* (6 to 8 weeks at 29-32°C before the first colonies appear), amplification of the bacterial genome is the fastest and most sensitive

method of diagnosis. Detection of specific sequences of *M. ulcerans* is a reliable tool for confirming bacteriological diagnosis of Buruli ulcer on clinical samples. The time taken by confirmation varies from 48 to 72 hours, thus shortening the time needed for specific diagnosis, which as a rule requires several weeks where culture is concerned. In the light of the other etiological factors responsible for chronic ulcers in the endemic area, its use is necessary in case-detection campaigns which are the basis of any strategy to control the disease. Confirmed case diagnosis is indispensable for proper case management and to ensure the reliability of results for improved epidemiological use. The Buruli CRN in Côte d'Ivoire is in the process of approving real-time PCR (simultaneously targeting 2 or 3 targets) for diagnosis of clinical and environmental samples to identify the reservoir and understand the diseases' mode of transmission. It is also introducing methods for typing the strains circulating in Côte d'Ivoire. Detection of the *M. ulcerans* genome on clinical samples at the CNR–Buruli IPCI between 2000 and 2009

YEAR	TARGETS	NUMBER	NUMBER OF PCR	Number positive	Detection rate
2000	IS2606 and IS2404	245	490	85	34.70 %
2001	IS2404	179	179	57	31.84 %
2002	IS2404	119	119	38	31.93 %
2003	IS2404	101	101	57	56.43 %
2004	IS2404 nested PCR	199	398	105	52.76 %
2005	IS2404 nested PCR	168	336	128	76.20 %
2006	IS2404 nested PCR	176	352	74	42 %
2007	IS2404 nested PCR	348	696	215	61.78 %
2008	IS2404 nested PCR	1008	2010	843	83.90 %
2009	IS2404 nested PCR	1448	2896	1110	76.65 %

Buruli ulcer case confirmation in Cameroon

Presenter: Dr Sara Eyangoh

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In 2009, the mycobacterial laboratory at the Centre Pasteur in Cameroon (CPC) received samples (swabs, sera and biopsies) from 361 suspected cases of Buruli ulcer for analysis. For each patient, 2 to 4 swabs or 2 fine needle aspirations (FNA) were sent to the laboratory. A total of 963 swabs, 68 FNA, 2 biopsies and 1 nodule were analysed. The samples were collected and transported by nongovernmental organizations providing the Control Programme with support for the treatment of patients; Médecins Sans Frontières-Suisse (MSF) in Akonolinga health area and Aide aux Lépreux Emmaüs Suisse (ALES) in the other health areas (AYOS, BANKIM and BONGUE NGOANTET). At the laboratory, the samples were examined under the microscope, by amplification (PCR) of IS 2404 and by culture on Löwenstein Jensen solid media. Of the 361 suspected cases, 191 were from Akonolinga health area and 79 (41.4%) of these were confirmed by PCR; 170 were from the other health areas (Ayos, Ngoantet, Bankim and Bongue) and 44 (25.9%) of them were confirmed by PCR. Analysis of FNA is a fairly recent development and began in July 2009. Currently, these samples are taken only by teams under the supervision of ALES.

Detailed data on suspected cases of Buruli ulcer

Out of 361 cases, 170 were female, 167 male and the sex of 25 suspected cases was unspecified on the forms submitted. The age of the cases varied from 1 to 86 years, with an average age of 26.39.

Distribution of the suspected cases in the health areas was as follows:

- Akonolinga health area: 191 /361 cases (52.9%),
- Ayos health area: 61/361(17%),
- Bankim health area: 59/361 (16.3%),
- Ngoantet health area: 42/361 (11.6%),
- Bongue health area: 8/361 (2.2%).

A total of 314 of the suspected cases presented an ulcer (87%), in most cases on the limbs: 71.9% on the lower limbs and 22.8% on the upper limbs. The other sites affected were the head, trunk and neck.

Confirmed cases

Of the 361 suspected cases analysed, 123 (32.88%) were confirmed by PCR. Of the 191 suspected cases from Akonolinga health area, 79 (41.36%) were confirmed by PCR. Of the 170 suspected cases from the other sites, 44 (25.9%) were confirmed by PCR.

A total of 89 of the 123 cases confirmed by PCR had already been found positive by microscopy and 52 by culture; culture of some cases is still under way.

Comments/observations

Transport procedure

The laboratory was able to provide Bankim and Bongue, which are remote, with transport media for samples only in April. On account of the considerable distance between the sites and the laboratory, samples are sent once a month. The procedure for sending samples is not yet systematic and should be improved.

As for the other sites, Akonolinga, Ayos and Ngoantet, which are not remote, samples are cold stored dry and sent as soon as possible to the laboratory in cold boxes.

A three-page form has been introduced to ensure information on patients is sent to the laboratory and to make it easier to trace the results. However, the form needs to be revised to include all the information required by the WHO form.

Challenges and prospects

1. Improved sensitivity

Sensitivity of PCR is still quite low. In order to improve it, the different partners working in the field should harmonize sampling and collection procedures on all sites. An effort is being made to achieve this and in November 2009 different technical staff attended a training course on sampling and collection methods.

The laboratory intends to perform real-time PCR. At the end of the year, this technique was tested and validated using an Applied Biosystems 7300 device, and 2 kits have been ordered to enable the laboratory to introduce the technique for the benefit of patients.

2. Reimbursement of analyses

Between 2003 and 2008, analyses were carried out free of charge by CPC as part of its public health mission. On account of the financial problems affecting CPC, in 2009 MSF defrayed the full cost of analyses for patients from Akonolinga health area. We hope that in 2010 all the donors will support the laboratory to enable it to continue its diagnostic activity and with real-time PCT and to improve sensitivity.

In addition, donors are also urged to help the laboratory to purchase PANTA (a very expensive antibiotic cocktail) and used as a supplement in transport media and thus to improve transport of samples from remote sites.

3. Tuberculosis sensitivity test

At the request of MSF, we have introduced the sensitivity test for drugs to treat tuberculosis for patients with treatment failure or relapse and from whom strains were available. All the strains tested were sensitive to rifampicin and streptomycin. We do not currently test clarithromycin, in accordance with the wishes of MSF.

Fine needle aspiration for the diagnosis of *M. ulcerans* infection and for mycolactone detection

Presenter: Dr Laurent Marsollier

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Over recent years, management of Buruli ulcer patients has considerably changed with advances in antibiotherapy. Antibiotherapy is particularly effective on nonulcerative forms. However, the bacteriological diagnosis in the early forms is difficult because simple and non-invasive methods are not available. In this study, the diagnostic effectiveness of the Fine Needle aspiration was evaluated on early lesions. Our results showed that PCR from FNA samples, unlike Ziehl-Neelsen staining, is very sensitive on nonulcerative forms like other standard sampling methods (biopsy and punch biopsy). Furthermore, mycolactone was detected in aspirated liquid from lesions in mouse experimentally infected by *M. ulcerans* and in FNA from Buruli ulcer patients. This is a crucial observation to encourage the development of diagnosis test based on mycolactone detection. Moreover, mycolactone was never detected in aspirated liquid from a patient treated by antibiotherapy. To conclude, fine needle aspiration is a simple, fast, painless, accurate and inexpensive method of sampling and could be used for diagnosis of *M. ulcerans* infection.

This work was supported by the Fondation Française Raoul Follereau, Pasteur Institute network (PTR 212), Région Pays de la Loire and Inserm

Fine-needle aspiration: a step-by-step tutorial

Presenter: Dr Miriam Eddyani

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Fine-needle aspiration (FNA) is increasingly used for obtaining diagnostic material from nonulcerative Buruli ulcer lesions because it is little invasive. However, the success of the technique is very dependent on a correct execution. Although FNA seems to be simple, several crucial details in the procedure are easily overlooked. For this reason a step-by-step tutorial was developed consisting of a video and manual for distribution both in English and French in Buruli ulcer endemic countries. The tutorial will allow medical personnel to learn to correctly perform FNA with a satisfactory diagnostic success. FNA can be used for direct smear examination directly after aspirating and can be stored and transported in a liquid transport medium for PCR analysis and culture.

First round of External Quality Assessment (EQA) of molecular detection of *Mycobacterium ulcerans* in clinical specimens

Presenter: Dr Miriam Eddyani

Authors: Miriam Eddyani, Koen Vandelannoote, Pim de Rijk, Françoise Portaels

Institute of Tropical Medicine, Antwerp, Belgium

In 2009 the first External Quality Assessment (EQA) programme was organized. Its primary aim was to assess the proficiency of laboratories in the detection of *Mycobacterium ulcerans* DNA. Participating laboratories were asked to process the EQA panel by the test routinely used in their laboratory for molecular detection of *M. ulcerans* in clinical specimens. Eleven out of the 17 participating laboratories (64,71%) returned results. The participating laboratories were located in 15 countries (on 4 continents) and represented reference, academic and hospital laboratories.

The EQA panel for the detection of *M. ulcerans* DNA consisted of 34 suspensions. Suspensions were selected in such a way that they would allow an assessment of sensitivity (false negatives), specificity (false positives), and inter-laboratory reproducibility. All suspensions were sent in double to assess intra-laboratory reproducibility.

The proportion of correct qualitative results varied between 50.00% and 100.00% by sample (**inter-laboratory reproducibility**). The proportion of correct qualitative results varied between 57.58 and 100.00% by laboratory. Only four out of the eleven participating laboratories had more than 90% concordant results and they were all reference laboratories. Seven (63.64%) laboratories reported **false positive** results (5.00-45.00%), indicating problems of **specificity** most probably due to DNA contaminations. Six (54.55%) laboratories reported **false negative** results (14.29-78.57%) indicating problems of **sensitivity**. **Intra-laboratory reproducibility** was assessed by shipping all suspensions in double. Reproducibility within laboratories varied between 58.82 and 100.00%.

There is a **great variation** between laboratories in the quality of the molecular detection of *M. ulcerans* from clinical specimens. The laboratories with few correct results all had low intra-laboratory reproducibility indicating that their false results were due to mistakes in manipulations rather than to the techniques used. This impression is reinforced by the observation that the different extraction methods and PCR assays used by the participating laboratories showed great variations in correct results. Moreover, reference laboratories had more concordant results than academic and hospital laboratories, another argument for the great variation to be most probably due to **laboratory performance**.

Implications of the weak outcome of the majority of respondent laboratories may be that:

- 1) patients suffering from illnesses other than Buruli ulcer receive inappropriate treatment;
- 2) patients with Buruli ulcer are erroneously considered as suffering from other illnesses;
- 3) epidemiological data on Buruli ulcer are unreliable;
- 4) conclusions drawn from clinical as well as environmental studies are doubtful.

We therefore recommend that:

- 1) **funds** are released by WHO and/or other funders to continue this important quality assessment programme on a yearly basis and to reinforce laboratories with weak results;
- 2) the diagnosis of Buruli ulcer by **microscopy** is reinforced. By direct smear examination (DSE) 2/3 of PCR positive specimens can be confirmed. DSE is a cheap, sensitive and fast diagnostic method that can be applied easily in Buruli ulcer endemic areas without the need for expensive and sophisticated equipment. Moreover, in most Buruli ulcer endemic countries systems are in place to control the quality of DSE by national tuberculosis programmes;
- 3) laboratories with high numbers of false positives do not embark on environmental studies as long as no measures have been taken to avoid DNA contaminations.

Development of new and improvement of current diagnostic methods of *M. ulcerans* at the Noguchi Memorial Institute for Medical Research

Presenter: Professor Daniel Boakye

Authors: Charles Quaye, Dzedzom de Souza, Lydia Mosi, Phyllis Addo, Anthony Ablordey, Dorothy Yeboah-Manu and Daniel Boakye

Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

Diagnosis of *Mycobacterium ulcerans* disease (Buruli ulcer) has been one of the priority areas towards controlling this debilitating disease. Notwithstanding the progress made over the years, diagnosis in most cases is still based on clinical symptoms at the point of care with confirmation made in well equipped laboratories away from those sites. Research at the Noguchi Memorial Institute for Medical Research is aiming to develop new methods and/or improve existing ones to bring diagnosis to the point of care and also provide a means for detecting pre-patent infections. Two of such methods; DNA Loop Mediated Isothermal Amplification (LAMP) and the use of microscopy to determine the presence of *M. ulcerans* from human biopsies in amoeba species are presented and others discussed.

Diagnosis and detection of Buruli Ulcer: the potential of cross-disease, integrated diagnostic platforms

Presenter: Dr Cristina Gutierrez

Foundation for Innovative New Diagnostics, Geneva, Switzerland

Although diagnosis of Buruli Ulcer by PCR has been shown to have a sensitivity of 90% or higher, this disease mainly affects people living in poor rural areas, where access to diagnostic facilities that can perform PCR is limited and difficult. In these areas, other diseases of public health importance, such as malaria, human African trypanosomiasis (HAT) and TB are also prevalent. Typically, the most peripheral areas of the health system are only served by microscopy, at best. However, diagnostic services are often among the most neglected areas of the health system in resource-limited settings, and light microscopy, although easy to perform, requires extensive support through systems for Quality Assurance and a reliable supply chain in order to ensure reliable diagnosis.

Recently, there has been renewed interest in laboratory strengthening as a global health priority. The Maputo Declaration of 2008 calls on national governments to “support laboratory systems as a priority by developing a national laboratory policy within the national health development plan” and “to develop national strategic laboratory plans that integrate laboratory support for the major diseases of public health importance, including HIV, TB and malaria”. The declaration also calls on countries to establish a tiered laboratory network (Fig 1) in which laboratories at different levels of the health system have defined roles based on their capacity and resources.

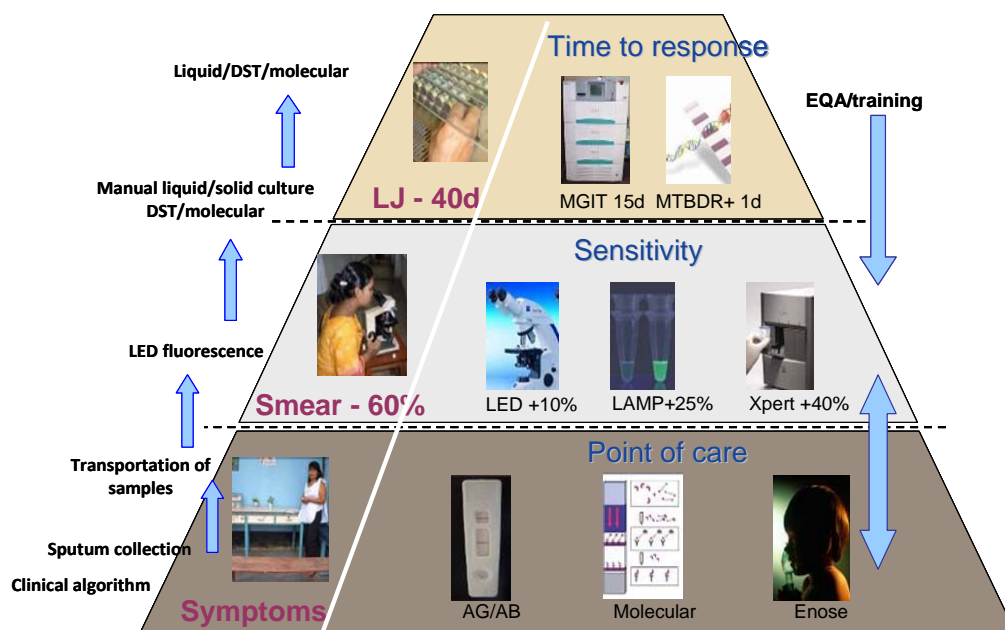


Figure 1: Tiered laboratory network (TB example)

Optimizing investment in laboratories can be achieved by utilizing technological platforms that can be used across diseases. In the case of Buruli Ulcer, FIND (Foundation for Innovative New Diagnostics) has been working on a manual technology for nucleic acid amplification (LAMP platform), which is intended for the diagnosis of TB, and for deployment in laboratories where currently smear microscopy is used. The same platform has also been adapted for the diagnosis of malaria and HAT. As the causative agent of Buruli Ulcer is *Mycobacterium ulcerans*, the LAMP platform has great potential for adaptation to the diagnosis of both diseases – and then be made available in diagnostic facilities closer to the areas where patients need access to early diagnosis to prevent morbidity and needless suffering.

Development of a simple, inexpensive, point-of-care test for *Mycobacterium ulcerans* based on direct chemical detection of mycolactone

Presenter: Professor Pamela Small

Authors: Thomas Spangenberg¹, Katrina Jackson¹, Lydia Mosi², Heather Williamson², Pamela Small^{2*} and Yoshito Kishi¹

¹ Harvard University, USA,

² University of Tennessee, USA.

Despite the recent development of successful antibiotic treatment for Buruli Ulcer, patients are often diagnosed late, and molecular methods for confirmation of *M. ulcerans* in patient tissue are unavailable outside of major reference labs. Although clinical diagnosis of Buruli ulcer in late stage disease is quite accurate, clinical diagnosis of the earliest forms is problematic. There is a crucial need for the development of a simple, inexpensive diagnostic method adaptable even for clinics in remote areas. We have shown in earlier work that the major virulence determinant of *M. ulcerans*, mycolactone A/B, is present in tissue at all stages of *M. ulcerans* disease. Thus, detection of mycolactone A/B in tissue could provide the basis for a reliable diagnostic test. However, the chemical nature of mycolactone as an immunosuppressive lipid has so far impeded efforts to produce a diagnostic based on an anti-mycolactone antibody. For this reason, we have studied a method based on direct chemical detection of mycolactone.

Mycolactone A/B is comprised of a core lactone and a pentaenoate fatty acid side chain. We felt that the 1,3-diol present in the fatty acid side chain might provide us with a selective handle to modulate mycolactone A/B. The plan we envisioned consists of three steps: (1) excitation of the pentaenoate chromophore present in the fatty acid side chain, (2) energy transfer from the excited pentaenoate to a fluorogenic acceptor selectively bound to the 1,3-diol, and (3) detection of the fluorescence emission from the acceptor. With this plan, we screened a wide range of aromatic boronic acids, leading us to identify 2-naphthylboronic acid as the best reagent. However, spectroscopic studies suggested that this process involves the excitation/emission of the pentaenoate chromophore itself rather than fluorescence energy transfer as originally planned. In this method, mycolactone is detected as the fluorescent spot, the color (green-yellow) of which corresponds to the fluorescence spectrum of the pentaenoate in cyclohexane. Interestingly, when 2-naphthylboronic acid is attached to the 1,3-diol present in the pentaenoate moiety, the fluorescence intensity is significantly enhanced. Thus, the boronic acid functions as an enhancer of the fluorescent emission, rather than a fluorogenic acceptor.

It is of particular interest that all the mycolactones A/B, C, and D produced by the human pathogen *M. ulcerans* are detected by this method, whereas mycolactones E and F from frog and fish pathogens are not. This analytical method consists of four steps: (1) to prepare the lipid extract from a tissue sample using a simple hand-operated homogenizer and a CHCl₃/MeOH extraction procedure, (2) to elute the crude lipid extract on thin layer chromatography (TLC), (3) to dip the TLC plate in a 0.1 M solution of 2-naphthylboronic acid in acetone and heat for 5-10 seconds to 100 °C, and (4) to detect any mycolactone present with a hand-held UV lamp. This method allows us to detect 2-5 ng of mycolactones A/B, C, and D in animal or fish tissue doped with synthetic mycolactones. All of the chemicals, equipment and supplies required are available commercially at modest prices. This method is operationally simple and should be adaptable even for clinics in remote areas. Results described here provide proof of concept for direct detection of mycolactones in tissue. Further studies are planned to evaluate the potential of this method for diagnosis of Buruli ulcer in human lesions.

LABORATORY CONFIRMATION

Laboratory case confirmation of Buruli ulcer using PCR in the Republic of the Congo

Author : Dr Damas Obvala

National leprosy/Buruli ulcer control programme

Buruli ulcer is widely present in the Republic of the Congo. The first cases were detected in 2004, and case-management activities began in 2005 after the adoption of the basic documents of the national programme. Specific treatment using antibiotics began in September 2006.

In 2008 and 2009 the total number of cases was 271. Case confirmation in the laboratory used AFB (11 cases - 9%) which is less used and less reliable than PCR, which was used for 91% of cases.

Samples from suspected Buruli ulcer cases came from areas in which the disease is endemic, and were taken by health workers responsible for the treatment centres (CDTUB) or in the course of awareness-raising/case-detection campaigns.

A total of 53.9% of the samples came from Kouilou (the disease epicentre) and Pointe Noire departments, 14.4% from Bouenza department and 10.5% from Niari department.

Data for each case were entered on a single test request and included the patient's name and forename, their sex, place of residence, the type of sample, the site from which the sample was taken, the date, the type of test requested and the reason for taking the sample. The same information was included on the results form.

In 2008 and 2009 ten sets of samples were taken and sent to the reference laboratory in Angers (France); the time between submitting the samples and receiving the results was at the most two weeks and at the least five days.

In 2008, out of a total of 124 cases of Buruli ulcer, samples were taken from 55 cases (43.6%), 24 of which were positive under PCR (43.6%). The commonest sampling method was the swab (81.8%), followed by fine needle aspiration (12.7%). A total of 45.4% of samples were from children.

In 2009, out of a total of 147 cases, samples were taken from 76 cases (51.7%), 35 (46.8%) of which were positive under PCR. The commonest sampling method was the swab and samples were taken from 32 children (42.1%).

The reasons for the requests for PCR included the following:

- Case confirmation at the time of detection: 96.5%
- Case monitoring during treatment: 1%
- Evolution on completion of treatment: 2.5%
- Relapses: 0%

In all, during these two years 131 samples were taken for PCR from an aggregate total of 271 cases of Buruli ulcer, a sample rate of 48.3%; 59 cases (49%) were positive under PCR. Swabs were taken from 117 cases (89.3%), followed by AFB (7.6%).

During the period, the number of cases from which samples were taken increased gradually, as did the number of positive cases.

The main constraint was lack of funds and problems of access to some areas and treatment centres. In 2010, the main focus should be organizing the sample collection network by improving supervisory training rounds, ensuring supplies of the necessary equipment and improving links with the reference laboratory through the new partnership between the national public health laboratory in Brazzaville and the laboratory in Angers.

Optimization of the isolation of *Mycobacterium ulcerans* by improving conditions of collection, preservation and transport of clinical samples

Authors: Aka N., Ekaza E., Coulibaly B., Coulibaly-Ngolo M.D., N'Guessan K.R., Ngazoa-Kakou S., Ehué P., Assandé J-M., Yapo-Crézoit A. and Dosso Mireille

Introduction

After tuberculosis and leprosy, *Mycobacterium ulcerans* or Buruli ulcer infection is the commonest human mycobacterial infection in humid tropical areas. Its resurgence presents a challenge to the scientific community, especially in countries where the disease is endemic. The disease's particularly striking symptoms and characteristic epidemiology make it relatively easy to identify in a clinical context. The remaining difficulty is isolation in the laboratory to obtain strains for thorough investigation of the biology of *M. ulcerans*. In contrast with tuberculosis mycobacteria, in vitro cultivation of *M. ulcerans* is not easy. It is a slow-growing bacterium and isolation by culture in polymicrobial clinical samples is not easy. Isolation of the first strain of *M. ulcerans* in Côte d'Ivoire dates from 1994. That achievement has subsequently been built upon by several trials. This presentation describes the different periods and technical procedures employed. The objective is to provide laboratories with simple procedures to enable them to optimize isolation of *M. ulcerans* from polymicrobial clinical samples.

Material and methods

Between 1994 and 2009, a series of tests was carried out on samples of skin exudates to isolate *Mycobacterium ulcerans*. From 2 to 4 swab samples were collected from each ulcer and placed in a tube containing 2 ml of preservation and transport medium. The first medium was composed of Dubos to which PANTA. was added. These samples were transported to the laboratory at between +4° et 8°C, where they were either immediately cultivated or preserved for 2 to 4 weeks at a temperature of -20°C. The modified Petroff method was used to decontaminate the samples. The second medium comprised 0.75% cetylpyridinium chloride (CPC). The third medium was composed of Middlebrook 7H9 supplemented by 0.75% CPC. The samples were preserved at room temperature for 24 hours or between +4° and +8°C for 2 to 5 days. They were centrifuged at 3000 rpm for between 10 and 20 minutes and the pellet was recovered with sterile distilled water for cultivation on Lowenstein-Jensen medium. The sample was incubated at 32°C for 6 months.

Results

1994-2001: 204 samples were collected on Dubos and PANTA; 67 were positive under Ziehl-Neelsen and 7 produced colonies of *M. ulcerans*. The contamination rate was 23%. **2002-2004:** 116 exudates were prepared using 0.75% CPC; 59 were Ziehl positive, 86 contained *M. ulcerans* DNA and 16 produced colonies. The contamination rate was 6%. **2005-2009:** 150 samples were collected and transported on 0.75% 7H9+CPC. 73 were Ziehl positive, 96 contained IS2404 and 36 produced colonies of *M. ulcerans*. The contamination rate was below 5%.

Conclusion

Different test cultures at IPCI have demonstrated the contribution of an association of CPC and 7H9 as a preservation and transport medium for isolation of *M. ulcerans*. This medium is suitable for isolating *M. ulcerans* from polymicrobial clinical samples.

Keywords: Buruli ulcer; *Mycobacterium ulcerans*; bacterial isolation; Middlebrook ; cetylpyridinium chloride (CPC) ; Institut Pasteur Côte d'Ivoire.

Capacity-building for diagnosis and surveillance of Buruli ulcer in Côte d'Ivoire: development of real-time PCR

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Buruli ulcer is caused by the mycobacterium *M. ulcerans*. It manifests itself in skin lesions and the lack of early diagnosis and treatment leads to severe disabilities. Currently, the emergence of Buruli ulcer infections in Africa, and in particular in Côte d'Ivoire, makes it necessary to provide more rapid diagnosis to meet the needs of the populations. In 2009, 1910 samples were analysed; 76.65% of them were positive under conventional PCR using the IS2404 sequence.

On account of the increase in the number of clinical samples (1100 in 2008 and 1910 in 2009), the discovery in December 2009, of a new disease focus in the vicinity of Abidjan (Adiopodumé) and the need to evaluate bacterial load of positive samples, it became essential and a matter of priority to develop real-time PCR in order to meet the needs of the diagnostic laboratory.

A total of 55 samples (clinical, environmental, local strains and reference strains) were analysed using real-time PCR by comparing the IS2404 insertion sequence and the mycolactone ketoreductase sequence (KR) targets. A total of 46 samples (83.3%) were positive in real time for both targets. In order to evaluate bacterial load, a solution of DNA containing 2.5×10^5 genomes made it possible to achieve a detection threshold of 0.25 genomes for both samples.

Our results show that real-time detection is efficacious as a means of detection on all types of sample for targets IS2404 and KR. The detection threshold is 0.25 *M. ulcerans* genomes, with Ct of 39.21 (IS2404) and 37.55 (KR) respectively.

Detection of environmental samples proved ineffective and a number of activities are under way involving insects and water from infected areas. Coordinated and improved surveillance together with control measures are being discussed with the Buruli ulcer research institute of the Institut Pasteur in Cote d'Ivoire.

Real-time PCR offers a number of advantages for Buruli ulcer surveillance and diagnosis. The capacity, rapidity and sensitivity of PCR improve diagnosis and make it possible to respond to new disease foci.

Report on laboratory confirmation of Buruli ulcer cases, VIDRL, 2009

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Laboratory protocol

All specimens from suspected cases of Buruli ulcer (BU) received at the Victorian Infectious Diseases Reference Laboratory (VIDRL) are tested for *Mycobacterium ulcerans* using an in-house real-time PCR assay targeting IS2404 (Fyfe et al. 2007) and stained for acid-fast bacilli using the Ziehl-Neelsen method. Specimens that are PCR-positive for *M. ulcerans* are put up for culture at 31°C using solid (Brown and Buckle slopes and chocolate slopes) and liquid media (MGIT broth).

Specimens tested

In 2009, VIDRL performed *M. ulcerans* PCR on 525 specimens from 414 individuals. Most specimens were from humans, but a small number of specimens from wild and domestic animals were also tested. The majority of specimens were swabs (63%) or fresh tissue (32%) (Table 1). Fifty-four (10%) of the 525 specimens tested, which represented 29 new human cases and two new animals cases, were positive for *M. ulcerans* DNA. The demographic and clinical characteristics of these cases are provided below.

Table 1. Specimens tested for *M. ulcerans* by PCR, VIDRL, 2009

Specimen type	PCR result			Total
	Positive	Negative	Inconclusive	
Swab	22	306	1 ^a	329
Tissue	23	145		168
Tissue - paraffin sections	6	11		17
Aspirate		2		2
Pus		2		2
Blood clot	1			1
MGIT broth	1			1
Pooled tissue/swab		1		1
Not specified	1	3		4
Total	54	470	1	525

^a NB. Four other specimens from this patients tested negative

Although the number of positive specimens as a proportion of all specimens tested is low, we do not believe that cases of BU in Australia are going undiagnosed, because (i) the sensitivity of the PCR is extremely high and (ii) many specimens are tested as a precautionary measure to exclude BU (as opposed to being tested to confirm a strong clinical suspicion of BU). Therefore, those specimens that tested negative for *M. ulcerans* are highly unlikely to be unconfirmed cases of BU.

Confirmed BU cases

All 29 (100%) human cases of BU notified to the Victorian Department of Health in 2009 were confirmed by PCR \pm smear and/or culture (see BU02 form for details). The number of cases of BU diagnosed in 2009 was slightly lower than in 2008, but higher than in 2007 (Fig. 1). The median age of cases was 50 years (range: 3–89) and 16 (55%) were male (Fig. 2). Twenty-one (72%) cases presented with ulcers and 13 (45%) cases had lesions on the legs (Table 2). As in previous years, most cases (n=22, 76%) were linked to seaside towns on the Bellarine Peninsula, an area approximately 60 km southwest of Melbourne. The remaining 24% of cases were linked to areas with previous cases of BU, including east Gippsland, the southeast bay side suburbs of Melbourne and the Mornington Peninsula.

Fig. 1. Laboratory-confirmed cases of BU, 2000-09

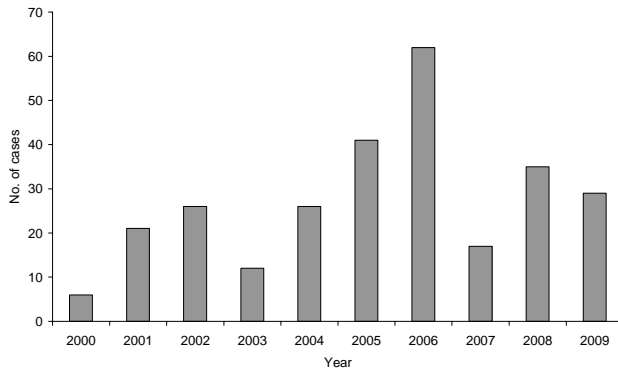


Fig. 2. Age and sex of laboratory-confirmed cases of BU, 2009

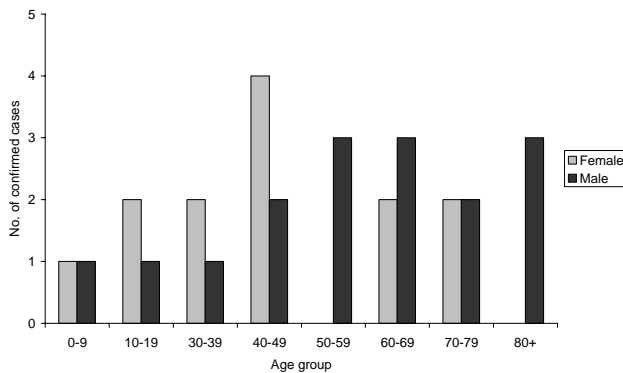


Table 2. Clinical site and manifestation of confirmed BU lesions, 2009

Site	Clinical form					Total
	Ulcer/s	Cellulitis	Lump	Wound	Not specified	
Leg	12		1			13
Ankle	1				2	3
Arm	1			1		2
Elbow	2					2
Back	1					1
Buttock	1					1
Foot		1				1
Neck					1	1
Not specified	3				2	5
Total	21	1	1	1	5	29

Reference

Fyfe JA, Lavender CJ, Johnson PD, Globan M, Sievers A, Azuolas J, Stinear TP. Development and application of two multiplex real-time PCR assays for the detection of *Mycobacterium ulcerans* in clinical and environmental samples. *Appl Environ Microbiol.* 2007 Aug;73(15):4733-40.

Report on Benin National Reference Laboratory activities, 2009

Author : Dr Dissou Affolabi

In 2009, 558 samples from 360 patients were received mainly from three Buruli ulcer (BU) treatment centres (Allada, Lalo and Zagnanado). Of these patients, 54% were male, 46% female and 60% had age below 15 years. Lesions were mainly ulcers (64%) followed by plaques (25%) and samples were tissues (48%), swabs (40%) and fine needle aspirations (12%).

174 (31%) were microscopy positive and 384 (69%) microscopy negative. Culture was performed on all these specimens but is complete so far for 476 specimens. Among these specimens, only 70 (16%) were culture positive. PCR was carried out on 544 specimens collected from patients suspected of having BU: 275 (51%) were PCR positive, in which 163 (59%) were also microscopy positive. Only seven samples (1%) were microscopy positive but PCR negative. The distribution PCR positive specimens per center is as shown in Table 1.

Table 1: PCR results by treatment centers

Centres	Specimens	Number PCR+	%PCR+
Allada	267	147	55%
Lalo	133	38	29%
Zagnanado	128	79	62%
Others (Nigeria, ...)	16	11	69%
Total	544	275	51%

Table 2. PCR results by types of specimens

	PCR		Total N (%)
	Positive N (%)	Negative N (%)	
Tissue/Biopsy	147 (57.4)	109 (42.6)	256 (100)
Swab	103 (46.6)	118 (53.4)	221 (100)
Fine needle aspiration	25 (37.3)	42 (62.7)	67 (100)
Total	275	269	544

In 2008, there were 601 samples from 476 patients with lesions mainly ulcers (63%) and plaques (23%). Tissues represented 62% of specimens while swabs represented 33% and fine needle aspirations only 5%. Rate of positivity on all specimens was 14% with culture, 30% with microscopy, and 54% with PCR. Delay in receiving samples from BU treatment centres that was a concern in 2008 has greatly improved in 2009.

Due to the high concordance between PCR and microscopy in smear negative specimens, perspectives are to establish a well functioning laboratory network between peripheral laboratories and the national reference laboratory (NRL). Therefore, microscopy can be used in peripheral laboratories for diagnosis and only smear negative samples will be sent to the NRL for PCR. A team from the NRL has recently supervised peripheral laboratories. The next step will be training for technicians on microscopy and an establishment of a regular program for supervision, quality control and training.

Report of the laboratory confirmation of cases from the CDTUB Pobé, Benin

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In 2009, 420 diagnostic samples (corresponding to 301 patients suspected of *M. ulcerans* infection) from the Centre de Dépistage et de Traitement de l'Ulçère de Buruli of Benin were subjected to IS2404 PCR at the Centre Hospitalier Universitaire of Angers. 180 samples (corresponding to 141 patients) were confirmed positive to *M. ulcerans* infection by PCR.

Confirmed patients to *M. ulcerans* infection

Out of 301 patients, 141 (47%) were confirmed positive by PCR to *M. ulcerans* infection (table 1).

Out of 141 confirmed patients, 61 (43.5%) were female.

Out of the 141 confirmed patients, 28 (19.75%) presented with ulcer, 25 (17.5%) with plaque, 7 (5%) with osteomyelitis, 3 (2%) with nodule, 2 (1.5%) with oedema, 59 (42%) with mixed forms, 17 (12.25%) with other forms (table 2).

The lesions were classified according to World Health Organization categories as follow: 24 (17%) category I (< 5 cm), 57 (40.5%) category II (between 5 cm and 15 cm), 42 (29.75%) category III (>15 cm), and 18 (12.75%) of unfurnished data concerning the category (table 2).

Positive samples

180 samples have been collected, corresponding to 1.3 samples per patient. 46 (25.5%) samples have been collected by biopsy method (cutaneous and bones biopsies), 56 (31%) by Fine Needle Aspiration method, 72 (40%) by swab method (2 swabs corresponding to one sample per patient) and 6 (3.5%) samples consisted of pus collection (table 3).

Table 1

	Number of patients	Percentage
PCR positive	141	47%
PCR négative	156	52%
Lost samples	4	1%
Total	301	100%

Table 2

Form	Number of patients	Category I	Category II	Category III	Unfurnished data
Ulcer	28 (19,75%)	6 (4,25%)	10 (7%)	12 (8,5%)	0
Plaque	25 (17,5%)	6 (4,25%)	13 (9,25%)	3 (2%)	3 (2%)
Oedema	2 (1,5%)	0	1 (0,75%)	1 (0,75%)	0
Osteomyelitis	7 (5%)	0	0	6 (4,25%)	1 (0,75%)
Nodule	3 (2%)	3 (2%)	0	0	0
mixed forms	59 (42%)	9 (6,5%)	32 (22,75%)	18 (12,75%)	0
other form	17 (12,25%)	0	1 (0,75%)	2 (1,5%)	14 (10%)
total	141 (100%)	24 (17%)	57 (40,5%)	42 (29,75%)	18 (12,75%)

Table 3

	Number of samples	Percentage
Biopsy (cutaneous and bones tissues)	46	25,5%
Swab	72	40%
Fine Needle Aspiration	56	31%
Pus	6	3,5%
total	180	100%

Report on laboratory confirmation of Buruli ulcer cases in Congo-Brazzaville

Author : Dr Laurent Marsollier

In 2009, 87 diagnostic samples (corresponding to 78 patients suspected of *M. ulcerans* infection) from Congo Brazzaville were subjected to IS2404 PCR at the Centre Hospitalier Universitaire of Angers. 38 samples (corresponding to 32 patients) were confirmed positive to *M. ulcerans* infection by PCR.

Confirmed patients to *M. ulcerans* infection

Out of 78 patients, 32 (41%) were confirmed positive by PCR to *M. ulcerans* infection (table 1).

Out of 32 confirmed patients, 19 (59.5%) were female.

Out of the 19 confirmed female patients, the range of age was 2.5-70 years; the average age 29.5 years.

Out of the 13 confirmed male patients, the range of age was 5-34 years; the average age 16 years.

Out of the 32 confirmed patients, 31 (97%) presented with ulcer, 1 (3%) with nodule (table 2).

Positive samples

38 samples have been collected, corresponding to 1.2 samples per patient.

2 samples (5.5%) have been collected by Fine Needle Aspiration method and 36(94.5%) by swab method (up to 2 swabs corresponding to one sample per patient) (table 3).

Table 1

	Number of patients	Percentage
PCR positive	32	41%
PCR négative	46	59%
TOTAL	78	100%

Table 2

Form	Number of patients	Percentage
Ulcer	31	97%
Plaque	0	0%
Oedema	0	0%
Osteomyelitis	0	0%
Nodule	1	3%
Total	32	100%

Table 3

	Number of samples	Percentage
Fine Needle Aspiration	2	5,5%
Swab	36	94,5%
total	38	100%

Confirmation of the presence of *M. ulcerans* in samples analysed at the CNR-Buruli in Côte d'Ivoire en 2009

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The interest generated by *M. ulcerans* infection is attributable to its sharp global resurgence over more than a decade, and particularly in Africa. In Côte d'Ivoire, the number of cases has regularly increased and the disease now affects all regions in the country. In 2009, 1448 samples suspected to contain *M. ulcerans*, the pathogen responsible for Buruli ulcer, were analysed at the national Buruli ulcer reference centre at the Institut Pasteur in Côte d'Ivoire. The samples were tested using nested PCR to detect the IS2404 sequence, which was found in 1110 of the suspect samples.

The suspect samples

Of the 1448 suspect samples received, (for the number of individuals, see the presentation by the national Buruli ulcer control programme in Côte d'Ivoire: PNLUB-CI), 639 (44.13%) were from females and 741 (51.17%) from males, while the sex of the donors of 70 samples (04.83%) was unspecified.

A total of 229 (35.83%) of the 639 samples provided by females came from patients aged between 4 and 87 years, while the age of donors of 410 samples (64.17%) was unspecified.

Of the 741 samples provided by males, 282 (38,05%) came from donors aged from 7 months to 88 years while the age of the donors of 459 samples (61,95%) was unspecified.

Of the 70 samples from donors whose sex was unspecified, 11 (15,71%) were from donors aged from 2 to 40 years and 59 (84,29%) from donors whose age was unspecified (for mean ages, see PNLUB-CI).

The suspect samples came from different localities in the following regions: Lacs region (centre) 329 (22.72%), Vallée du Bandama region (centre) 288 (19.89%), Zanzan region (north-east) 190 (13.12%), Bas-Sassandra region (south-west) 177 (12.22%), Lagunes region, including Abidjan (south) 157 (10.84%), Haut-Sassandra region (west) 96 (6.63%), Sud-Bandama region (south) 80 (05.52%), Fromager region (west) 56 (03.86%), Savanes region (north) 41 (02.83%), N'Zi Comoé region (east) 16 (01.10%), Marahoué region (centre-west) 14 (0.96%), Sud-Comoé region (south-east) 03 (0.20%) and Worodougou region (north-west) 01 (0.07%).

A total of 1309 of the samples (90.40%) were from ulcers, 59 (04.07%) from nodules, 05 (0.34%) from plaques and 03 (0.20%) from œdemas. The type of lesion was unspecified for 72 samples (04.97%).

Samples in which presence of the *M. ulcerans* was confirmed

Nested PCR targeting IS2404 was positive in 1110 (76.65%) of all the human samples received for diagnosis of *M. ulcerans* (for the number of cases, see PNLUB-CI).

Of the 1110 positive samples, 577 (51.98%) were from male patients, 499 (44.95%) from females and the sex of 34 was unspecified (03.07%).

Two known types of samples were received for analysis, those obtained by fine needle aspiration (FNA) and those obtained by swabs. Some of the samples that reached us provided no indication of the method used to collect them. No biopsy samples were delivered to the laboratory for analysis.

Of the samples obtained by FNA, 60 were analysed and 38 (63.33 %) tested positive using nested PCR IS2404.

Of the samples obtained by swabs, 1316 were analysed and 1004 (76.29 %) tested positive using nested PCR IS2404.

Of the samples where the method of collection was unknown, 72 were analysed and 68 (94.44 %) tested positive using nested PCR IS2404.

Of the 577 positive samples from males, the age of 197 (34.15%) patients was between 7 months and 88 years (for mean age, see PNLUB-CI), the age of 380 donors (65.85%) was unspecified.

Of the 499 positive samples from females, 164 (32.87%) donors were aged between 3 and 87 years, and the age of 335 (67.13%) was unspecified.

Of the 34 samples from donors whose sex was unspecified, 3 donors (08.82%) were aged 07, 10 and 24 years and the age of 31 (91.17%) was unspecified.

Distribution by region of the positive samples was as follows: Lacs region (centre) 267 (24.05%), Vallée du Bandama region (centre) 217 (19.54%), Zanzan region (north-east) 158 (14.23%), Lagunes region including Abidjan (south) 110 (09.90%), Bas-Sassandra region (south-west) 109 (09.81%), Haut-Sassandra (west) and Sud-Bandama (south) regions 75 each (06.75%), Fromager region (west) 43 (03.87%), Savanes region (north) 37 (03.33%), N'Zi Comoé region (east) 12 (01.08%), Marahoué region (centre-west) 05 (0.45%) and Sud-Comoé (south-east) and Worodougou (north-west) regions 01 each (0.09%).

A total of 1000 (90.09%) samples were from ulcers, 38 (03.42%) from nodules and 03 (0.27%) from plaques; 01 (0,09%) sample was from an oedema and in the case of 68 (06.12%) samples, the type of lesion from which the samples were taken was unspecified.

Other types of sample

We received 33 bacterial strains and 82 samples of batches of aquatic insects for confirmation of the presence of the *M. ulcerans* genome.

Examination of the 33 bacterial strains by nested PCR for the IS2404 sequence confirmed its presence in all the strains, a positive rate of 100%.

As a result of the breakdown of the supply chain providing the supply of extraction kits, it was impossible to test any of the samples of insects this year. Orders for DNA extraction kits are being processed.

External quality control:

We participated in the « First round of external quality assessment of molecular detection of *Mycobacterium ulcerans* in clinical specimens » organized by WHO in collaboration with the Institute of Tropical Medicine in Antwerp, Belgium. We are awaiting the results of that quality control exercise.

Problems encountered

No (epidemiological) information slip was provided with the samples we received, and because family names are identical in different regions and several different samples may be taken from the same person, PNLUB-CI is alone capable of indicating to how many individuals the different samples received correspond.

Most of the samples (1097 or 75,75%) were dry swabs left for several months at room temperature before being sent to the laboratory for diagnosis. In order to ensure the best possible diagnostic results from these samples we were obliged to incubate the swabs in sterile water for molecular biology for one night at 37°C before extracting the genetic material after having transferred the swab into the same water. Despite these precautions, 79.29% (268/338) of the negative samples were from dry swabs. However, the laboratory tested a transport medium (Middelbrook 7H9 +CPC) which it has made available for the sample-collection network in order to improve its performance. This medium is also suitable for cultivation of clinical samples in order to isolate clinical strains for studies of sensitivity, virulence and molecular epidemiology.

The outlook for confirmation

In view of the large number of samples received for confirmation of Buruli ulcer in Côte d'Ivoire and for investigation of the environmental reservoir of *M. ulcerans*, we have acquired real-time PCR equipment (Applied Biosystems 7300 and 7500 Real Time PCR system). On 10 November 2009, we began developing real-time PCR diagnosis, first of all on bacterial strains isolated from culture of human samples. A total of 33 strains were tested to detect the IS2404 sequence and the ketoreductase (kr) sequence using real-time PCR. The target sequences were detected in all the strains tested. This new technique will be used for routine confirmation of human cases and to detect *M. ulcerans* in the environment. Thanks to this technique, it will be possible not only to avoid lengthy manipulation but also possible post-amplification contaminations from nested PCR. It will also be possible to enhance detection sensitivity. We also intend to participate in training for staff from treatment centres in how to complete epidemiological forms, sampling technique and use of the transport medium provided.

Summary Report on Laboratory Confirmation of Buruli ulcer cases at KATH, Kumasi 2009

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Since January 2009 samples suspected to be Buruli ulcer were received from Tepa Government hospital (54) in the Ahafo Ano North District, Nkawie hospital (112), Agogo Presbyterian hospital (59) and other places that included Amansie Central (25), Assin Akropong(1), Wasa Akropong(1), Brenso Chipo (1), Kojo Absong (1), Cape Coast (2), Assin Wurakese(3) and Komfo Anokye Teaching Hospital (17). The characteristics of the patients confirmed from these areas are shown in table 1.

Samples from the Ahafo Ano North district were transported to the microbiology laboratory of KATH by the outreach treatment team members that visit the Tepa Government Hospital whilst those from other areas were transported by health personnel in the respective districts.

Sample types included swabs, FNA and punch biopsies and were processed by the Ziehl-Neelsen technique, culture and PCR. A positive result of any of the above techniques was considered a positive result.

Patient characteristics

Suspected Buruli ulcer cases

Samples from 277 suspected Buruli ulcer patients were submitted for analysis.

There were samples from 130 male and 147 female. Overall the age range was 1 to 86 years with a median age of 14 (see table 1).

Confirmed Buruli ulcer cases

Table 1: Characteristics for those confirmed as Buruli ulcer

Total # patients	180	
Sex	#	%
Male	75	42%
Female	105	58%
Age		
Median		13
range		1 to 86
Site of lesion		
Head & Neck	4	2%
Upper limb	88	49%
Thorax	4	2%%
Abdomen	0	0%
Lower limb	84	47%
Clinical Form		
Nodule	81	45%
Plaque	18	10%
oedema	10	6%
Ulcer	71	39%

Test Results

Table 2: shows details of sample type and test results for the various centres

TEPA	TEST	FNA			Swab			Punch biopsy		
		No.	+	-	No.	+	-	No.	+	-
N=54										
	PCR	11	8	3	16	9	7			
	CULTURE				22	15	7	12	8	4
	ZN				31	8	23	16	5	11
AGOGO										
N=59	PCR	28	18	10	21	19	2			
	CULTURE				1	1		45	32	13
	ZN				1	1		47	24	23
NKAWIE										
N=112	PCR	14	11	3	41	36	5			
	CULTURE				24	15	9	5	2	3
	ZN				65	11	54	7	0	7
OTHERS										
N=52	PCR				25	21	4			
	CULTURE				24	13	11			
	ZN				31	5	26			

Table 3. Samples processed by the PCR technique

PCR	FNA			Swab		
	No.	+	-	No.	+	-
TEPA	11	8	3	16	9	7
AGOGO	28	18	10	21	19	2
NKAWIE	14	11	3	41	36	5
OTHERS				25	21	4
TOTAL	53	37	16	103	85	18

156 samples FNA and Swabs were processed by the IS2404 PCR technique of which 122 (78%) were positive and 34 (22%) were negative.

Table 3 shows that overall samples for 277 patients were processed by either ZN, culture or PCR of which 180 (65%) were confirmed as Buruli ulcer

Table 4. Samples confirmed or unconfirmed as Buruli ulcer

District	Confirmed		Unconfirmed		Total
	Number	%	Number	%	
TEPA	35	65%	19	35%	54
AGOGO	51	86%	8	14%	59
NKAWIE	59	53%	53	47%	112
OTHERS	35	67%	17	33%	52
TOTAL	180	65%	97	35%	277

Challenges

1. There was great difficulty with legibility of patient labels and patient details were often incomplete.
2. Some of the unconfirmed specimen were are a result of samples being submitted in unidentified transport medium that could have contributed to inhibition in those samples
3. In the greater part of the year the laboratory had problems with support for key reagents and consumables to cope with the number of samples that were brought for processing from all surrounding districts. This resulted in delays in processing and submission of results to the various centres. Personnel were overstretched but there was no support to train additional laboratory hands.

Recommendations

- There should be a scale up of training in specimen collection, transportation and documentation in all affected district
- Control programmes should be encouraged to have a defined budget for laboratory diagnosis outside research budgets so there will be no break in laboratory diagnosis when research funds are run out.

Report on laboratory confirmation of BUD cases at the KCCR, Ghana, 2009

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In 2009, 129 diagnostic samples from 117 BUD suspects from seven treatment sites were subjected to IS2404 PCR at the Kumasi Centre for Collaborative Research in Tropical Medicine, 48 BUD cases were confirmed by PCR.

BUD suspects

Out of the 117 BUD suspects 63 (53.9%) were female.

The range of age was 1month–85 years; the arithmetic mean of age was 31.3 years, and the medium 19 years.

Among the 63 female BUD suspects, the range of age was 2–85 years; the arithmetic mean of age was 26.9 years, and the medium 14 years.

Among the 54 male BUD suspects, the range of age was 1month–78 years; the arithmetic mean of age was 35.6 years, and the medium 24 years.

The BUD suspects were living in the following regions: 94 (80.3%) in Ashanti Region (5 different districts), 15 (13.7%) in the Central region, and 7 (6.0%) in the Western Region.

All but one 116 (99.1%) of the 117 BUD suspects were new cases. The only recurrent case 1 (0.9%) was from the Ashanti Region.

Out of them, 95 (81.2%) presented with ulcers, 11 (9.4%) with nodules, 7 (6.0%) with plaque and 4 (3.4%) with oedema.

Confirmed BUD cases

Out of the 117 BUD suspects, 48 (41%) were PCR confirmed as **BUD cases**.

Out of the 48 confirmed cases, 25 (52.1%) were female.

The range of age was 2–71 years; the arithmetic mean of age was 28 years, and the medium 11 years.

Among the 25 female BUD cases, the range of age was 2–55 years; the arithmetic mean of age was 25.9 years, and the medium 9 years.

Among the 23 male BUD cases, the range of age was 2.5–71 years; the arithmetic mean of age was 30.1 years, and the medium 13 years.

For the 48 BUD cases, 38 (79.2%) were living in the Ashanti Region, 7 (14.6%) in the Central Region and 3 (6.2%) in the Western Region.

All 48 BUD cases were new cases.

Out of them, 29 (60.4%) presented with ulcers, 8 (16.7%) with nodules, 7 (14.6%) with plaque and 4 (8.3%) with oedema.

The duration of disease among the 48 BUD cases was as follows: 15 (31.3%) less than 1 month, 14 (29.2%) 1–2 months, 14 (29.2%) 3–5 months, 3 (6.3%) 6–11 months, and 1 (2.1%) 14 months. One (2.1%) was not stated.

The lesions were classified according to WHO categories as follows: 10 (20.8%) category I, 18 (37.5%) category II, and 13 (27.1%) in category III. From 7 (14.6%) BUD cases, the exact category of lesion was not known.

Samples

Out of altogether 129 diagnostic samples 51 (39.5%) were PCR positive.

Out of 67 swab samples 22 (32.8%) were PCR positive.

Out of 57 FNA samples 27 (47.4%) were PCR positive.

Out of 53 tissue biopsies 21 (40.0%) were PCR positive.

Comments

1. Negative cases observed were as high as 60.5% (78) . This could be due to antimycobacterial treatment before collection of specimens especially in the case of ulcerative lesions.
2. Again, this observation could be due to the fact that the suspected cases were NOT BU cases to start with.
3. From the results, FNAs still remain a good tool for the laboratory confirmation of ulcerative and non-ulcerative lesions.

Challenges

1. For the year under review, specimen collection had to be suspended in October due to the unavailability of key reagents for processing..
2. Another major challenge was the poor quality of specimen sent for analyses and the improper filling of BU04 forms.
3. We encourage the various health facilities to collect suitable specimens and endeavor to send their samples as soon as possible instead of keeping them for long periods before sending them to the laboratory.

Report on laboratory confirmation of Buruli ulcer cases in 2009 by the Institute of Tropical Medicine (ITM), Antwerp, Belgium

Authors : Portaels F, Eddyani M, de Rijk P, Fissette K, Van Aerde A, Rigouts L

In 2009, we have received specimens from 8 different countries: Benin, DRC, Gabon, Guinea, Italy (from Cameroon), New Guinea, Nigeria and Sierra Leone. A total of 544 specimens were analyzed, 510 by direct smear examination (ZN), culture and PCR and 34 by PCR only.

The table gives the results for ZN and PCR. Culture results are not yet available since the tubes are kept for 12 months at 32°C.

Laboratory analyses:

Direct smear examination: Ziehl-Neelsen (ZN) staining

Culture: on Löwenstein-Jensen

PCR: IS2404 PCR on gel

	PCR			ZN *			swab	biopsy	FNA	TOTAL
	+	-	%+	+	-	%+				
Gabon	15	28	34,9	14	29	32,6		43		43
Sierra Leone		7	0		7	0	4	3		7
New Guinea		2	0		2	0	2			2
Guinea		14	0	3	11	21,4	14			14
Cameroon (Italy)		8	0		8	0		8		8
Nigeria	7	9	43,8	7	9	43,8	16			16
Benin	63	96	39,6	46	79	36,8	11	111	37	159
DRC	74	221	25,1	76	219	25,8	202	56	37	295
										544

* For some specimens, ZN results were not available

**ANTIBIOTIC TREATMENT
AND
OTHER TREATMENT**

Four cases of rifampicin-based oral antibiotic treatment for Buruli ulcer: three successes and one apparent failure

Presenter: Dr Claire Gordon

Authors: Claire L Gordon, John A Buntine, John A Hayman, Caroline J Lavender, Janet A Fyfe, Mike Starr, Paul DR Johnson.

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Intramuscular streptomycin combined with oral rifampicin for 8 weeks ("SR8") has become standard care for patients with Buruli ulcer in Africa. In Australia, we use oral rifampicin-based antibiotic regimens, most often combined with conservative surgery, but the efficacy of oral antibiotics alone has yet to be established. We describe four Australian patients with *Mycobacterium ulcerans* disease (Buruli ulcer) three of whom were treated with rifampicin-based oral antibiotic therapy for 4-6 weeks prior to surgical resection. In two patients, excision specimens were sterile on culture. In a third patient, histology of excision specimens was negative for acid-fast bacilli, but culture and PCR were not performed. All 3 of these patients were relapse free at 12 months. A fourth patient, a 3 year old girl with a PCR and culture positive Buruli ulcer on her upper thigh, was treated with only oral rifampicin and clarithromycin syrup for 8 weeks, and did not undergo surgery. One month after ceasing antibiotics, the lesion that had initially reduced to a small nodule, became inflamed and then discharged. Discharge material was positive by smear and PCR for *M. ulcerans*, culture is currently pending. The first 3 cases suggest that oral antibiotic combinations may be able to replace SR8 in some situations, but the 4th case suggests treatment failure. Alternatively, this apparent failure may be due to an inflammatory response to residual but dead bacteria.

***Mycobacterium ulcerans* (Buruli ulcer) disease in French Guiana: development of therapeutic practice in the period 2004-2009**

Presenter: Professor Pierre Couppié

Authors: Couppié P¹, Dufour J¹, Papot E¹, Hotz C¹, Guédénon F¹, Fior A², Queuche F³, Sainte-Marie D¹

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Introduction

Until recently scant guidance was available on the treatment of *Mycobacterium ulcerans* disease. Surgery was the standard option. In 2004 WHO outlined recommendations on the use of first-line antibiotic treatment using rifampicin and an aminoglycoside (streptomycin or amikacin) in combination (1). The object of this study is to assess medical practice in the dermatology unit in the light of these recommendations.

Material and Methods

This is a retrospective study of the case-files of patients attending our dermatology unit in connection with *Mycobacterium ulcerans* infection in the period 2004-2009. Patients with *Mycobacterium ulcerans* infection confirmed by at least one method (PCR, Ziehl-Neelsen stain, culture, clinical pathology) were included. An evaluation was made of the annual proportion of patients treated by means of antibiotics and surgery, respectively.

Results

32 new cases of *Mycobacterium ulcerans* disease were diagnosed at our dermatology unit in the period 2004-2009. Most cases were adult males; 97% were category I or II. PCR was positive in 94% of cases. The proportion of patients treated using antibiotics increased from 8/17 (47%) of cases in 2004 to 13/13 (100%) in 2006. The combination of rifampicin and amikacin has gradually been replaced by a combination of rifampicin and clarithromycin. The proportion of patients not requiring surgery increased from 5/17 (29%) in 2004 to 11/11 (100%) since 2006. Contact has been lost with three patients. All monitored patients have made a complete recovery.

Discussion

The antibiotic treatment recommended by WHO is a combination of rifampicin (10 mg/kg/day orally) and an aminoglycoside (streptomycin or amikacin 15 mg/kg/day by intramuscular injection) for 2 months. This treatment is relatively cumbersome because aminoglycoside must be injected intramuscularly every day for 2 months. Studies are being undertaken in Africa on the efficacy of a rifampicin-clarithromycin combination (12 mg/kg/day orally). Given that the strains of *Mycobacterium ulcerans* isolated in French Guiana are susceptible to clarithromycin, and given the problems of administering amikacin outside hospital settings (in France amikacin is only used in hospitals), a rifampicine-clarithromycin combination is now the first-line antibiotic therapy in Guiana.

Conclusion

Antibiotic treatment of *Mycobacterium ulcerans* disease is now the norm in French Guiana. Rifampicine and clarithromycin used in combination is the first-line treatment.

Reference: 1. WHO 2004. Provisional guidance on the role of specific antibiotics in the management of *Mycobacterium ulcerans* disease (Buruli ulcer).

Decentralization of antibiotic treatment using a combination of rifampicin and clarithromycin

Presenter: Dr Phillip Humphris

Introduction

In the context of managing Buruli ulcer (BU) disease, MSF has been working in partnership with the Akonolinga health district to treat BU patients at the district hospital, health centres and in the community.

With a view to reducing morbidity and disabilities caused by BU, MSF has developed an ambitious outpatient treatment strategy for certain patients, resulting in earlier treatment, fewer defaulters, and reduced time in hospital. Another core element of our strategy is the transfer of functions from the centre to the periphery.

Implementation

Patients are selected according to the following precise medical criteria: category 1 wounds with no complications, children accompanied by a responsible adult, in a health area within Akonolinga health district.

The decentralized treatment of BU patients has been made possible by an alternative antibiotic treatment combining rifampicin and clarithromycin (R/C); wound care is provided by health centres.

Results

To measure the results, a system of monitoring has been put in place that uses a register, an antibiotic treatment and follow-up supervision form and a patient treatment card.

The indicators applied are the number of new cases using R/C therapy, the percentage of cured patients relative to the total number of patients in R/C treatment and the percentage of relapses relative to the number of cured patients in R/C treatment.

Since August 2008, 21 patients have been treated, wholly or partly, on a decentralized basis at the Abem health area. Of these, 16 were recruited in the health area and 8 were referred from hospital.

Of 13 patients in treatment with a clarithromycin component (9 on a decentralized basis and 4 in hospital), 12 provided positive specimens (or 92.3% confirmed cases).

The cure rate is 60% (6 patients left the programme cured)

1 patient relapsed less than 12 months after completing the antibiotic treatment.

Challenges

- Monitoring of patient compliance with antibiotic treatment
- Transfer of functions to the peripheral centres.

Solutions

- Introduction of pill dispensers.
- Availability of reserve doses of antibiotics to ensure uninterrupted treatment, even if the patient does not report to collect medicines as scheduled.
- Regular training of nurses at health centres.

Conclusion

A combination of rifampicin and clarithromycin enables BU patients to be treated without admission to hospital, or reduces the length of hospital stay when admission is indicated.

Benin's experience in implementing specific antibiotic treatment: Results and prospects

Presenter: Dr Christian Johnson

Authors: R.C. Johnson, G.E. Sopoh, Y. Barogui, A. Chauty, J Aguiar

Introduction

As in other endemic countries, the national control programme in Benin has followed WHO recommendations on treating BU with a combination of streptomycin and rifampicin since 2005. This treatment has been administered in detection and treatment centres (of which there are 4) and peripheral dispensaries (32). The skills of treatment workers are maintained at the requisite level through regular training and supervision. The BU detection and treatment centres ensure that treatment sites are regularly provided with the appropriate inputs.

Method

A retrospective study focusing on analysis of BU02 and BU01 forms at BU detection and treatment centres.

The number of patients in each category and the data on antibiotic treatment were analysed. The relapse rate per centre was calculated and the treatment outcomes noted.

Results

The study showed that, since 2005, a total of 2941 patients in Benin have been regularly treated and documented according to the protocol recommended by WHO. Of these, 68% have been cured by surgery and 32% by nonsurgical methods. Most of the patients who have been cured without recourse to surgery belong to category 1, i.e. have lesions measuring less than 5 cm in diameter. The completion rate for antibiotic treatment remains high at over 95%; the relapse rate is under 5% nationally. Overall, however, poor clinical responses were observed among category 3 patients. The reasons are still unclear. Following decentralization of treatment, a significant proportion of patients begin their treatment close to their homes before having an operation at a detection and treatment centre.

Problems and prospects

Decentralization is an effective tool for the success of antibiotic treatment, but requires significant logistical and human resources (bimonthly supervision). In addition, selective strike action in the health service has disrupted treatment. The issue of the appropriate time for surgical intervention has still not been clarified and requires further research. Another concern for the programme is national standardization of dressing techniques.

Conclusion

Specific antibiotic treatment has improved the management of BU in Benin. A total of 2941 patients have been documented in Benin since 2005. The case files show that category 1 patients have good clinical responses. Research is needed to explain the factors determining clinical responses in patients with category 3 lesions and the best time for surgery in patients undergoing antibiotic treatment.

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

Presenter: Dr Richard Phillips

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Introduction

As part of a programme to develop an oral antibiotic regimen treatment for Buruli ulcer, a small non-blinded randomised controlled trial is in progress to compare the clinical and microbiological response to standard rifampicin/streptomycin for 8 weeks (RS8) with rifampicin/streptomycin for 2 weeks followed by rifampicin/clarithromycin for 6 weeks (RS2RC6) in patients with small Buruli lesions. This study will further determine if mycolactone reduction in Buruli lesions correlate with development of the immune response. This is an interim analysis of 83 patients recruited to the study by January 2010.

Methods

Between July 2009 and January 2010 patients were recruited from the Ahafo Ano North, Atwima and Asante Akim North districts of Ghana and active Mu disease was diagnosed by microscopy for AFB, culture for *M. ulcerans* (Mu) and PCR on 4 mm punch biopsies, fine needle aspirates or swabs. Subjects with Buruli lesions that were <15 cm in maximum diameter were randomised to receive RS8 or RS2RC6. Two further biopsies were obtained both at week 6 and 12 if the lesion was still ulcerated. One biopsy was cultured for Mu by a semi-quantitative method and the other was stored for measurement of mycolactone concentration. Whole blood was obtained at the same time points for stimulation with Mu antigens and quantification of cytokine IFN γ release. Clinical response was assessed 2 weekly by measuring the surface area of lesions until healing was complete. Subjects will be followed up monthly for 12 months after treatment.

Results

Out of 83 patients recruited all of whom had a positive PCR for Mu, 40 were randomised to receive RS8 and 43 to receive RS2RC6. The two groups were well matched demographically. All lesions were <15cm in diameter but 4 in the RSRC group and 1 in the RS only group had multiple lesions classified as category III. In January 2010, 74 patients had completed antibiotic treatment and there was no difference in the proportion healed in each group after 4, 8, 12, 16 or 20 weeks.

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

Conclusions

In this interim analysis the evidence suggests that there will not be any difference in healing of small Buruli lesions using RS8 compared with RS2SC6 but the final outcome will not be available for a few months. The number of recurrences up to 12 months in the two groups will be crucial in this analysis since clarithromycin is bacteriostatic for Mu compared to the bactericidal action of aminoglycoside antibiotics. Interpretation of the culture data is not possible until tissue mycolactone concentrations have been measured and the immune response to Mu evaluated serially. It remains possible that although some lesions are still culture positive during and after antibiotic treatment, the organisms are unable to produce mycolactone in significant quantities and this will be investigated in ongoing studies.

Acknowledgement

Support is gratefully acknowledged from the European Foundations Initiative on Neglected Tropical Disease (NTD)

Potential of rifapentine in the standard treatment for Buruli ulcer

Presenter: Dr Deepak Almeida

Deepak V. Almeida, Paul J. Converse, Si-Yang Lee, Eric L. Nuermberger, and Jacques H. Grosset. Johns Hopkins University Center for Tuberculosis Research, Baltimore, MD, USA

Background

The standard WHO recommended treatment for Buruli ulcer (BU) is rifampicin (R) plus streptomycin (S) injections given daily (7 days a week) for 8 weeks. Rifapentine (P), a rifamycin derivative with a long half life has shown promising results in treatment shortening experiments in the murine model of tuberculosis (TB) infection. In a previous experiment, P given alone was found to be much more active than R given alone. We hypothesized that the substitution of P for R in the standard treatment of BU would help in treatment shortening.

Material and methods

355 Balb/C mice were infected in the right hind footpad with *Mycobacterium 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 25 days with mice randomized to one of the 4 treatment groups which were, (i) untreated controls, (ii) SR given 5 days/week (5/7) (iii) SR given 7 days/week (7/7) and (iv) SP given 5 days/week (5/7). R and P were given at a dose of 10 mg/kg by oral gavage and S was injected subcutaneously at 150 mg/kg.

For each treatment group, the duration of drug administration was 1, 2, 3 or 4 weeks. On treatment completion 5 mice were sacrificed for CFU and 20 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 ≥ 2 , i.e., exhibiting definite swelling and inflammation of the foot.

Results

The day after infection the CFU counts were $3.73 \pm 0.12 \log_{10}$. At the start of treatment, the mean \log_{10} CFU count/footpad was 4.88 ± 0.51 . At the end of one week treatment, the \log_{10} CFU counts in untreated controls were 5.72 ± 0.68 and there was not much difference between different treatment groups as the \log_{10} CFU were 3.81 ± 0.45 in SR 5/7, 3.82 ± 0.19 in SR 7/7; and 3.24 ± 0.45 in SP 5/7. By the end of 2 week however the CFU counts in mice treated with SP 5/7 had dropped to 1.6 ± 0.96 with 1 out of the 5 mice culture-negative for CFU, while those in SR 5/7 and SR 7/7 were 3.32 ± 0.5 and 2.87 ± 0.33 , respectively. At the end of 3 weeks, all SP treated mice were negative for CFU, while in SR 5/7 it was $1.5 \pm 0.43 \log_{10}$ CFU and in SR 7/7 it was $1.3 \pm 0.87 \log_{10}$ CFU with 1 out of 5 mice culture-negative. At 4 weeks all SP 5/7 mice were culture negative, while in SR 5/7 the CFU count was $1.4 \pm 0.74 \log_{10}$ CFU and in SR 7/7 it was 0.52 ± 0.59 with 2 out of 5 mice culture-negative. All mice are now under observation for median time to footpad swelling, in other words to determine the curative potential of each regimen.

Conclusion

The combination of SP given 5/7 shows promising activity in CFU reduction and converted all mice to culture negativity by the end of 3 weeks compared to the standard regimen of SR 5/7 or 7/7 which were still culture positive at the end of 4 weeks of treatment.

‘Paradoxical’ immune-mediated reactions to *Mycobacterium ulcerans* during antibiotic treatment; a result of treatment success not failure

Presenter: Dr Daniel O'Brien

Authors: Daniel P O'Brien^{1,2}, Micheal Robson³, Peter Callan⁴, Anthony McDonald⁴

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Background

Recent evidence has demonstrated that antibiotics have an effective role in treatment of *Mycobacterium ulcerans* and their use is now recommended. However clinical deterioration during antibiotic treatment can occur and may be interpreted as treatment failure leading to further expensive and potentially disfiguring surgery, and a change in antibiotic regimens or a prolongation of their course.

Case Descriptions

We present clinical descriptions of the treatment of *Mycobacterium ulcerans* from an endemic area in the Bellarine peninsula of south-eastern Australia where initial improvement on antibiotic therapy was followed by a paradoxical worsening in the clinical appearance. This was initially interpreted as treatment failure, but we believe was subsequently shown to result from an immune mediated reaction to effective antibiotic treatment.

The evidence for a paradoxical reaction in our cases included: a) persisting *Mycobacterium ulcerans* organisms in affected tissue following initial surgery, b) initial clinical improvement in the *Mycobacterium ulcerans* lesions on antibiotics followed by significant clinical deterioration 1-2 months after their commencement, c) negative cultures of aspirates and excised tissue at the time of the paradoxical reactions suggesting that remaining mycobacteria were not alive as would be expected in a lesion deteriorating due to uncontrolled infection, d) a lack of mycobacteria seen on microscopy of the excised tissue at the time of the paradoxical reaction compared with active lesions that usually show large numbers of extracellular mycobacteria, and e) the presence of significant inflammatory reactions on histopathological examination of excised tissue.

We propose that rather than a progression of the lesions due to failure of antibiotic treatment, these cases represent an adverse consequence of effective antibiotic treatment. It is likely that antibiotics facilitate this immune reaction by killing the mycobacteria and therefore reducing the production of the immunoinhibitory exotoxin mycolactone and also by liberating mycobacterial antigens from dead organisms. This allows an intense immune reaction to occur with a paradoxical worsening of the clinical state. Recognition of this phenomenon can prevent unnecessary antibiotic regimen changes and potentially obviate the need for, or reduce the extent of, further surgery.

Conclusions

Clinicians should be alert to the possibility of paradoxical reactions occurring during the antibiotic treatment of *Mycobacterium ulcerans*. We would recommend when initial improvement on antibiotic treatment is followed by clinical deterioration that if possible clinicians perform histopathological examination and mycobacterial culture of involved tissue to assess for the possibility of a paradoxical reaction.

Assessment of rate of healing of Buruli ulcer

Presenter: Dr Stephen Sarfo

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Introduction

Several methods have been used to assess the response of Buruli ulcer to antibiotic treatment the most common of which are time to 50% or complete healing which does not take account of the initial size of the lesion. The rate at which Buruli ulcers heal may depend on the type of lesion, the burden of infection with *M. ulcerans* and the nutritional state of the patient among other factors. In theory it is expected that ulcers will heal at the same rate once infection has been eliminated. Our studies have shown that some lesions remain infected after antibiotic treatment but they have nevertheless healed without recurrence. We have used serial measurements of the mean diameter of ulcers to study the rate of healing in a series of patients during treatment with antibiotics as recommended by the WHO.

Methods

Mean diameter was calculated by measuring the maximum diameter of the ulcer and the diameter at right angles to that from tracings taken at the start of treatment. Rate of healing was calculated by dividing the initial mean diameter by the number of weeks to complete healing giving a rate in millimetres per week. The patients were part of a series undergoing standard antibiotic treatment with rifampicin 10mg/kg and streptomycin 15mg/kg daily for 8 weeks. Mann-Whitney's U-test was used to compare the median rate of healing.

Results

42 patients had ulcers that could be measured reliably, confirmed to be BU by PCR. Fourteen were category I (<5cm), 22 category II (5-14.9cm) and 6 category III (≥15). The median time to healing of category I, II and III ulcers were 12 weeks (range 4-15), 11 weeks (4 – 24) and 31.5 weeks (6 – 39). Rate of healing varied between 1.6 and 28.7 mm/week with a median of 4.6 mm/week. For category I, II, and III ulcers, the median rate of healing was 3.1 mm/wk (range 2.6-9.3), 6.4 mm/wk (4.3-22.0) and 7.0 mm/wk (2.2-28.7) respectively with category I ulcers healing significantly more slowly than category II (p<0.05) (figure 1).

In order to see whether all ulcers healed at the same rate after completion of antibiotic treatment, 8 ulcers that could be measured reliably at 8 weeks or more were analysed separately. Most ulcers healed more rapidly after antibiotic treatment but there was considerable variation in the rate; median healing rate 2.0 mm/wk (0.4–8.6) from 0 to 8 weeks and 4.0 mm/wk (1.4–9.0) after completion of antibiotics (p>0.05) (Figure 2).

Conclusion

Healing rate of Buruli ulcers varies both during and after antibiotic treatment and further work is required to investigate the causes of delayed healing. It may be important to assess rate of healing when comparing the efficacy of different antibiotic regimes.

Figure 1

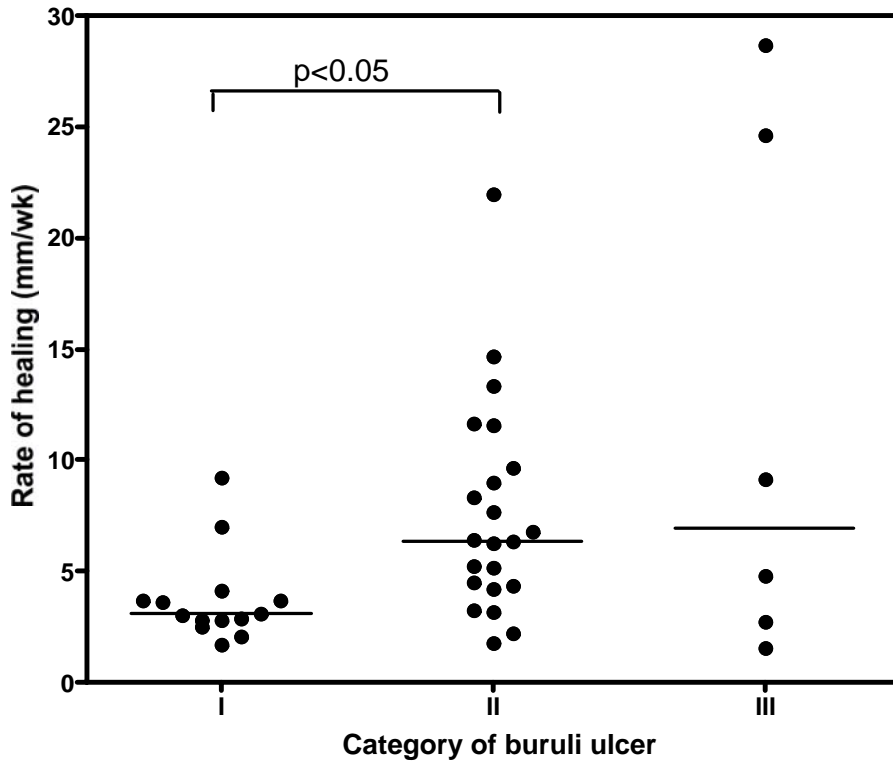
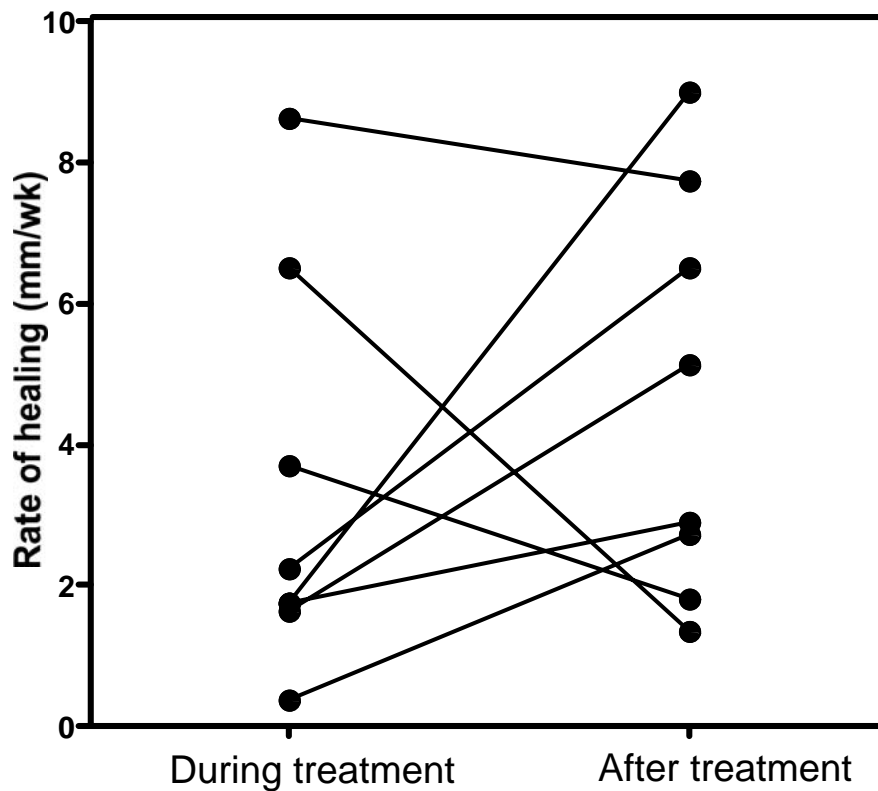


Figure 2



Antibiotic use in the management of Buruli ulcer: Rapid assessment of treatment outcome; Ghana, 2009

Authors: Edwin Ampadu¹; William Opare²

¹Programme manager;

²Technical Assistant, National programme

Introduction and rationale

Ghana initiated antibiotics treatment of Buruli ulcer the WHO recommended medicine in 2005. The national advisory committee on Buruli ulcer supported the move after the WHO produced provisional guidelines for use.

Six hospitals started and today we have over 49 treatment centres [*hospitals=20 and health centres/ community clinics=29*] giving a wider scope of decentralization.

For this report, the national office carried out an assessment work in 14 Buruli ulcer treatment facilities which give us 70% of total cases detected in a year for the last five years. This covered 4 of the 6 endemic regions of the country; Ashanti, Eastern, Central and Greater Accra.

The facilities include :

1. Agogo Presbyterian Hospital, Agogo
2. Amasaman Hospital, Amasaman
3. Nkawie Hospital, Nkawie
4. St Martin's hospital, Agroyesum
5. Dunkwa Hospital, Dunkwa
6. Tapa hospital, Tapa
7. St John's Community clinic, Via Dunkwa
8. Asuboi, Health centre, Asuboi
9. Pakro health centre, Pakro
10. Obom health centre, Obom
11. Presby community clinic, Assin Nsuta
12. St Peters; Catholic hospital, Jacobu
13. Kojo Ashong Community Clinic
14. Global Evangelical Mission Hospital

Implementation

Within the span of four years all the 49 treatment centres have been trained in case management, use of antibiotics and early case detection. These were made possible through the efforts of the national programme and some of the local and international NGOs; World Vision International, Health Foundation, Ghana, Nestle Ghana Ltd, ANESVAD, MAP international.

Training

In all an average of 4 major training per year were carried out over the last 4-5 years. We have covered over 50 treatment centres and districts and currently we have 49 in full scale treatment

Logistic support

The national office provided Antibiotics, syringe and needle, water for injection, specimen containers, and surveillance forms to support case management.

Monitoring activities

The strategy adapted since the introduction of antibiotics is to decentralize monitoring activities and visits. The national teams occasionally carried out some visits to ensure that case management is carried out and to identify and address issues challenging the disease control activities.

Treatment outcomes analysis

Total Buruli ulcer cases recorded national	851
No of cases from the 14 selected Rx centres reviewed	692
Cases from the studies facilities that were documented	69%
Cases that completed their treatment [56 doses in 70 days]	55.58%
Cases that that healed without surgery	22%
Cases that got healed without joint movement limitation	20%
Defaulters' recorded	1%
Those lost to follow ups	6.32%

Trend of dosing

1-10 doses of WHO recommended 56 doses antibiotics	5.48%
10-30 doses of WHO recommended 56 doses antibiotics	13.69%
30- 55 doses of WHO recommended 56 doses antibiotics	10.95%
56 doses of WHO recommended 56 doses antibiotics	57.9%

Any serious side effect

Treatment failure were seen in 2 patients who were later diagnosed as having HIV
Occasional dizziness and when the dosage is reduced patients are ok.

Challenges

Administrative

Drug administration [injections, dosing and chatting] needs some improvements
Out patients on treatment often default with trivial reasons.
Patients difficult to trace if they default
High attrition rate of health staff from the endemic centres

Patients' access to care

1. Geographical Access to treatment

High proportions of patients continue to have difficulties to access treatment. Communities are very far and inaccessible to treatment centres.

Cases continue to report late.

2. Financial access to treatment

Even though health insurance now covers 'Buruli ulcer', most patients remain poor and cannot afford the insurance premium

Most BU treatment facilities don't offer inpatients feeding services and patients have to do it themselves.

Other challenges

Filling of client cards and other forms poorly done in most cases- Not completely filled

Decision as to when to carry out surgery while on antibiotics is a challenge to some clinicians. Some want completion of medication before surgery even when the lesion is responding enough. Others too want to commence surgery but the question is when while treating?

Proposed solutions

1. As a result of high staff attrition, there is the need to constantly build human capacity and encourage them to stay
2. In order to encourage better health seeking practices particularly among the rural folks in areas where Buruli ulcer is common, there is the need to assist communities pay for health insurance premium to enable them easily and early access healthcare.
3. Initiate early detection programmes to minimize severity of lesions presented
4. More local NGOs encouraged to initiate rural health activities to support disease control.
5. Increase pro poor interventions to support victims of Buruli ulcer
 - i) Creating enabling environment for community easy access to treatment. [enabling patients to receive treatment despite no matter what]

Conclusions

The antibiotics treatment is proving very effective particularly for early lesions; however, national programme continues to record large proportion of patients reporting with late stages lesions. Each case detected early could effectively maximize the antibiotics use without surgery. The cost is minimal and the duration could be very short.

The main intervention is to step up early case detection interventions and encourage district health directorates to support this initiative without waiting for national programme to initiate. This is our ultimate!

The treatment of Buruli ulcer with antibiotics: Simultaneous monitoring of patients at a number of diagnostic and treatment centres

Author : Dr Alphonse Um Boock

Introduction

Since 2005 the management of Buruli ulcer has been revolutionized by the introduction of a treatment using a combination of streptomycin and rifampicin. Previously, the sole option was surgery with all its attendant problems.

This advance has made it possible to reduce the number of relapses nationwide and improve access to treatment through the increasingly popular option of decentralized care.

In Cameroon, the (unpublished) national survey conducted by Um Boock et al. in 2005 pinpointed **x** foci, namely:

1. The Nyoung basin in the central region.
2. The Bankim health district in Adamaou province.
3. The Mbongwe health district in the south-west region.
- 4.

For best possible implementation, diagnostic and treatment centres have been established and made operational in these areas.

Decentralized care has led to a large increase in the number of diagnostic and treatment centres, and bearing in mind the great distances that separate them, it has proved necessary to monitor treatment on the basis of well-organized coordination between the various centres. A toolkit has been developed to improve the management of patients at all levels of care; accordingly, this presentation aims to share the experience of Cameroon with other stakeholders involved in the management of Buruli ulcer.

Organization of BU management in Cameroun

The management of Buruli ulcer in Cameroon follows the principles recommended by WHO, namely:

- Awareness-raising
- Suspected cases identified clinically and confirmed by Ziehl Neelsen stain and PCR.
- Treatment with antibiotics for 8 weeks depending on the case, with the options of rehabilitation or surgery.

Forms recommended by WHO (BU01 and BU02) are used.

Treatment support activities are available such as nutritional supplements and making up lost school time, specifically at referral diagnostic and treatment centres such as Ayos and Akonolinga.

The Cameroonian system is increasingly decentralized; all levels of the health pyramid are involved in the treatment of patients:

Peripheral level:

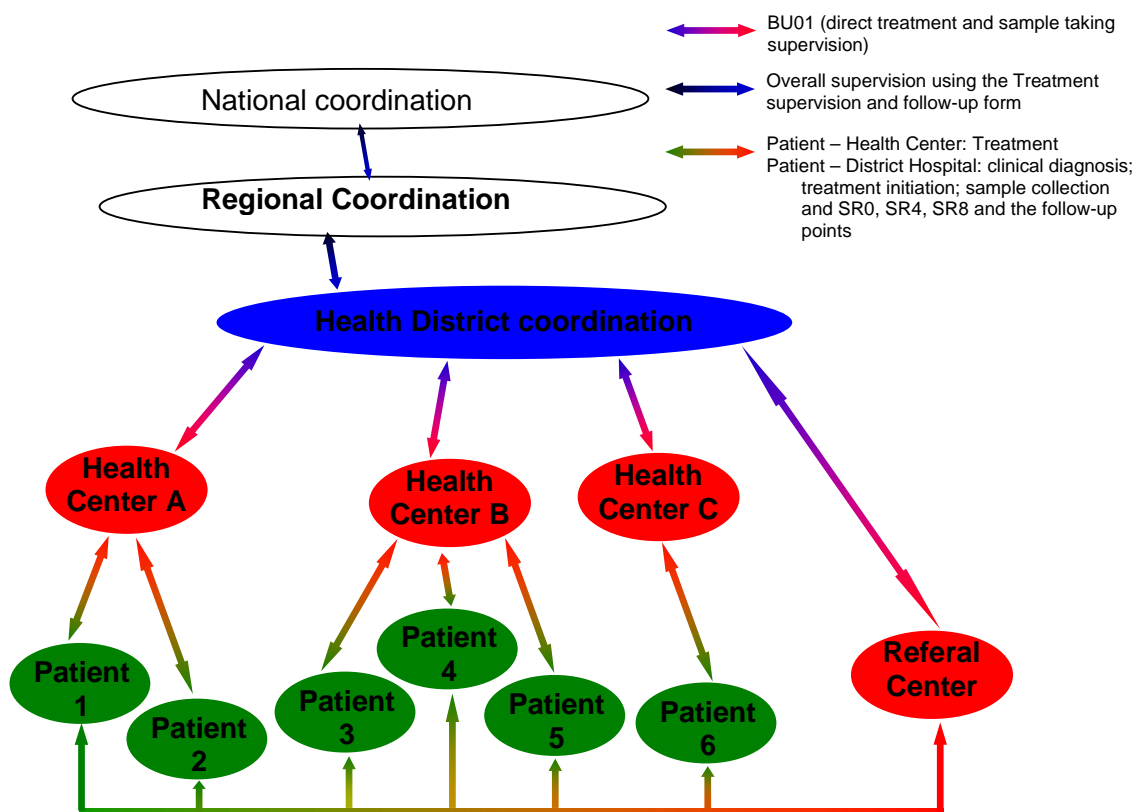
Health centres.

Responsible for raising awareness and identifying suspected cases. Apply dressings and administer antibiotic treatment.

May also supervise functional rehabilitation if required.

Cases requiring surgery are referred to the district hospital.

Organizational plan of coordination and supervision



Referral level:

Manages patients referred by health centres or patients reporting spontaneously.
Performs skin grafts and complex rehabilitation activities.

Health district service level:

Collates data transmitted by treatment centres and provides supervision. Transmits processed data to the regional health authority.
May also supervise diagnostic and treatment centres if required.

Health region

Conducts regional overview and supervises endemic health districts.

National level

Receives national data and supervises reporting facilities.

Strengths of the system:

- Decentralization brings treatment closer to beneficiaries
- Accountability of health stakeholders at all levels
- Increase in the number of cases under treatment

Weaknesses:

Poor data quality, complicating supervision by higher levels
Decline in quality of treatment

Challenge:

Maintain high-quality treatment through well-structured supervision

To this end we have devised a new patient management system that not only indicates the number of patients at each diagnostic and treatment centre but also provides information about their treatment.

Use of the treatment and follow-up supervision form

The form is completed every month at diagnostic and treatment centres (only the section shaded in grey) and forwarded to the district level, together with the result of the Ziehl Neelsen stain and the clinical examination.

The health district is the first level of coordination and can therefore complete the other parts of the form. The form completed at the district level is sent to the region. The health region is thus able to schedule follow-up treatment without interfering in the work of the health district.

The same procedure is used at the national level, where the national overview is prepared.

Advantages of the form

A tool that provides monthly and simultaneous information on various patient cohorts at different centres according to their recruitment throughout their follow-up period.

Avoids supervision scheduling interference at different levels of the health system.

Results

	No. of cases detected	PCR	Lost to treatment	Cured without sequelae
2008	382	30% (n=103)	10% (n=37)	70% (n=242)
2009	432	82 % (n=354)	1% (n=6)	82% (n= 364)

Percentage of cases confirmed by PCR:

There have been more confirmations by PCR this year because all the cases were relatively familiar to all levels of supervision. Previously, following up confirmation was quite problematic because the data were opaque and difficult to process at the various levels of monitoring and supervision.

Number of cases lost to treatment:

A decline was noted in 2009 thanks to this very systematic follow-up. Most defaulters were tracked down and resumed treatment.

Cures without sequelae:

The quality of treatment has improved overall.

Conclusions

Given the pyramidal structure of the national health system, the decentralization of BU treatment imposes quite a considerable coordination/monitoring/supervision burden, without which the quality of treatment would be severely jeopardized.

The introduction of this comprehensive approach has enabled us to remedy the problems observed in 2008.

Improvement in the quality of treatment of lesions needs to be controlled and adapted

The collective team work of nursing staff and 2 student nurses from Geneva, enabled the development of simple reference tables for daily use of available products at all stages of treatment of wounds. The Buruli d'Akonolinga Ulcer Pavillon in Cameroun.

Authors: Dimitry Madoery and Paul Bobbink, 3rd year student nurses, Bachelor of the Haute Ecole de Santé, Geneva (HEdS-Ge) in collaboration with Dominique Bidet-Dazin, Head of Studies HES (HEdS-Ge)

Introduction

Pain has no useful goal if it is not taken into consideration in the treatment. It can even bring about the exhaustion, both physical and mental, of the patient so that he will not follow regularly his treatment or return for consultations. It could hamper the recovery or at least prolong the healing time.

During the training course of 6 weeks at the The Buruli d'Akonolinga Ulcer Pavillon in Cameroun, one of their objectives was directed towards evaluating pain during treatment of the wounds and how to help the medical staff to reduce the pain. They had a certain control of the subject following an experience in the field of pediatrics in Geneva.

What was done

Firstly, the students were attentive and respected the way of working of their colleagues. The stages were:

- observation, evaluation of the available material and medication, for dressing wounds with the team
- animation of sessions on remarks on actions performed, seminars with exchanges of knowledge and practice
- suggestion of tools for evaluating pain and their use

Results obtained

- participation of medical staff and the team's anesthetist on their thoughts on the subject:
- utilisation more or less supported by evaluation cards
- self evaluation and reflexion on difficulties encountered
- direction of the team towards the less evasive choice possible, more particularly for children

Challenge

- duration of training course for working on a certain subject
- different cultural approaches to pain
- differences in access to resources

Proposed solutions

- improve the use of a shower for removal of dressings which can be a moment of acute pain
- elaboration of two reference tables on the proper use of (essential) available medication for reducing pain – one for adults and the other for children

Conclusions

This work is supported by the referent doctor UB of MSF, Geneva, who could assure the long term continuation.

The importance of continuing to support the work for change in the funding of treatment of wounds (asepsis, prevention of secondary infections, good use of products at the right moment,...) whatever the conditions and means at disposal.

Contribution following the training project for treatment of wounds at the Buruli d'Akonolinga Ulcer Pavillon in Cameroun and the impact of the training course of the two nursing students of the Haute Ecole de santé of Geneva

Authors: Dominique Bidet-Dazin, Head of Studies HES (HEdS-Ge) and Dimitry Madoery et Paul Bobbink, 3rd year Bachelor student nurses, of the Haute Ecole de Santé, Geneva (HEdS-Ge)

Introduction

The channel for training in nursing care at the Haute école de Santé de Genève (HEdS-Ge) and its camerounaise partner, l'Université des Sciences Infirmière, (University of Nursing Sciences) Yaoundé have established a training course for the exchange of knowledge on the treatment of wounds and their scares.

In 2009, during a training course of 6 weeks at the Ulcer Buruli Pavillon, Akonolinga in Cameroun, two nursing students benefitted from being the first to experience the course in a structure and approach to wounds very different from those they experienced during the course of their studies. This first stay proved positive for the programme for improving the responsibility for changing dressings, put in place with the collaboration of MSF Geneva.

What was done

After preparation in Geneva, led by a clinical nurse for wounds of the HEdS-Ge the referent doctor UB of MSF, Geneva, the two 2 second year Bachelor students in nursing care left with the objectives of supporting the programme put in place:

- asepsis during changing of dressings:
- hospital hygiene and prevention of infection:
- integral part of pain for treatment of wounds.

These points had been discussed and made evident during the last stay of the instructors. They had received the same training as the adviser in Cameroon based on the fundamentals of healing in a humid atmosphere.

Results obtained

- the duration of the course was longer as the presence of the instructors allowed a real collaboration between the medical staff
- important involvement needed of the students allowed them to progress in the treatment of wounds by adapting to a humid environment
- a dynamism was created in the heart of the team of the Pavillon UB thanks to the collective work for searching for a solution for a quality in nursing care

Challenges for initiating the course

- do not lose sight of the first goal of the programme which is to introduce ways of improving the initiation of the course
- to allow student nurses from Geneva to integrate into a programme already in progress dealing with a different and unknown subject
- give them sufficient support during the course to help them aim for working on building exchanges between nursing students.

Suggested solutions

The first idea was above all to support exchange during the two training sessions in 2008. The 2 students were able to help the medical staff to be more precise in applying the basic principles, such as asepsis, in redressing wounds, but also to find the best ways to continue the work in a precise manner.

Conclusions

The interest of this first course was that training be given over certain period of time but does not mean that the hoped for and initiated changes will continue, time given for daily work in the field is indispensable in order to bring real help to the teams of curers in “difficult” circumstances. The results brought by the different partners showed this. We hope to continue these stays.

Hyperbaric oxygen therapy in the treatment of Buruli ulcer

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Context

Tissue loss due to *Mycobacterium ulcerans* and surgical excision of Buruli ulcer is normally managed by dressings that promote granulation of the subcutaneous tissues, followed by skin grafts.

Common phenomena during the scarring process are SECONDARY INFECTIONS, GRAFT REJECTIONS, and DELAYS IN SCAR FORMATION, all of which can prolong hospitalization.

Considering the benefits of hyperbaric oxygen therapy (HOB) in the scarring and secondary infection process, it has been introduced into the BU treatment protocol.

Method

We have assessed the contribution of HOB in the treatment of BU using precisely defined scarring process criteria in two cohorts of patients participating in a case control study. The patients received 45 cycles of oxygen therapy at 2.5 atm.

Results

- Shorter duration of pre-grafting period: Mean duration of pre-grafting period was 9 days in the exposed group (A) compared with 14 in the control group (B).
- Skin grafts were accepted in 91.16% of cases in the exposed group compared with 72.27% in the control group.
- Secondary infection was observed in one patient in group A compared with 3 in group B.
- Mean scarring time was 35 days ($28 < Q < 44$) in group A compared with 45 days ($37 < Q < 62$) in group B.
- No keloids were observed in group A, whereas keloids were observed in 24% of cases in group B.
- Use of HOB avoided more amputations in cases of bone and joint involvement.

Major challenges and future action

- The principal problems were:
 - Cost of procuring oxygen
 - Maintenance of equipment
 - Reliability of electrical supply
- Future action:
 - Increase sample size
 - Management of soft and bony tissue conditions of infectious or ischaemic origin.

Conclusion

Use of HOB accelerates the process of scarring of tissue lost to Buruli ulcer by promoting:

- Good tissue granulation prior to graft neovascularization
- Protection against secondary infections after excision of necrosed tissues
- Good acceptance of grafts

Degree thesis about use of oxygen ozone for treatment of “Buruli Ulcer”

Author : Mrs Fiammetta Benetton

Miss. Silvia Romagnoli, in July 2008, decided to draw up her degree thesis in “Medical Professions in Nursing Sciences” using the experiences and the scientific data supplied by “O₃ For Africa” ONLUS.

This document is the first official scientific treatise about our work and it has taken on particular significance because it is written in the university area.

The nursing protocol is registered at the Polytechnic University of Marche, Faculty of “Medicine and Surgery”. The complete title is: “Implementation of a nursing protocol about the use of oxygen ozone therapy for Buruli Ulcer in the Ivory Coast”.

This scientific literature discovered that:

Diagnosis

In an endemic area, an experienced health worker can diagnose Buruli ulcer, keeping account of a series of clinical reasons.

The following clinical/epidemiological characteristics are important diagnostic indices:

- Most of the patients live or have lived in a known endemic area;
- Most of the patients are children under the age of 15;
- About 85% of the lesions are found in the limbs;
- Lesions in the lower limbs are twice as common as those in the upper limbs;
- The non-ulcerative lesions are almost always painless whereas the ulcerative ones are only painful in the presence of a secondary infection of the lesion;
- In the ulcerative lesions, without secondary infections or superinfections, constitutional symptoms (such as fever) are not present;
- It does not affect the lymph nodes.

The diagnosis of *Mycobacterium ulcerans* is confirmed when at least two of the following laboratory tests are positive:

- Ziehl-Neelsen microscopic test;
- Positive *Mycobacterium ulcerans* culture, which takes at least 6-8 weeks or more;
- Histological study of an excisional biopsy sample;
- PCR (Polimerase Chain Reaction) for the DNA of the *Mycobacterium ulcerans* (Agbenorku 2001).

Therapy

Until a short time ago the only therapy that had been used for curing Buruli ulcer was surgical treatment, which was carried out by excising the lesions with a large margin in order to guarantee complete removal of the visually infected tissue or by amputating the limb.

However, recent studies have established the use of some antibiotics for managing this disease.

The need for a change is clear from two observations in particular: On a practical level, surgery is only accessible to a fraction of patients, as can be deduced from the high number of people with large ulcerations due to the advanced state of the disease from *Mycobacterium ulcerans*, especially in tropical rural areas where the disease is endemic.

On a scientific level, it has been shown that the infection from *Mycobacterium ulcerans* stretches beyond the margins of the lesions, into the healthy tissue, where surgeons usually excise for removing the lesion, hence a relapse is likely, despite good surgical technique. In fact, the relapse rates after surgical intervention vary from 6% to 17% according to the type and extension of the lesion and the surgeon's experience.

Publications on controlled clinical studies for the antibiotic treatment of Buruli ulcer are very rare and, until a short time ago, anecdotal evidence of doctors with experience in managing this disease claimed that antibiotics used against other diseases from mycobacteria were ineffective.

In vitro, *Mycobacterium ulcerans* has shown to be susceptible to rifampicin, aminoglycosides, macrolides and chinolones.

Sensitivity tests carried out on infected mice have actually shown that, after treatment with antibiotics, the lesions became smaller and that the total number of *Mycobacterium ulcerans* cells in the tissue reduced. The combination of rifampicin with amikacin or streptomycin was the most effective in preventing a relapse after 12 weeks of treatment, suggesting a bactericidal effect, even if the *Mycobacterium ulcerans* culture did not confirm this study.

Subsequent experiments have shown that, after 7 weeks of treatment with the rifampicin and amikacin combination, no living bacterium was left in the tissue of the laboratory mice.

As well as the efficacy, another reason for the combination of more than one drug is to prevent pharmacoresistance, such as that which occurred after therapy experiments with just rifampicin, which developed mutants for resistance to that drug.

Recently, the combination of rifampicin with streptomycin (table 1) has been evaluated for its ability to kill *Mycobacterium ulcerans* at the start of the human lesion. Treatment with rifampicin 10 mg/kg by mouth and intramuscular streptomycin 15 mg/kg was administered for 0 or 2 or 4 or 8 or 12 weeks and the lesions were cut and excised in order to carry out a culture. The results were very clear and showed that the culture of the tissue cut from the ulcer without treatment and from the one which had treatment for just 2 weeks were positive for *Mycobacterium ulcerans*, whereas the others were not. This indicates that treatment for at least 4 weeks with this combination of antibiotics could be useful for managing nodules and plaques (the only two types of lesions included in this study).

Table 1. Dosage of rifampicin and streptomycin based on the patient's body weight. Source: OMS 2004.

Weight of patient (kg)	Rifampicin (300 mg/tablet) ^b		Streptomycin ^c (1 g/2 ml)	
	Dose (mg)	No. of tablets	Dose (g)	Volume (ml)
5-10	75	0.25	0.25	0.50
11-20	150	0.50	0.33	0.70
21-30	300	1.00	0.50	1.00
31-39	300	1.00	0.50	1.00
40-54	450	1.50	0.75	1.50
> 54	600	2.00	1.00	2.00

Further encouragement came about through the clinical observation and measurements, which showed that no lesion increased in size during treatment and that, in actual fact, most had reduced in size.

However, this data has not shown that the disease from *Mycobacterium ulcerans* can be cured by antibiotic treatment, because the lesions were excised in any case; only the ideal duration for the treatment can be identified.

Subsequently, the OMS advisory group on Buruli ulcer issued some guidelines for clinicians suggesting the administration of rifampicin and streptomycin for out-patients of all ages, with early lesions, for 8 weeks, reserving surgical intervention for just two scenarios: surgical incision for patients who need it for lesions that keep growing despite antibiotic therapy and, skin grafts for accelerating the healing process of large ulcers.

These guidelines have been formulated based on the evidence of patients treated in Benin; they have shown that most ulcers healed but not necessarily after 8 weeks and that the relapse rate is less than 3% a year after the end of the treatment (Wansbrough-Jones 2006; Table 2).

Table 2: Categories and treatment aims, level of diagnosis and health care system required.
Source: OMS 2004.

Cat	Form of disease	Treatment	Primary aim	Secondary aim	Level of health-care system	Diagnosis
I	Small early lesions (e.g. nodules/papules/plaques & ulcers less than 5 cm in diameter)	For papules & nodules, if immediate excision & suturing is possible, start antibiotics at least 24 hours before surgery & continue for a total of 4 weeks Otherwise treat all lesions in this category with antibiotics for 8 weeks	Cure without surgery except for simple removal of dead tissue	Reduce/prevent recurrence	Community, health centres & district hospitals (see Section 13.6)	Strong clinical diagnosis & laboratory
II	Non-ulcerative & ulcerative plaque & oedematous forms Large ulcerative lesions more than 5 cm in diameter Lesions in the head & neck region particularly face	Treat with antibiotics for at least 4 weeks, then surgery (if necessary), followed by another 4 weeks of antibiotics	Reduce the extent of surgical excision	Reduce/prevent recurrence	District & tertiary hospitals (see Section 13.6)	Strong clinical diagnosis & laboratory
III	Disseminated/mixed forms such as osteitis, osteomyelitis, joint involvement	Treat for at least 1 week before surgery & continue for a total of 8 weeks	Reduce <i>M. ulcerans</i> infection & dissemination before and after surgery	Reduce/prevent recurrence Reduce the extent of surgical excision	District & tertiary hospitals	Strong clinical diagnosis & laboratory

There are no effective prophylactic measures. The BCG vaccination seems to have some protective effects, but the brief duration of the protection provided would require repeated cycles of vaccination in the populations at risk. Health education is therefore extremely important for curing pre-ulcerative lesions (Youngsteadt 2008, Costa 2006).

Outcome

“Nursing protocol about the use of oxygen ozone therapy of Buruli Ulcer in the Ivory Coast”

STEP 1: ASSESSMENT.

Before being taken in charge each patient must undergo a clinical test and, where possible, a bacteriological test. The clinical test allows for the stage of the disease and the appearance of the lesions to be defined, in terms of extension, depth, presence or not of necrosis, colour of the tissue and smell.

The lesions are then measured and photographed in order to get an idea of the effectiveness of the therapy.

The bacteriological test is needed to verify the presence of *Mycobacterium ulcerans* and any association with other superinfections.

STEP 2: PREPARING THE EQUIPMENT.

Material required:

- Gloves;
- Distilled water
- Non-sterile gauze;
- Plastic tube to be connected to the ozone therapy apparatus;
- Polyethylene bag;
- Plaster;

STEP 3:

Connect the plastic tube to the ozone therapy apparatus; open the bottle of distilled water and make the ozone bubble through the water for about 5 minutes at a concentration of 30 µg/ml to disinfect the water.

STEP 4:

Wear the gloves and with the ozonated water clean the ulcer well and then leave it covered in gauze soaked in ozonated water for another 5 minutes.

STEP 5:

Remove the gauze, then wrap the area of the lesion with the polyethylene bag; insert the tube connected to the ozone-therapy machine and hermetically close the bag with the plaster.

STEP 6:

The ozone is insufflated directly into the bag through the tube at a concentration of 30 µg/ml until it is completely full.

This creates an ozone chamber in which the ulcer remains confined for about 20 minutes.

STEP 7:

Then remove the bag, possibly ventilating the room during this step. In fact, ozone is only dangerous if it is inhaled, as it dries out the mucosa of the respiratory tracts.

STEP 8:

After removing the bag, the ulcer must be entirely covered in gauze soaked in the ozonated water and covered with a plaster. This medication must be carried out daily, whereas the ozone-therapy sessions are repeated every two days. If possible, the medication is to be carried out using gauze that has been pre-soaked in hyaluronic acid and sodium salt, effective in facilitating tissue repair processes; this gauze can also stay in place for two days, until the following session.

SURGERY

WHO Integrated Management for Emergency & Essential Surgical Care (IMEESC) tool for capacity building

Presenter: Dr Meena Cherian

Emergency and Essential Surgical Care program, Department of Essential Health Technologies, Health Systems & Services, World Health Organization, Geneva, Switzerland

The WHO Integrated Management for Emergency & Essential Surgical Care (CD) has been developed by the WHO Emergency & Essential Surgical Care programme aimed at strengthening capacities at district and sub-district level of care. It targets policy-makers, managers, and health-care providers (surgeons, anaesthetists, non-specialist doctors, health officers, nurses, technicians and paramedics).

The WHO IMEESC toolkit is introduced in countries through WHO and Ministry of Health for use in the development of policy, training curriculum, emergency equipment lists for resuscitation, needs assessment, and as teaching tools (video, self learning, power point presentation, agenda, trainer's guide, report, best practices), research, monitoring & evaluation, and quality & safety. The tool is used to teach safety during clinical procedures, infection control and HIV prevention as well as management of emergency surgical care in disaster situations.

This tool contains WHO recommendations for minimum standards in emergency, surgery, trauma, obstetrics and anaesthesia at first-referral level health-care facilities, as follows:

Teaching & Reference Guidelines: Surgery, Emergency, Obstetrics, Anesthesia, Trauma & Orthopedics Surgical Care at the District Hospital manual; Surgical Care at the District Hospital manual HTML; Surgical Care at the District Hospital teaching slides; Evaluation of Self Learning

Training Workshops: Trainer's Guide; Model Agenda; Participants Evaluation; Sample Report of Training Workshop

Best Practice Protocols: Clinical Procedures Safety; Hand Washing Techniques; Waste Disposal for Clinical Procedures; Burn Management; Post operative care guidance; Post-operative Pain Management; HIV Prevention Protocols

Disaster Management Guidelines: Best Practice Guidelines on Emergency Surgical Care in Disaster Situation

Equipment Lists & Needs Assessment: Essential Emergency Equipment Generic List; Anaesthetic Infrastructure/Supplies; Needs Assessment for Essential Emergency Room Equipment

Policy Management: Aide-Memoire: Surgical & Emergency Obstetrical Care; Training Curriculum on Emergency & Essential Surgical Skills; Organization & Management

Quality & Safety: Safe Surgery & Safe Anaesthesia Protocols; Obstetric Safety Protocols; Monitoring & Evaluation

Research Tool: WHO Situation Analysis to Assess Emergency & Essential Surgical Care

CD Training Videos: General principles of wound management; Fracture management using traction and plaster; Open fractures, tendon injuries and soft tissues injuries; Fractures and dislocations of the upper limb; Fractures and dislocations of the lower limb and pelvis; Fractures in children; Head and spinal injuries

« Gaps in training of BU surgeons: the plans of the Institute for Reparatory Surgery (ICR), Abidjan »

Presenter: Dr Patrick Meredith

Authors: Prof. H. Assé, ICR, Abidjan, Côte d'Ivoire; Dr. P. Meredith, MF, Suisse

Surgery remains the **preferred option** for treating BU despite the very significant contribution made by antibiotic therapy. But surgery to correct this disease is still discounted by surgeons in endemic countries despite efforts to incorporate BU into the « minimum package of activities » of surgical units.

In Côte d'Ivoire, the total number of BU cases since the Yamoussoukro Declaration is estimated to be in excess of 25 000, with a rate of increase of approximately 2000 new cases a year. 60% require surgery as a first resort and 30% experience disabling sequelae. A significant number of cases therefore require reparatory treatment.

10 surgeons are currently trained to perform first resort surgery (approx. 1400 cases) and 2 are trained to deal with sequelae (approx. 500 cases). The latter operate on 50 cases a year at the ICR. There is clearly a shortage of first and second-resort surgeons.

Accordingly, with effect from 2007, the Institute for Reparatory Surgery (ICR) and the Meredith Foundation (MF) have developed a training programme for surgeons at 4 different levels:

- 1) Medical students: BU awareness-raising courses. Use of the library, premises and materials to complete doctoral theses on BU. Instruction in surgical techniques essential in the treatment of BU (excision and grafting).
- 2) Local general surgeons: 9 surgeons have been trained in the theory and practice of BU surgery, based on a WHO model course at Agogo.
- 3) BU surgeons: MF is covering the cost of a 1-month skills upgrading internship at the ICR for surgeons specializing in BU in their respective countries. The course aims to deepen the surgeons' technical knowledge via hands-on experience. They receive instruction in performing simple flaps and 2 pedicle flaps, thereby enabling them to perform some of the surgery necessary to correct sequelae. This is followed by an annual bursary designed to cover the costs of an assistant, whom the beneficiary will train to perform simple surgical techniques. The first beneficiary was Dr A. Condé in August 2009.
To mark its 10th anniversary in 2010, MF has pledged to offer 10 internships at the ICR (1 per semester).
- 4) Specialization in plastic and reconstructive surgery : 3 assistant physicians posts have been provided for at the ICR, where the above-mentioned skills can be directly applied in the treatment of BU sequelae.

Our plan is to establish a network of competent centres in endemic countries for training at tertiary level by increasing the number of annual internships and attracting funds for this purpose (5000 Swiss francs per internship).

By following this strategy we hope to improve the surgical management of BU and its sequelae.

Reconstructive surgery in Buruli ulcer: Benefits of field training missions

Presenter: Dr Rémy Zilliox

Authors: Dr Rémy Zilliox, Lyon (France); Dr Christian Johnson, Cotonou (Benin); Dr Ghislain Sopo, Allada (Benin); Dr Annick Chauty, Pobé (Benin); Dr Marie Françoise Ardant, Pobé (Benin); Dr Yves Barogui, Lalo (Benin); Dr Jean Gabin Houezo, Allada (Benin), Dr Ambroise Adeye, Pobé (Benin), Dr Ange Dossou, Allada (Benin), Dr Odry Agbessi, Cotonou (Benin)

The surgical management of Buruli ulcer (BU) is normally by means of plastic surgery in the form of excisions and grafts. This is especially true in the management of sequelae, when recourse is had to all manner of interventions including strips and expansions. Orthopaedic intervention is required where there is bone or joint involvement.

In many countries affected by UB, treating physicians are always keen to improve their surgical skills by learning new techniques that they can apply in daily practice. Unfortunately, postgraduate training of this kind is often hard to come by for want of time or financial resources.

In Benin, for example, at the proposal of the director of the National Buruli Ulcer Control Programme in Cotonou and under the auspices and with the assistance of WHO, a surgical training mission has been organized for the second year running at Pobé, Allada and Lalo hospitals.

By attending consultations, surgical demonstrations, case briefings and dressing sessions, each physician can thereby obtain answers to his or her questions.

First of all, the instructing surgeon may be in a position to provide innovative equipment or high-performance consumables, which are often in extremely short supply in many countries and hospitals.

The consultation provides an opportunity to discuss surgical indications in a collegial manner, but sometimes it is difficult to turn away patients seeking reparative surgery, particularly in connection with burns sequelae, who cannot understand the differential diagnosis for BU.

Even though the surgical demonstrations are performed under difficult conditions (shortages of equipment, power cuts, etc.), they must be fully comprehensive and use reliable and reproducible techniques.

The case briefings recapitulate core concepts such as hygiene and asepsis, theoretical aspects of surgical techniques, dressings, splints, orthopaedic appliances and physiotherapy.

The handling of postoperative care is a mark of confidence in the medical teams.

This type of personalized training in the field is available in all affected and interested countries, and is more cost-effective than large conferences that oblige all the participants to travel, including the patients themselves.

Capacity-building in training and management for the BU programme in Togo

Presenter: Dr Joerg Nitsche

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General information

Specimens were collected from approximately 1500 BU cases in Togo in 2005. These were merely suspected cases, unconfirmed by laboratory tests. Whereas neighbouring countries such as Ghana and Benin had already heeded the WHO recommendation to establish a National Buruli Ulcer Control Programme (PLNUB) several years previously, Togo had yet to implement a health programme for the detection, management and treatment of BU in affected areas.

In 2006 DAHW took the decision to include BU in its leprosy and tuberculosis programme as a third mycobacterial disease, with a view to capitalizing on this well-established and approved logistical and infrastructure network.

Implementation

The DAHW UB project was launched in September 2007 in the Maritime region in collaboration with Tsévié Hospital, which pilots national activities. Following the inauguration phase, the project was regularly monitored as follows:

1. Training workshop for

- hospital personnel, focusing on clinical diagnosis, management and current treatment;
- surgeons, focusing on surgical treatment and appropriate techniques for taking specimens by swab, FNA, punch biopsy and excision;
- physicians and surgeons in other district hospitals;
- the 40 leprosy and tuberculosis inspectors, enabling them to better recognize ulcerative cases such as BU after individual history-taking and examining the clinical aspect of the lesion;
- Villagers with a view to participation in BU awareness-raising;
- Training at the University of Lomé Mixed Faculty of Medicine and Pharmaceuticals

The following initiatives have also been introduced:

2. Standardized operating procedures

- Techniques for collecting specimens,
- Procedures for treatment of BU
- Use of microscopy at hospital laboratories,

3. Essential COLLABORATION with the external reference laboratory in Munich (DITM) for PCR diagnosis

- DETECTION activities in the region, AWARENESS-RAISING in schools
- BETTER care for disabled patients provided by HANDICAP INTERNATIONAL
- SCHOOLING in a hospital setting for young patients.

Results

Management, laboratory diagnosis and treatment are all in accordance with standardized WHO directives. Tsévié hospital has been designated the National Buruli Ulcer Reference Centre (CNRTUB). Thus, during the past 2 years, BU has been detected in 149 suspected patients, of whom 83 (55.7%) tested positive using PCR.

Challenges and future action

- The need to complete forms correctly is policed by ongoing and rigorous inspections (including by the PLNUB)
 - BU01 questionnaires and individual lab forms
- Intensification of case-detection, better treatment
- A laboratory equipped to perform PCR testing is being established in TOGO
- In the next few years, it will be necessary to meet the challenges of BuruliVac.

Conclusion

A two-year collaboration between DAHW, DITM/Munich and the PNLUB has resulted in the establishment in Togo of a well-regarded programme in accordance with WHO guidelines on managing BU patients.

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Pattern of Sequelae after Buruli Ulcer from a surgical point of view

Presenter: Dr Thomas Fischer

Plastic- Reconstructive- and Aesthetic Surgeon, Bern, Switzerland

Under the patronage of FAIRMED, formerly known as ALES, we perform surgical missions in Ayos, Cameroun, once a year, starting in 2007. In three surgical missions we consulted 172 patients with sequelae of Buruli Ulcer (BU) and operated on a selection of 66 Patients of medium and high complexity.

As literature about the biology and nature of the sequelae of BU from a surgical point of view is lacking we had to learn about this disease and the extent of its destruction step by step. We started our activities with the information that this disease tends to result in superficial tissue destruction, comparable to a third degree burn wound, which proved to be right only in selected cases.

Most certainly according to the biology of the *Mycobacterium ulcerans* we find a certain pattern of affection. We all know that the extremities are preferably involved. Among these we find mainly affections on the dorso – medio - lateral aspect of the elbow, in this area frequently involving deep layers including muscles of the flexor pronator group and / or the supinator – extensor - Group. In this area we frequently find a destruction of the triceps brachii tendon. Another area of predilection is the dorsum of the wrist and hand, frequently with destruction of the extensor tendons. On the lower leg we find destructions in the knee area as well as on the dorsum of the lower leg and foot. We never saw an affection of the palm of the hand or the planta pedis.

In our observation the destruction tends to affect deeper layers in areas of bradytrophic tissue like the extensor tendon complex of the dorsum of the hand and wrist, the dorsum of the foot or the patella region as well as the aponeurotic origin of the flexor pronator group on the elbow or the triceps surae. This pattern might reflect the preference of the *Mycobacterium ulcerans* for lower body temperature and lower oxygen partial pressure.

Frequently we find secondary contractures of adjacent but uninvolved joints as a consequence of muscle destruction and consecutive tendon contracture as for example the pes equinus due to a partial destruction of the triceps surae. Or we find contractures due to the loss of extension forces as for example in the knee where we find a contraction deformity due to the destruction of the (extending) patella-tendon.

In the evolution of our surgical technique and experience we started by splitting contracted scars and covering the defect by skin grafts or flaps. Over the time we had to learn that tissue destruction goes deeper than we thought and that severe contractures are a complex combination of different factors. As a consequence surgical corrections of complex sequelae of BU frequently need extensive scar dissection, Arthrolysis and Neurolysis – sometimes even Arteriolyse as well as tendon transection or elongation. And last but not least adequate tissue coverage. All this in combination with an adequate aftercare with intensive physiotherapy.

My presentation is a conclusion of clinical and surgical observations with no entitlement of scientific value.

« Proposal for classification of surgery for BU and its sequelae and treatment strategy »

Authors: Dr Patrick Meredith and Professor Henri Assé

WHO guidelines on the surgical treatment of the early stages of BU are clear. Following antibiotic treatment, the excision and suturing of nodules or even plaques is the standard approach. At the ulcerative stage, extensive debridement with two-stage grafting has shown promising results. However, coverage is different in cases where the tendon or joint is exposed because the graft no longer suffices. Likewise, the treatment of sequelae requires more than a superficial approach in view of the extent of tissue involvement.

At the ulcerative stage, extensive debridement with two-stage grafting has shown promising results. However, coverage is different in cases where the tendon or joint is exposed because the skin graft no longer suffices. Likewise, the treatment of sequelae requires more than a superficial approach. In order to be effective, the management of BU must be both preventive and curative. Preventive through better coding of the initial surgical treatment, and curative through the application of reparatory procedures that take into account the skin, muscular and aponeurotic contractures associated with this disease.

Routine examination of the exposed tissues at the ulcerative stage and the extent of involvement in sequelae would facilitate triage of patients with a view to the necessary treatment, enabling patients to be referred to the centre best suited to the treatment of their lesions. A flowchart is proposed for patient triage and treatment depending on the stage of the lesion or sequelae.

It is hoped that, in endemic countries, national BU programme coordinators will compile a register of centres and their specialisms in order to give effect to this triage. Where obvious gaps are noted, capacity-building strategies could be developed with assistance from WHO. In this way the surgical management of BU patients can be enhanced.

PREVENTION OF DISABILITY

Benefit of prevention of disability (POD) in a community-based Buruli ulcer (BU) control programme: Case study of the programme organized by Kimpese Evangelical Medical Institute, DRC

Presenter: Dr Désiré Imposo

Authors: Imposo Bofunga¹, Linda Lehman², Phanzu Mavinga¹, Kongawi Kinda², James Oehrig²

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Introduction

POD is an essential component of treatment for BU patients. When properly applied, it enables patients who have been cured to resume their daily activities, regain their autonomy and boost their quality of life. Under our control programme, POD was introduced in 2006 after hospital teams had received initial and subsequent training. POD was initially introduced at the hospital, involving patients and their carers, with a view to encouraging self-management upon discharge.

POD implementation in the community

The region covered by our programme comprises 2 health areas subdivided into 20 health districts. 56 nurses, 2 supervisors and 4 physicians based at health centres have undergone prior basic training in BU and attended a number of awareness-raising sessions. However, they have not benefited from specific training in POD.

Following the decentralization of BU control activities in September 2009, staff learnt how to assess limitation of movements for recording purposes on forms BU01 and BU02 and to encourage early movement of joints. In addition, they were taught how to monitor patients caring for themselves in the community.

Results

Thanks to the expertise of the occupational therapist, assessment of POD during supervisory visits reveals that this activity has not been properly integrated into the work of health centres. Some patients with incipient or mild limitation of movement have been classified on forms BU01 and BU02 as experiencing no limitation of movement. Consequently, certain cases must be referred to hospital following complications owing to inadequate POD treatment.

An ad hoc form was used for this assessment. Errors arose because the evaluation of the range of movement was not performed in comparison with the healthy limb.

Challenges

- Improve the POD skills of physicians, supervisors and nurses at health centres.
- Make available tools for more effective implementation of POD.
- Involve the new physiotherapist (the former physiotherapist having left the General Referral Hospital) in supervisory visits and training sessions in the community.

Proposed solutions

- Members of the programme team are endeavouring to raise awareness of POD among community health workers and provide appropriate training.
- A document promoting the wider use of POD **, taking the form of a 10-step guide, has been reissued to this end.
- After training, the new physiotherapist will initially work at the hospital before undertaking work in the community.

*EVANGELICAL MEDICAL INSTITUTE

** Document prepared by Linda Lehman

The role of community intermediaries in preventing disabilities caused by Buruli ulcer: from experience at Ayos to that of Ngoantet

Presenter: Ms Valérie Simonet

Authors: Valérie Simonet, Prévention des Incapacités, FAIRMED ; Dr Joseph Ekoum, national representative, FAIRMED Cameroun

Introduction

Thanks to the establishment of a rehabilitation service at Ayos, in Cameroon, since 2004 people affected by Buruli ulcer have benefited from case management covering all aspects of disability prevention. However, the question of their follow-up after discharge from hospital immediately arose, on account of the potential risk of loss of mobility during the period of scarring, which lasts approximately one year. The development of a means of providing them with follow-up in their village has also made it possible to shorten their stay in hospital and discharge them earlier, albeit accompanied and equipped with knowledge of how to prevent disabilities.

Implementation

A strategy to provide follow-up for persons affected by Buruli ulcer has been jointly developed by the Ministry of Health in Cameroon and Aide aux Lépreux Emmaüs Suisse (now known as FAIRMED). One of the solutions considered was to use and enhance the existing network of integrated health centres and community relays in order to involve them in disability prevention.

In 2005, officials responsible for the integrated health centres in Ayos district were briefed on prevention of disabilities caused by Buruli ulcer. However, on account of the relative inaccessibility of the facilities, especially for regular follow-up, the need for local support from community intermediaries soon became apparent. In 2006, they were provided with initial training in Ayos district; the purpose of the one-day training course was to enable them to identify a Buruli ulcer, to familiarize them with its case management and to explain to them their role in disability prevention.

Sharing tasks among the different actors and selecting them for their skills were probably the main challenges that had to be addressed in order to provide follow-up. Another important challenge was to interest health workers in these interventions and to ensure they fully understood their importance; depending on which health problems had priority in the villages, it was to be expected that the concept of disability prevention might prove hard to explain. A further challenge was the distances that have to be travelled on occasionally hazardous roads, either by the patients attending the health centres or by the health workers or community intermediaries to visit the patients' homes.

Results

At the end of 2007, an evaluation of follow-up activities in Ayos district showed that communities were little involved in follow-up, whereas they had been deeply involved in case-detection after they had been trained: the number of cases detected and referred by the community intermediaries had increased significantly, and the majority had been confirmed as cases of Buruli ulcer.

However, after discharge, neither the integrated health centres nor the community intermediaries provided the patients with follow-up and their families had neither the information nor the knowledge required properly to prevent loss of mobility. In addition, it was not possible for the occupational therapists from the referral centre to make many of the house calls that were initially planned.

Analyse these results clearly showed that the roles of the different actors (occupational therapists from the referral centre, officials at the integrated health centres and community intermediaries) had not been clearly defined, leaving gaps in the follow-up procedure. It was also clear that the community intermediaries did not really understand what they were actually supposed to do when they visited the

patients and their families during their rounds. Theoretically, some of them had to walk almost 20km to cover their area, and they pointed out this difficulty; however, when the evaluation was carried out, most of the persons concerned were in the same village or in a neighbouring one, and remoteness could not be invoked. The occupational therapists from the referral centre poorly appreciated the importance of teaching families how to prevent disabilities after discharge from hospital; actually carrying out treatment procedures took precedence over educating patients and their families about treatment. Lastly, the strategy proposed (house calls and supervision of the integrated health centres by the occupational therapists from the referral centre) encountered too many obstacles at too many levels (cost, availability of vehicles and trained staff, state of the roads etc.): it was impossible for it serve as a realistic means of providing follow-up.

Solutions proposed

The measures proposed in 2007 and 2008 relied on three main pillars:

1. Family-based case-management (training patients and their family to provide treatment)
2. Priority for the provision of follow-up by the community intermediaries (a role in supervising, providing encouragement and ensuring linkage with the integrated health centres)
3. Replacing the previous strategy with a regular and reliable flow of information between the communities, the integrated health centres and the district (adjustment of monitoring and of the conditions for referral and counter-referral).

With this in mind, a new follow-up procedure that includes precise conditions for referral and counter-referral has been defined. A form has been introduced for counter-referral and monitoring patients in the village. The new training course for community intermediaries, which was inaugurated in a pilot scheme in the spring of 2009 in Ngoantet health area, has been simplified and focuses on proper use of the form. It lasts two days to allow time to experiment and to satisfy actual needs in the field. A very simple short question-and answer handbook has been produced for use in the training course and to help the community intermediaries in their work in the field. One of the sections of the counter-referral and follow-up form is a chart showing items to be checked with statements alongside boxes which the community intermediary simply has to tick. A document for use in supervising the performance of the intermediaries has also been prepared. The first data collected on this new procedure are encouraging and raise the issue of how to define indicators for proper follow-up.

A basic, primary-care level, 3-day POD training program: methods and follow-up 4 months after training

Presenter: Ms Linda Lehman

Authors: Linda Lehman, Yao Aubin, Koffi Didier, Koffi Paul, Konan Konan N'guessan, Yao Aubin, Julien Aké, Henry Assé, Paul Saunderson

Brief introduction(background)

Up to now, POD training for Buruli ulcer programs has generally been carried out at a sophisticated level, designating the management of complex problems to physiotherapists in tertiary centers. There is a need for simple POD measures to be undertaken by health workers, persons affected by BU and caregivers, for all new cases, wherever they are being treated, which for most patients is in a primary-care setting.

What was done (implementation)

Having developed a simplified chart summarizing key points important to POD in BU, we tested its application in a 3-day training session in Ivory Coast. Participants were a mixed group of caregivers (doctors, nurses, community health workers, patients and family members). Four months after the training, it is proposed to examine a sample of cases in the community where the training was done, and a second sample in another community where such training was not given. This comparison will give some indication of the value of simple POD interventions in the primary-care setting.

What was achieved (results)

The training course itself will be described briefly, with the results of the 4 month follow-up.

What were the challenges during the implementation

The challenges were to show that simple measures can be very effective in preventing disability in most cases, and to expand the training so that this becomes a routine part of BU management.

What are your proposed solutions to these challenges

Rather than running special training of trainers (TOT) courses, it is proposed to carry out a series of simple, community-based training sessions, country-by-country, involving all levels of health staff who can then run similar training sessions throughout the country. This is expected to lead to the inclusion of POD activities within routine BU case management, and empower persons affected by BU and their families to prevent or lessen disability at the community level. In addition, health workers can observe the positive results when persons affected by BU and their families participate in POD training sessions.

Conclusions

We have designed a simple POD training program for use at the primary care level. Comparison of results between areas where training was done or not done will be presented. Trainers learn how to conduct such sessions through a participatory and interactive teaching method.

Overview of the Disability Prevention and Physical Rehabilitation Programme (PIRP) in Côte d'Ivoire and Benin, 2004-2009

Presenter: Ms Verónica Malda

1. Brief introduction: (general remarks).

In the treatment of lesions caused by *Mycobacterium ulcerans*, physiotherapy plays a crucial role in preventing and reducing the effects of lesion healing ; the real risk of loss of elasticity in the soft tissues (skin, muscles, tendons) resulting in limitation of movement is one of the principal areas of application of this medical discipline.

The ANESVAD Foundation has been involved in BU control since 1999. In 2004 we incorporated disability prevention and physical rehabilitation (PIRP) into patient management as a new component of our strategy.

2. Achievements (implementation)

We have collaborated with Fabrizio Bonifacio to plan and implement the PIRP programme in Côte d'Ivoire and Benin. The first phase involved fact-finding visits to identify BU detection and treatment centres and assess their needs in terms of personnel, infrastructure and facilities from the perspective of PIRP.

At the end of this initial phase, 5 detection and treatment centres in Côte d'Ivoire and 5 more in Benin had been visited and identified. At that point, a PIRP programme was scheduled for implementation with the aim of preventing and minimizing the sequelae that appear with varying degrees of seriousness if proper treatment is not administered. The components of the programme are:

- Theoretical and practical training
- Assistance with the establishment or upgrading of facilities and equipment
- Development of educational materials

3. Results

PIRP training

24 individuals were trained at the identified centres in Côte d'Ivoire and 14 at the identified centres in Benin.

Facilities and equipment

5 detection and treatment centres have installed new physiotherapy rooms and a workshop for the manufacture of orthopaedic appliances and 3 centres have adapted existing facilities. All the centres have been supplied with basic materials manufactured from locally sourced materials and with specific instruments to improve PIRP activities, resulting in high-quality instruments manufactured at low cost.

Development of educational materials

All the centres included in the programme have received at least one copy of each of the educational materials developed, chiefly training aids.

When the 2004-2008 strategic plan of the Beninese National Buruli Ulcer Control Programme was assessed in July 2008, the PIRP component was also taken into account. Below are some of the results:

- The rate of disability on admission to the 3 facilities with available data decreased from 29% in 2003 to 22% in 2007. The steepest decline was recorded at the Lalo detection and treatment centre where the proportion fell from 41% to 14% between 2003 and 2007. At the other detection and treatment centres at Zinvié and Zagnanado, the rate remained virtually unchanged.

- Comparing the proportion of patients with disability on admission to the proportion of cured patients without any sequelae on discharge, significant progress was recorded at the Lalo and Zagnanado detection and treatment centres, where the rate increased from 17% to 95% and from 50% to 98% respectively.

4. Challenges to implementation

Three main challenges were observed in implementation:

- **Adapting a method of physiotherapy for joint recovery during the healing period.**
- **Creating a good working relationship and a climate of trust between the physician, the surgeon, the nurse, the therapist and the patient.**
- **Obtaining patient acceptance of the positioning protocol before and after the skin graft.**

5. Proposed solutions

- **At each centre**
 - Each centre must pledge to ensure that the rules governing hospital admissions are properly adhered to.
 - The centre must prioritize the skills of its team, offer training and propose review of protocols.
 - The management must above all seek to establish or maintain good working conditions.
 - The attitude of workers at the centre is vital for patient management.
 - The management of the centre must monitor and promote collaboration among health workers.
- **At national programme level**
 - Promote knowledge-sharing among centres.
 - Organize and support workshops for physiotherapists.
 - Greater supervision of the application of the treatment and the medical protocols followed at each centre. Check data obtained from patient.
 - Promote and expedite surgical interventions.
 - Organize pilot PIRP training projects at decentralized dispensaries using local resources and personnel who have already undergone PIRP training.
 - Support the creation of the PIRP focal point at national level.
- **At partner level**
 - Training review visits at centres where training has already been carried out.
 - Support PIRP activities at decentralized dispensaries
 - Improve educational materials
 - Create new instruments to improve treatment

6. Conclusion

We reaffirm that PIRP is an essential component of the BU control strategy. The PIRP programme requires continued support to obtain real results and have an appropriate impact in Côte d'Ivoire and Benin. ANESVAD will thus continue to support PIRP activities in both these countries.

Problems posed by training for control of Buruli ulcer: a contribution by the Luxembourg Follereau Foundation (FFL)

Presenter: Dr Emile China

Authors: Emile CHINA, Ghislain SOPOH, Christian JOHNSON, Robert KOHLL

Buruli, ulcer is a neglected tropical disease which is responsible for considerable pain and disability, especially among children. There used to be no medical treatment for the disease apart from surgery.

Since the Yamoussoukro declaration and the establishment of the Global Buruli Ulcer Initiative, (GBUI), the Luxembourg Foundation (FFL), and its local partner the Beninese Raoul Follereau Association (ARFB), have been resolutely committed to the control effort alongside the Ministry of Health of Benin. This commitment has resulted in the construction of the Buruli Ulcer Treatment and detection Centre (CDTUB) in Allada, which was inaugurated in 2002, and has continued up to the present day through the support provided to the Centre for the comprehensive implementation of the strategies recommended by the World Health Organization in order to reduce suffering among the affected population. Significantly, thanks to this support and the commitment of the its personnel, the Centre has been highly successful: it treats on average 250 cases each year and its performance indicators have improved year after year. The Centre's success allows us to look confidently ahead, along with the actors involved in the PNLLUB.

Since the implementation of GBUI, understanding of the disease has made significant progress and medical treatment is now available, holding out the possibility of better integration within primary health care through the decentralization of care for patients.

In spite of these achievements, staff working in the field still lack knowledge in a number of areas, such as confirmation of diagnosis, medical case management, surgery and epidemiological surveillance. Nevertheless, a considerable effort has been made in this respect. For example, in Benin several categories of players, including community intermediaries, teachers, health workers, physicians and surgeons (in basic plastic surgery techniques) have taken training courses. A number of advocacy activities have targeted decision-makers, the political and administrative authorities and officials of nongovernmental organizations at both the national and international levels.

The results of these activities are apparent in the decline in the disease's incidence and a reversal in the trend by category of cases detected and treated, testifying to improved control of the disease.

We are convinced that theoretical and practical training of greater numbers of basic health care staff in Buruli ulcer diagnosis and case management, laboratory confirmation and investigation is of vital importance for the control of an endemic disease as complex as Buruli ulcer, in terms of its physiological and pathological features, its clinical manifestations and its treatment. Although individual efforts are being made in the different countries, we believe that the challenge that faces us for the future, if we are to improve the quality of care for Buruli ulcer patients and better to control the epidemic, is to draw up, approve at the international level and implement training curricula suited to each specific actor and to determine quality standards.

Because they have understood this need, FFL, ARFB and the CDTUB in Allada have designed and built an ad-hoc infrastructure which they are willing to make share with the international community. The infrastructure comprises an integrated training unit within the health-care facility, equipped with audio-visual, communication and logistic resources that are ideally suited to adult continuing-education methods. Integration within the health care facility is an added advantage because it facilitates practical activities. In the near future, the availability of a guest house to provide on-site accommodation for participants will be a further improvement that will enhance the effectiveness of activities.

This training unit has already twice been tested; it has served for an international training course (Benin, Ghana and Cameroon) on sampling techniques for laboratory confirmation of Buruli ulcer cases and as the venue for an international workshop to validate data-collection tools as part of the socio-economic Buruli Stop project. Both activities were extremely successful.

It is now up to the international community to make use of this valuable resource which Benin has made available to it, with the support of FFL.

Reliability of assessing limitation of movement (LOM)

Authors: Linda Lehman, Koffi Didier, Koffi Paul, Yao Aubin, Konan N'guessan, Julien Aké, Henry Assé, Paul Saunderson

Brief introduction(background)

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

What was done (implementation)

At the start of a POD training session in Ivory Coast, we asked health workers to assess limitation of movement in BU patients. Results were compared to a gold standard established by an experienced occupational therapist and two doctors.

What was achieved (results)

A total of 14 trainees, ranging from doctors and nurses to community health workers, assessed the same 10 BU cases. Out of 140 total LOM responses, there were 31 responses (22%) that were incorrect and LOM was not identified.

What were the challenges during the implementation

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

What are your proposed solutions to these challenges

During the training courses already mentioned, we developed the following rules for examining LOM in BU cases. Health workers were asked to observe limb movements of both the affected and non-affected sides together and to compare whether the movement was the same or different. LOM was identified when the BU affected side demonstrated less movement than the non-affected side.

Conclusions

We have found that LOM may be missed in one fifth of cases in the field. In order to improve the accuracy of these assessments, we propose that some simple guidelines are used, and practiced during training and supervision activities.

Ten Tasks for Preventing Disability in Buruli Ulcer

“Tasks for people affected by Buruli Ulcer who want to prevent disability - I Can Do It!”

10 Tasks	Key Point 1	Key Point 2	When to start?	How often?	Expected Result
Task 1 Diagnosis & Treatment	Early diagnosis - find other BU cases as early as possible, before much damage has occurred	Take your treatment	Right away!	Daily for 8 weeks (56 days)	The germs are killed, but: <i>You need to do these other things to help the body heal completely</i>
Task 2 Hygiene	Wash body and wash clothes	Wash hands often	Now!	Daily	Stay clean Prevent infection
Task 3 Nutrition	Know what foods help the body to heal	Eat the best food you can	Now!	2-3 times daily	The body heals faster
Task 4 Wound & Skin Care	Clean with water Oil to keep skin flexible	Dress with clean cloth ¹ Avoid tight bandages, but encourage movement ²	As soon as the wound is discovered – even before the exact diagnosis is known	Daily, until healed	Wound heals Skin is soft and flexible
Task 5 Movement & Exercise	Try to make the affected part move just like the other side ²	Play games and do other normal activities ²	Start movement and exercise as soon as BU is diagnosed ²	Many times a day (about every 1-2 hrs)	Normal movement
Task 6 Position	When resting or sleeping, position the limb in order to stretch the wound or scar	Position to allow swelling to drain	At diagnosis, if there is any limitation of movement or any swelling	Daily	Avoids contractures Reduces swelling
Task 7 Reduce Swelling	Raise the affected limb and encourage movement	Bandage from the end of the limb and up	At diagnosis, if there is any swelling	Most of the day and night until there is no more swelling	Lessens pain and allows full movement
Task 8 Scar Care	Soak & oil	Massage, Stretch and Protect ¹	Once the wound has healed	Daily for 1- 2 years	Soft, mobile scar Full movement
Task 9 Participation	Participate in self-care Involve family members	Participate in home, school, work and social activities	Right away!	Daily	Live a normal life
Task 10 Extra Help	Know when you need help	Know where to go to get help Use phone or e-mail	When needed	When needed	Solve problems Improve functioning

¹ Consider applying light pressure with foam padding, ² No exercises to be done for 10 days after a skin graft; movement that is beneficial can be expected to cause some discomfort but forced movement causing more severe pain is harmful and should be avoided.

Lehman L & Saunderson P, American Leprosy Missions 20/08/2009, revision 20/02/2010

Simple, one-page scheme for POD

Authors: Paul Saunderson, Linda Lehman

Brief introduction (background)

Current manuals and guidelines for POD are too complex for many health staff and certainly for training community volunteers, patients and family members in POD within the health service and community.

What was done (implementation)

Initially, important POD tasks from the perspective of the person affected by BU and the health worker, were identified from several West African countries. We then summarized the main POD tasks in a simple, one-page table. This can serve as a training framework, indicating which critical topics need to be covered. The level of explanation and the information given on each topic will depend on the audience.

What was achieved (results)

It was possible to include all the key POD tasks on one-page, under 10 headings. Critical messages for each task aim to help health workers and volunteers focus their POD teaching on issues that persons affected by BU and their caregivers need to know and practice.

What were the challenges during the implementation

The challenge was to keep each statement as simple and straightforward as possible, but to allow for more detailed discussion where necessary. Trainers will need practice in facilitating discussions and in identifying opportunities to practice POD skills with persons affected by BU, and their caregivers. The ultimate challenge is to show that this simplified approach will lead to effective disability prevention.

What are your proposed solutions to these challenges

Providing opportunities for multidisciplinary teams to learn through presenting, discussing and doing key POD tasks together, will develop their communication skills and the confidence that simplifying the main POD tasks can produce very beneficial results.

Conclusions

We have designed a simple table of POD tasks, which will enable these important interventions to be performed by many different caregivers, including the patients themselves and family members, so that most disability from BU in future can be prevented.

Prevention of disability

Author : Mr Grégoire Tiencheu Tchokouago

Introduction

Buruli ulcer frequently causes functional disabilities that may preclude a patient from engaging in occupational or daily living activities.

Mycobacterium ulcerans generally affects the lower limbs.

When *Mycobacterium ulcerans* affects the feet, this can prove to be more disabling than any at other site since the patient may be unable to walk.

Physiotherapy can be used to prevent disability.

Implementation

All patients treated for BU under the Akononlinga project have the option of rehabilitation by physiotherapy if needed.

Each new patient will initially be assessed to identify functional impairment. Following this assessment, a rehabilitation programme will be drawn up and implemented.

Depending on the extent of disability, a number of rehabilitation techniques may be used.

Results

The indicators used are the duration of treatment the percentage of recoveries, and improvements or treatment failures following physiotherapy.

Of 34 affected in the area of the ankle, 24 have benefited from type I rehabilitation and 10 from type II rehabilitation.

Outcomes	Patients	%
Recovery	24	70.6
Improvement	08	23.5
Failure	02	5.9
TOTAL	34	100

Positive outcomes have been observed in 94% of rehabilitated patients.

Treatment is generally long, in excess of 90 days for 25/34 patients (73.5 %).

Challenges

- Duration of the treatment (more than 3 months for 73% of patients)
- Patients' motivation to perform regular exercises
- The therapist's ability to assess the patient's functional capacities with a view to selecting the treatment option.
- Rehabilitation outcomes depend on the seriousness of the patient's disability at the time of treatment.

Solutions

- Routine assessment by the physiotherapist of all new patients and early management of impairments.
- Propose exercises in ludic form to motivate patients, especially children.
- Ongoing training for physiotherapists.

Conclusion

Rehabilitation by means of a high-quality, tailored course of physiotherapy facilitates the prevention of disabilities arising from BU-related complications in the majority of cases.

OTHERS

Yaws in Ghana: WHO Sponsored Pilot Treatment Survey in Eastern Region

Presenter: Dr Agana Nsiire

Program Manager, National Yaws Elimination Program

Introduction

Yaws, a disfiguring disease of skin, cartilage and bone, caused by *Treponema pertenue*, was eliminated from 46 countries including Ghana during WHO led campaigns from 1952 to 1964. From the 1970s however yaws started to resurge in Ghana because there was no strong health system to maintain the gains. Efforts have since been made by the Ministry of Health to control the disease but these were poorly resourced and not sustained. Yaws is now reported from all 170 districts in Ghana except a few in the Greater Accra Region. Studies in 2008 show some schools with prevalence up to 19.5% (National Yaws Elimination Program Annual Report 2008). The trend, mainly from routine reporting and school surveys, from 1995 to 2009 is shown in the table below.

Table 1. Yaws Cases Reported in the last 15 years

Year	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Cases	52398	38000	30456	16152	4096	10966	11131	46799	37560	49917	27280	19515	25969	28080	35528

In 2008 a National Yaws Elimination Program was formed to coordinate efforts towards elimination by 2014. A national Technical Advisory Committee was put in place. In support of this effort, the WHO funded a pilot study in 3 districts (West Akim, Birim Central and Birim South) in Eastern Region to gain experiences to scale up elimination activities. Findings of this pilot treatment survey are presented in this abstract.

Pilot implementation activities

Implementation involved planning with the region and the districts concerned (thus sensitizing them in the process), developing data tools and training manuals (structured for a one day session at any level), training, advocacy, social mobilization, case and contact treatment, supervision, monitoring and a rapid assessment of the project. A one day training of trainers and supervisors from region, districts and sub-districts in one session followed by a one day training of the rest of sub-district staff and volunteers in the various 13 sub-districts took place in September 2008. Yaws focal points were identified at all levels to work in an integrated fashion with other services. Social mobilization involved official correspondence, inter-sector meetings and personal contacts with Education and Local Government authorities, schools and communities.

Field activities spread over 10 weeks were carried out by sub-district teams of three (1 injector and 2 volunteers) in basic schools using tally sheets, registers for contact tracing, an assessment form and an adverse drug reaction form provided by the Food and Drugs Board. Selective, juvenile or total mass treatment was used in schools according to prevalence. In the communities cases and household contacts were treated. The teams enjoyed vital cooperation of the teachers and community volunteers. District, regional and national staff supervised and after the field work a team from national carried out a rapid assessment and gave useful feedback.

Results

In all 4,006 clinical cases and 67,146 contacts were treated. A total of 547 schools were visited and 208,413 children examined. Yaws cases were found in half of the schools visited (51%). School prevalence ranged from 0.0% to 19.5%. Average sub-district prevalence ranged from 0.23% to 3.69% while district prevalence stood at 0.98% to 1.12%. Overall average prevalence estimate was 1.92. These results are based on clinical diagnosis by trained health personnel with no laboratory confirmation.

Of the total number of cases 61% were not in the schools but in the communities. Eight minor adverse drug reactions were registered and investigated by the Food and Drugs Board. Apart from the results obtained important lessons were gained which have been used in preparing the program strategic plan for 2010 – 2014.

Conclusions

Yaws is prevalent in Ghana and efforts so far have been inadequate to eliminate it due to inadequate resources. A pilot study in the Eastern Region of Ghana gives evidence that there is enough moral commitment by the immediate stakeholders (health workers, the Ghana Education Service, the Local Government and communities) to fight the disease. Useful lessons have also been learnt to deal with it more effectively. What remains is the material and financial commitments of government of Ghana and our larger partners.

Yaws in the Lomie health district of the East Region of Cameroon

Presenter: Dr Earnest Njih

Authors: Earnest Njih Tabah¹, Alphonse Um Boock²

¹National committee for leprosy, Buruli ulcer, leishmaniasis and yaws control; ²Regional Bureau for Africa of FAIRMED (former ALES)

Introduction

Yaws, a disease that was eradicated, is re-emerging in the Congo basin. It is thought to be endemic among the pygmy population who inhabit the dense equatorial rain forest of the Congo basin. Gerstl and collaborators reported a prevalence of 4.7% in the general population of the rural Wasolo health zone in the Equator Province of the north Democratic Republic of Congo. Damas Obvala reported 646 cases of yaws among the pygmies of the Likouala and Sangha divisions of Congo. The Sangha division of Congo shares a common border with the Upper Nyong and Bomba and Ngoko divisions of the East region of Cameroon. All of the divisions cited are located in the Congo Basin and are home to the pygmies.

In 2008 and the first semester of 2009, cases of yaws were notified by health authorities of the Abong Mbang, Lomie and Mbang health districts of the East region of Cameroon, with Lomie notifying the highest number of cases. All of the cases notified were among the Baka pygmies.

We carried out a mass screening of yaws and treatment of clinical cases of yaws among the Baka pygmies in the Lomie health district from the 27th of October to the 2nd of November 2009. Lomie health district is one of the 14 health districts in the East Region of Cameroon. It has a population of 36581 inhabitants, of whom 5211 are the Baka Pygmies. These pygmies in this health district live in 35 villages (pygmy camps).

Objectives and Methods

The objectives of this screening activity were to determine the magnitude of yaws among the pygmies in the Lomie health district and to gather information favoring its propagation in this population, in view of establishing a proper control strategy.

In collaboration with the Lomie health district authorities, the pygmy villages were identified and a programme of visit was drawn and communicated to the villages in advance. Two teams were constituted and trained on the clinical diagnosis and treatment of yaws. After the training, the teams went into the villages for mass screening and treatment. In each village, villagers were gathered at the village town-hall or school premises and each villager present was examined following a standardized checklist for clinical signs suggestive of yaws. Those with yaws were each given an injection of benzathine penicillin, and a blood sample taken from those who were 6 months or older, for TPHA analysis.

Results

Thirty five (35) pygmy villages were visited, where 822 persons were examined. 167 (20.3%) cases of yaws were detected in 22 of the 35 villages. For the 167 cases of yaws, 61% were males and 80% were children below 15 years of age. The mean age was 11.9 years (min= 3months, max=55 years). The average number of contacts was 6 (min=2, max17). Early yaws constituted 94% of the cases. Out of 143 blood samples collected, 40(28%) were positive for TPHA test.

Follow-up

The 167 patients screened and treated were followed-up by the district medical officer and his team. Within a period of 3 weeks, 149 (89.2%) of the treated cases had healed.

Challenges

The pygmy population of the Lomie health district lives in a very difficult geo-economic and socio-cultural environment. The unhygienic living conditions, abject poverty, high illiteracy rate, lack of potable water, insufficient health infrastructure and the attachment of this population to the warm and humid equatorial rain forest favor the transmission of yaws. The expensive nature of organizing mass screening and treatment campaigns also poses a big challenge.

In perspective

There is need to survey the remaining health districts in the East Region where pygmies live. At the end of these surveys, the National Comity for Leprosy, Buruli ulcer, Leishmaniasis and Yaws Control in the Ministry of Public Health would have a clear picture of the situation yaws, to be able to develop an effective and efficient control strategy.

Control of Yaws in the Republic of the Congo in 2009

Presenter: Dr Damas Obvala

Coordinator, national leprosy/Buruli ulcer control programme

In the Republic of the Congo, yaws is endemic to the warm humid forest departments of Likouala, Lékoumou and Sangha. The populations most affected are ethnic minorities (pygmies or babenga) who make up approximately one third of the population in the departments, where almost 65% of the population is affected.

In January 2007, a global plan for the elimination of yaws was developed by WHO, followed by the preparation of an integrated leprosy-yaws project in the Congo in June of the same year.

The project's overall objective is the eradication of yaws among the population affected (pygmies or babenga) by 2013, with as its specific objectives mass community mobilization and awareness-raising in respect of yaws, detection and treatment of all cases of yaws and all contacts with benzathin penicillin and promotion of general hygiene among the affected communities.

The main activities are improving awareness of yaws among the populations and detection of cases and of contacts, training/retraining for health personnel and community health workers and promotion of hygiene.

In January 2009, two integrated leprosy and yaws awareness-raising, case-detection and treatment missions were organized in Likouala and Sangha departments. A total of 56 community intermediaries and 22 health workers were trained in case detection and management.

The mission's results include improved awareness among 51.6% of the population. A total of 646 clinical cases of yaws and 83 contacts were treated, 31.73% of them children. Primary yaws lesions accounted for 80.8% of cases. In 85% of cases, scarring was achieved within three weeks of injection of a single dose of delayed-action penicillin.

The main difficulties were problems of access to the pygmy populations, inadequate health infrastructure, the high cost of case-detection missions, the poor level of education of the pygmy populations, their attachment to the forest areas where geographical and environmental conditions favour the disease and the socio-cultural environment which is also favourable to the permanent presence of yaws.

The prospects include comprehensive improvement of the living conditions of the pygmies, improving education and awareness among them, the inclusion of yaws control in integrated health centres and the regular organization of mobile missions in endemic areas.

Yaws control is undoubtedly one of the major challenges we face. We must all combine our efforts to avoid the marginalization of the disease so that it ceases to be a public-health problem in our country.

Improving Buruli ulcer control in Songololo Territory, Democratic Republic of the Congo.

Presenter: Dr Phanzu Mavinga Delphin

Kimpese Evangelical Medical Institute, Bas-Congo, DR Congo

Overview

Songololo Territory is one of the most active foci of Buruli ulcer (BU) in the Democratic Republic of the Congo. The functional disabilities caused by BU have significant socioeconomic consequences for patients and their families. In 95% of the 64 patients admitted to the Kimpese General Referral Hospital/Evangelical Medical Institute in connection with BU during the period 2002-2004, the disease had been detected at the ulcerative stage; 36% of cases already presented with functional limitation at the time of diagnosis; and 23% had developed permanent disabilities. The average hospital stay was 89 days. Furthermore, an abnormally high fatality rate (19%) was observed. After more than three years of control measures undertaken by the Kimpese Evangelical Medical Institute BU project, covering the Kimpese and the Nsona Mpangu health areas, the morbidity and disability rates for BU remain high. Of 190 patients admitted to the hospital, 86% were diagnosed at the ulcerative stage and 20% made a recovery but with permanent disabilities. The object of this paper is to review the current state of knowledge on BU control and identify the progress, problems and limitations of the Buruli ulcer control project in Songololo, in order to suggest ways to enhance the interaction between the vertical control programme and core health services.

A combination of three methods has been used:

1. A review of the available literature on BU control, using vertical analysis as a conceptual framework;
2. Analysis of quantitative data from inpatients at the Kimpese General Referral Hospital/Evangelical Medical Institute in the period 2002-2007, using the conceptual framework for the evaluation of health programmes proposed by Habicht et al.;
3. Analysis of data from the BU prevalence survey conducted between June and August 2008 in Songololo Territory.

The analysis reveals that options for improving BU control are currently limited. The mode of transmission of *M. ulcerans* from the environment to humans is still not entirely understood. There is neither a specific vaccine for BU, nor a rapid diagnostic test that could detect the infection in asymptomatic patients. The current control strategy focuses on early detection and treatment through a combination of antibiotics, with or without surgery.

Data from patients treated at the Kimpese General Referral Hospital/Evangelical Medical Institute show that the average number of annual admissions has tripled, from 21 cases in the period 2002-2004 to 63 cases in the period 2005-2007. The proportion of female patients increased from 30% in the former period to 49% in the latter ($p=0.005$). In the latter period, there was a significant reduction in the recurrence rate ($p<0.001$), ulcerative forms at case detection ($p=0.041$), mixed ulcerative forms ($p=0.0038$), and the fatality rate ($p=0.0001$). The proportion of patients cured without complications increased markedly in comparison with the period before the project. ($p=0.001$). Just over half of patients (56.3%) had benefited from the specific antibiotic therapy. However, no significant difference was observed between the two periods with regard to the number of confirmed cases ($p=0.183$), cases of osteomyelitis ($p=0.302$), cases of restricted joint movement at the time of diagnosis ($p=0.119$), cases of recovery with restricted joint movement in all patients (however they were discharged from hospital) ($p=0.0496$), and even the number of patients deemed to be cured ($p=0.119$) or referred for surgery ($p=0.052$). The average duration of hospitalization (almost 90 days) was almost the same in both periods.

According to the prevalence survey, of 259 patients with progressive lesions (a prevalence of 109 per 100 000), 74% are ulcerated, 48.8% are category I, 31.5% are category II, and 19.7% are category III. Functional limitation is found in 23.9% of cases at case detection and confirmed by laboratory tests in 27.8% of patients.

We note, however, that since the beginning of this year, 25 patients have already been admitted to the Kimpese General Referral Hospital/Evangelical Medical Institute. This shows that the numbers admitted to hospital are just the tip of the iceberg.

To reduce the BU burden, which is chiefly attributable to late diagnosis, and to increase coverage of the at-risk population, we believe that the most effective method of coordinating BU control in Songololo Territory would be to pursue a vertical programme - either centralized or decentralized - and to incorporate control measures into existing multidisciplinary facilities in the Kimpese and Nsone Mpangu health areas. Decentralization and integration of control activities will facilitate access to case detection and offer better treatment to BU patients in the most peripheral areas of the health system.

Integration of decentralized Buruli ulcer control and treatment in Côte d'Ivoire

Presenter: Professor Henri Assé

National Buruli ulcer control Programme, Côte d'Ivoire

Buruli ulcer control and treatment in Côte d'Ivoire were for many years the preserve of a handful of specialist centres. The intensification of control efforts starting in 2006 led to the discovery that the level of endemicity in a number of health districts had initially been underestimated. The sudden extension of the endemic area has focused attention on the issue of patient access to specialized treatment (which is now too remote from users). Moreover, these centres with their already overburdened capacity were unable to meet the ever-increasing demand for treatment. Thus since 2007 we have opted for decentralization of BU control activities in order to afford all patients equal opportunities for case-detection and early treatment. This initiative, which seeks to integrate BU control into the minimum package of health district activities presupposes active involvement by first-line health facilities (rural health centres), general hospitals and specialized centres. This health network currently comprises 353 health facilities in 32 endemic districts.

109 health facilities reported and treated 2679 new cases of UB in Côte d'Ivoire in 2009. 75% of these patients benefited from immediate local outpatient treatment at a rural health centre leading to cure.

This strategy has several advantages:

- Earlier detection and treatment
- Outpatient treatment
- Strong involvement of public health facilities
- Facilities permanently staffed by skilled human resources
- Better coordination at health district level
- Adherence to national policy guidelines.

Considering the numerous advantages of decentralization and integration of BU control activities at the peripheral level, we would like governments and partners to invest more heavily in this strategy in order to develop it as rapidly as possible.

Assessment of the benefit of a decentralization strategy in the epidemiological surveillance of Buruli ulcer in the area covered by the Allada CDTUB in Benin

Presenter: Dr Ange Dossou

Authors: Ange DOSSOU¹, Ghislain SOPOH¹, JG HOUEZO¹, Yves BAROGUI², RC JOHNSON³

¹Buruli Ulcer Detection and Treatment Centre, Allada, Benin

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Context

Lengthy hospitalization and its consequences are a decisive factor in a patient's decision to seek treatment. To reduce the time spent in hospital and incorporate combination antibiotic therapy into the management of BU, the National Leprosy and Buruli Ulcer Control Programme has introduced «decentralized» treatment into the protocol for BU patients.

Following clearly and precisely defined criteria, 12 centres have been established in the area covered by Allada CDTUB.

Method

We assessed the impact of decentralized treatment through a number of BU epidemiological surveillance indicators during the period July 2006 to July 2009.

Challenges and future action

To build on the success of decentralized treatment, the challenges ahead are to:

- Increase the number of centres
- Improve prevention of disability
- Improve post-treatment scar care

To meet these challenges, we must:

- Improve the quality of care in peripheral centres
- Institute routine supervision of decentralized physiotherapists
- Organize periodic assessments of former patients.

Results

- 12 centres are operational, within the catchment area of Allada CDTUB.
- 65% of patients admitted during this period initiated decentralized treatment, the rate of increase varying between 54% et 86%.
- Most patients admitted on a decentralized basis present with early lesions: 76% of cases (cat 1 : 31%, cat 2 : 45%).
- Patients admitted directly to hospital present with advanced lesions: 53% of cases in category 3.
- The proportion of category 1 lesions increased between 2006 and 2009 from 18 to 33%, an average increase of 23%. This increase is at the expense of category 2 cases, which are on the decline (down from 50 to 32%, an average decrease of 43%). The proportion of category 3 lesions has remained almost stationary at around 34%.
- Non-ulcerative lesions represent 46% of admissions, with a rate of increase varying between 39 and 57%.

- Time elapsed before seeking treatment is relatively short among patients receiving decentralized care: 54% seek treatment before the 45-day mark compared with 31% of those admitted directly to hospital.
- Late recourse to treatment is typical of patients admitted directly to hospital, 61% of whom seek treatment after the 60-day mark.
- The rate of laboratory confirmation by PCR is 63%, with a higher rate for patients initiating decentralized treatment (65% among confirmed cases).
- Laboratory confirmation rates appear to be higher for category 2 and 3 lesions.
- The PCR confirmation rate has increased over the years, from 51% in 2006 to 84% in 2009.
- 31% of patients admitted to decentralized treatment achieved scar formation without surgery.
- The default rate is below 1%

Conclusion

Assessing the impact of decentralized treatment will enable us:

- To improve early detection
- To promote early treatment
- To improve the laboratory confirmation rate

A system of care featuring the total involvement of all stakeholders at the peripheral level will help to avoid treatment defaults.

It is essential, however, to assess the disability rate, the quality of care and above all the quality of scar formation without surgery.

Contribution by MAP International to Buruli ulcer control in Côte d'Ivoire and Ghana

Presenter: Mr Aubin Koffi Yao

Authors: Dr Julien Ake AKE; Aubin Koffi YAO; Konan N'guessan; Samuel BENEFOUR

I. Introduction

MAP International and ALM have been involved in Buruli ulcer control in Côte d'Ivoire since 2001 and in Ghana since 2007. In both countries, the intervention strategies pursued by MAP International and ALM seek to incorporate control strategies into the minimum package of activities of health facilities and health districts.

This paper presents the actions taken by MAP International and ALM in the area of Buruli ulcer control in Côte d'Ivoire and Ghana.

II. Implementation

The activities listed below have been conducted in the health districts of Tiassalé ; Oumé ; Toumodi ; Yamoussoukro and Daloa in Côte d'Ivoire and in N'koranza district in Ghana.

- Support for drafting policy documents and national guidelines on Buruli ulcer control;
- Drafting documents on training of health professionals and community health workers;
- Conducting training for district trainers;
- Provision of vehicles and computers to health districts;
- Support for the training of district health professionals by district trainers in techniques of case detection and collection of specimens for laboratory confirmation and medical treatment;
- Support for the training of community health workers by district trainers in community-based surveillance (early detection and referral of cases to health facilities).
- Equipping front-line health facilities and district referral hospitals with dressing, excision and grafting kits;
- Support for case confirmation.

III. Results

➤ In 5 health districts in Côte d'Ivoire :

- a. The number of health facilities offering treatment has increased from 13 to 91 or 7 times the number of facilities prior to the interventions;
- b. The number of health professionals providing proper treatment for BU has increased from **12 to 515** or approximately 43 times the number prior to the interventions
- c. The number of cases detected and treated annually has increased from 420 to 1313 or three times the number prior to the interventions
- d. Specimens have been taken in 340 detected cases with a view to carrying out PCR tests (25.8% of all detected cases). Of these 340 specimens, 265 tested positive (78%) .

NB : these statistics need to be cross-checked with the National Buruli Ulcer Control Programme

➤ In Nkoranza health district in Brong Ahafo region in Ghana

- a. The number of cases detected and treated annually has increased from 10 to 24 on average, i.e. double the number of cases reported prior to the interventions

IV. Difficulties encountered

- Community participation in Buruli ulcer control is severely hampered by poor motivation of community health workers and other local stakeholders (in Côte d'Ivoire).
- Coordination of case confirmation and availability of specific antibiotics remains a problem;
- Free treatment conflicts with the policy of recovering costs from health facilities.

V. Proposed solutions

- Encourage local development structures (municipalities, local councils, district assemblies, etc.) to become involved in BU control by establishing motivation schemes for community health workers;
- Strengthen collaboration with reference laboratories that are equipped to carry out PCR tests and are experienced in doing so;
- Outline a formal framework for implementing free treatment of Buruli ulcer in Côte d'Ivoire
- Make treatment coverage more widely available in Ghana through **universal health insurance**.

Early detection of Buruli ulcer in Akonolinga district of cameroon

Presenter: Dr Pablo Díaz Badial

Introduction

In the context of managing Buruli ulcer (BU) disease, MSF has been working in partnership with the Akonolinga health district to implement a community-based approach in all health areas to detect and treat BU.

The objectives of this approach are:

- To provide the public with more information about the disease and change attitudes towards it.
- To expand the search for new cases, especially early stages of the disease, with a view to reducing the duration of hospitalization and treatment and minimizing complications (infections, sequelae).

Implementation

MSF and its partners in the health district have recruited and trained a large number of community intermediaries and influential figures in the community (religious leaders, administrative chiefs, etc.), who have received instruction in awareness-raising techniques.

The community intermediaries and influential figures conduct ongoing awareness-raising activities on BU, including clinical signs of the disease, detection techniques and possible courses of treatment at health facilities.

Anyone suspected of having BU is referred to the MSF medical team for a consultation at a peripheral health centre or the Akonolinga district hospital with a view to clinical and/or laboratory confirmation of the disease and subsequent treatment.

Results

In 2009, 328 suspected cases were seen and 104 patients were diagnosed with BU, of which 96 were new cases and 8 were relapses. Among the new cases, 69% presented with a category I ulcer and 17 % with a non-ulcerative form (plaque or nodule).

Since 2007, the proportion of patients presenting with a category I ulcer or a non-ulcerative form has steadily increased, leading us to believe that a community-based approach is helpful in early detection of the disease.

Challenges

- The sheer size of the Akonolinga health district means that a large number of community intermediaries is required, which complicates the training and supervision of the teams.
- Access to health areas is sometimes difficult owing to the size of the health district and climate conditions.
- Large-scale awareness-raising presupposes the existence of medical staff who have been trained and are available at the various health centres.

Solutions

- The process of recruiting and training community health workers should be dynamic in order to promote awareness-raising in all health areas.
- The need for supervision presupposes specific human resources, human resource management skills, and also medical skills in order to be able to assess patients and take specimens if necessary without obliging patients to travel too far from their homes, or to re-attend.
- To maintain mobility despite poor roads and adverse weather conditions, motorbikes are used to visit health areas.
- Training of all nurses at health centres will also facilitate rapid patient assessment and specimen taking to diagnose suspected cases.

Conclusion

An extensive and active community network, in conjunction with a mass ongoing campaign of public awareness-raising and assuming the existence of mobile medical personnel within the community, considerably facilitates early detection of BU.

The contribution of mapping and georeferencing techniques (use of GPS) to control Buruli ulcer

Presenter: Dr Ghislain Sopoh

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Background

In order to control infections caused by *Mycobacterium ulcerans* the National Buruli Ulcer Control Programme employs an epidemiological surveillance system based on a combined health-sector, education-sector and community network. WHO has recommended the BU02 form and mapping based on the HealthMapper as tools for data collection and analysis.

By using these different tools, since 2003 Benin has built up a not insignificant data base that enables it precisely to describe the epidemiological situation in endemic areas in terms of time, place and individuals. This has made it possible to show distribution of Buruli ulcer by foci, without however the possibility of identifying the parameters determining its distribution and the level of prevalence of the disease.

Method

In order better to document these parameters, the Buruli ulcer detection and treatment centre (CDTUB) in Allada, has employed georeferencing and mapping techniques as a means of more closely studying distribution of Buruli ulcer in two communes in its area: the communes of Zê and Toffo. To this end, a team collected precise geographical coordinates for all the villages in the communes, including their altitude. Data routinely collected since 2005 on cases admitted to the centre were used to calculate prevalence of Buruli ulcer in each village.

Results

By projecting data for prevalence and altitudes onto a map, it is possible to observe that *the villages in which prevalence is high are essentially located at low altitudes.*

By using statistical techniques, we are also able to reveal this relationship, as they show that *the average prevalence of Buruli ulcer is 34.2 p. 10 000 inhabitants in areas whose altitude is below 80 metres, significantly higher than in areas whose altitude is 80 metres or above, where it is 6.8 p. 10 000 inhabitants (p= 0.03).*

If all the villages are taken included, simple linear regression of prevalence as a function of height shows the existence of a very weak correlation, with a coefficient of correlation of 0.20 and a coefficient of determination $R^2 = 4\%$. The constant is -0.0969 and the intercept 26.724. *In other words, expected prevalence at zero altitude is 26.724 cases per 10 000 inhabitants. This prevalence diminishes by 0.0969 per 10 000 inhabitants for each 1m rise in altitude.*

If we include only villages where the disease is endemic (prevalence $\neq 0$), there is a weak correlation, with a coefficient of correlation of 0.50 and a coefficient of determination $R^2 = 25\%$. The constant is -0.6967 and the intercept + 89.61. *In other words, expected prevalence at zero altitude is 89.61 cases pour 10 000 habitants. This prevalence diminishes by 0.6967 cases per 10 000 inhabitants for each 1m rise in altitude.*

Conclusion

Use of georeferencing and mapping tools has made it possible for us to show that **there is a linear relationship, albeit weak, between altitude and prevalence of Buruli ulcer**. Altitude could account for a proportion of 4% of variations in prevalence if we take into account all villages and in a proportion of 25% if we include only endemic villages. **Introduction of measurement of the altitude of villages into models for analysing environmental risk of Buruli ulcer could thus improve understanding of the factors accounting for the distribution of disease foci.**

Bridging the BU divide: surveillance, transmission, ecology, and control

Presenter: Mrs Ellen Whitney

Authors: Ellen Whitney, Julie Clennon, Shannon McClintock, and Lance A. Waller

Rollins School of Public Health, Emory University

Research and surveillance in Buruli ulcer and its causative agent, *Mycobacterium ulcerans*, typically focuses separately on national reports of incidence/prevalence, treatment options and protocols, detection of *M. ulcerans* in the environment, and testing transmission hypotheses. Increasingly, collaborative efforts allow research to focus on the interdisciplinary interactions between these fields of study and open possibilities of new understanding of the multiple components of the underlying disease process. In this presentation, we place ongoing research collaborations within the perspective of a wider system of disease, ecology, and surveillance. We explore linkage points defining both the ecology of the disease and the “ecology” of data generation, collection, and analysis. Based on this conceptual framework, we provide summaries of field work conducted in collaboration with the Ministry of Health of Ghana in the summer of 2008 and the fall of 2009 with specific emphasis on key elements of Buruli ulcer surveillance.

Findings include:

- Surveillance represents an important but often unappreciated aspect of Buruli ulcer research. While past efforts focus on the use of surveillance to generate national and regional counts of disease, new data collection and analysis options are available to monitor spatial and temporal changes in incidence and prevalence.
- Even the best surveillance systems can be enhanced as there are always gaps in data, which do not necessarily represent failure, but rather key features of the data collection process. Understanding the gaps in a surveillance system provides improved estimates of impact within a population and increases the robustness of research results relating to Buruli ulcer prevalence.
- Research regarding aspects of a surveillance system can inform, improve, and focus surveillance efforts.
- Effective Buruli ulcer control requires high quality surveillance, treatment, and research.

The Health Promotion approach to Buruli ulcer control in Allada/Zè/Toffo (ZS AZT) health area in southern Benin

Authors: Goudoté Paule Y., Houéto David, Deccache Alain.

The "BLUE HOPE" research-action project represents a trial intervention whose purpose is to improve early case management and to reduce prevalence of Buruli ulcer in the village of Azonmé.

The health promotion concept (HP) defines essential prerequisites for any improvement in health: "health depends on a number of conditions and resources: in particular, individuals need housing education, proper food, a certain income, a stable ecosystem, social justice and equity" (Ottawa Charter, 1986). Satisfying these conditions in a durable manner calls for community ownership and empowerment. Because Buruli ulcer is a disease associated with poverty, this project is based on the health promotion approach with the aim of entrusting the affected communities with responsibility for the process of analysing its causes and carrying out measures to control the disease. This paper describes the methodological approach adopted in selecting communities for the study.

Implementation

The project, which is scheduled to last 4 years (2009-2013), is being carried out as part of a PhD project and is made up of three components: a Buruli ulcer prevalence survey and psychosocial behavioural investigation involving 2 visits to the pilot and control villages (pre and post intervention), direct observation of changes in the pilot village during the intervention, comparison (pre and post intervention) and evaluation of the process.

After a preliminary survey conducted in the communes of Zè and Toffo in January 2010, two villages were selected for the study: Azonmé (in Toffo commune) and Hwouédota (in Zè commune).

Results

Azonmé and Hwouédota were selected as the «pilot » and «control» villages respectively. They are located in communes in Allada/Zè/Toffo, a health district in which Buruli ulcer is highly endemic.

Both villages satisfy the criteria required for our research and are comparable in terms of:

- The level of endemicity of the disease: in 2006 prevalence of Buruli ulcer in hospitals was 4.6 per 10 000 in Azonmé and 9 per 10 000 in Hwouédota.
- The size of their populations (in 2009, the population of Azonmé was 2923 and that of Hwouédota 2755).
- The vicinity of a body of freshwater used by the population.
- The proximity of a health centre (private, faith-based in Hwouédota and public in Azonmé).

Challenges

- Ownership on the part of the pilot community;
- The capacity of the populations to take responsibility for their own development;
- The willingness of the health professionals (investigator and collaborating health centres) to allow the communities to set priorities within the framework of the Blue Hope project.

Solutions proposed

- Improving the skills of the health professionals concerned to enable them to assure autonomy for the pilot community.
- Use of advocacy in building a multi-sectoral partnership to overcome the various obstacles identified by the community.

Keywords: Buruli ulcer, health promotion, participation, community empowerment, pilot and control villages.

Inpatient and outpatient support: an intercultural approach. Examples from some hospitals treating patients with Buruli ulcer

Author: Mr Samuel Kouassi Kouakou

1- Masters dissertation 2 Human and Social Sciences for research purpose with specialization in Education Science, Health and Social Promotion. University of Besançon, 2008.

Summary

Comparing African and French approaches to patient support from an intercultural perspective raises the question of the different systems of logic informing this issue. This study of the support available to BU patients reveals that this logic may be shaped by cultural factors, local realities or the inherent nature of health systems. Support for BU inpatients or outpatients in Africa offers various perspectives on the social and therapeutic relationship between physicians and patients, strongly influenced by the position that attending physician adopt vis-à-vis their patients. The roles of the « **nursing auxiliary** », « **cook** » and « **personal hygiene carer** » reflect a « peculiarly African » social reality that makes it necessary to take cultural dimensions into account in the management of disease, in addition providing information about the illness (including whether or not it is life-threatening). This study also postulates that inpatient and/or outpatient treatment may be improved and rendered more effective through a clearly articulated approach to patient support. Lastly, it suggests that carers of BU patients are de facto community intermediaries who disseminate a health education message while at the same time possessing traditional knowledge about the disease that should not be underestimated.

Patient support in the management of Buruli ulcer in Africa (Côte d'Ivoire): Research Project

Author: Mr Samuel Kouassi Kouakou

1- Masters dissertation in Analysis and Intervention in Education Systems, Department of Sciences and Education, Faculty of Psychology and Education Sciences (FAPSE), University of Geneva, 2009.

Summary

This research project focuses on social attitudes towards caring for patients in Africa (Côte d'Ivoire). It draws, on the one hand, on works demonstrating the need to break with the monopoly of the medical paradigm in the definition of disease (Schurmans & Charmillot, 2007). On the other hand, it refers to the epistemological clarifications of the comprehensive approach and qualitative methods cited by Charmillot & Dayer (2007) to shed light, from a comprehensive standpoint, on the social reality of caring for Buruli ulcer patients. The generation of empirical data via semi-directed interviews is envisioned using the comprehensive interview model developed by Kaufmann (1996). The study postulates that co-building of knowledge about patient care by all stakeholders in the management of Buruli ulcer, incorporating a cultural dimension, is necessary and useful for patient therapy. The study identifies a variation of the classical social link that becomes a three-way relationship (physician-patient-carer) and reveals the importance of patient care in the African context. Following Schurmans et Charmillot (2007), the study maintains that the simple application of anthropological, sociological and psychological knowledge about disease, preserving intact the predominance of the biomedical paradigm, is conducive to this combination of knowledge, skills and efforts directed against the common enemy of the diseased individual.

Project SCOBU

Authors : Professor Yuki Shimomura, Dr Kazuyuki Fukunishi

For the past five years, Kobe International University Project SCOBU has developed a pilot educational program for BU children in Benin and Togo.

The program provides BU children a proper primary education with the rigid administration and control by the ministry of health of Benin and Togo and the German Leprosy and Tuberculosis Relief Association (DAHWA).

In Cameroon, the program support also went for a BU student to enable to further his education.

We are continuing advocacy programs in Kansai, Western Japan area.

Advocacy programs have progressed considerably over the time and always made strength our fund.

Throughout our activities, we can confirm the theory how small NGO or NPO such as our group can do effective activity or support for the area where need help, is cooperation with International Organization such as United Nation or WHO and the Government where the organizations wishes support. Project SCOBU as a small non-medical group will continue our socio-economic challenges and wishes our small support can help BU children.

CONTROL

Epidemiological features of Buruli ulcer and surveillance system in Gabon in 2009

Author : Dr Louis Stanislas Bayonne Manou

1) Introduction

Buruli ulcer, or tropical lesion, is a disease caused by the *Mycobacterium ulcerans* mycobacterium. More than 40 cases are diagnosed each year, the main endemic focus being located in Moyen-Ogooué province. Because of the scale of the disease and its social and economic consequences, the authorities consider it to be a public health problem.

2) Activities carried out in 2009

- A survey in Ngounié province;
- Social mobilization of the population in Ogooué Ivindo;
- Social mobilization in Moyen-Ogooué province;
- Monitoring activities in Moyen-Ogooué province;
- Training and awareness-raising for health workers in Ogooué Maritime.
- Case confirmation and case management.

3) Results

a-1) Collection of samples and case confirmation in Ngounié (centre south region)

Ngounié province = 18 patients

The results of PCR and Ziehl Neelsen stain were negative

a-2) Collection of samples from cases in Moyen Ogooué province (centre region)

Moyen-Ogooué (Lambaréné) province = 23 patients

PCR was positive in 14 cases, i.e. 60.86%

Table N° I : Total number of cases diagnosed by sex and category (n= 41)

Sex \ Category	Cat. 1	Cat. 2	Cat. 3	Total
Male	5	9	12	26
Female	6	4	5	15
Total	11	13	17	41

Tableau N° II : Samples confirmed by PCR and ZN (n = 14)

Biologie \ Samples	Number of samples tested	Number of samples confirmed by PCR	Number of samples confirmed by ZN	%
PCR	41	14		34,14
Ziehl Neelsen	41		14	34,14

b) Awareness raising

We organized chats followed by discussions supplemented by visual aids, posters (1400 posters) and distribution of brochures (1350 brochures) for 60 to 70 % of the population of Moyen-Ogooué and Ogooué provinces in order to raise awareness among them. In order to cover a greater proportion of the population, we showed the WHO film *The Mystery Disease* at several locations.

c) Training

Twenty-five (25) health workers from Ogooué Maritime province were trained in clinical procedure, case notification, sampling techniques and case management.

d) Treatment

Of the total of 23 patients admitted to hospital in Moyen Ogooué province:

- 9 patients received specific antibiotic treatment and excision of the edges of their lesion
- 14 patients received specific antibiotic treatment and surgery (excision and grafts)

Results :

- Duration of treatment varied from 30 to 120 days, with an average of 75 days
- 2 patients were re-admitted to hospital (relapses) i.e. 8.6%
- After cure, 6 patients continued to present limitation of movement i.e. 26,08%
- The proportion of patients cured without sequelae was 73.92%

4) Challenges facing implementation

For administrative reasons, it was impossible to carry out a number of activities:

- Mapping cases of Buruli ulcer in Moyen-Ogooué province to improve surveillance of areas at risk;
- An active case-detection survey in Ogooué maritime province;
- Quarterly monitoring of activities;

Other challenges

- Momentary stockout of specific antibiotics
- Unavailability of a physiotherapist to treat muscle and tendon contractures;
- Poor compliance with antibiotic treatment (streptomycin) in the case of children;
- Failure of community intermediaries to follow up awareness-raising activities.
- Problems of access to riverside areas for lack of a boat

5) Solutions proposed

- Timely provision of the budget required for all activities before October of the current year ;
- Re-supply of the antibiotic stock at least three (3) months before the expiry date;
- Training a physiotherapist for Albert Schweitzer hospital;
- Providing the community intermediaries with resources to allow them to pursue the effort to raise awareness

6) Conclusion

If we exclude Sud Est and Sud Ouest health areas, which are as yet unexplored, the disease focus of Buruli ulcer is Moyen Ogooué province, and more precisely the department of Lambaréné,. The proportion of category 3 lesions is still high, making it necessary to continue the effort to raise awareness in the endemic area. Although medical and surgical treatment have been provided by the town's two hospitals, rehabilitation of muscle and tendon contractures remains a problem. The support provided by partners each year should reach us earlier to enable us to carry out all the activities scheduled for the year.

Buruli ulcer in the Central African Republic

Authors : Dr B.Boua, Dr E. Dolido

Introduction

Buruli ulcer (BU), a crippling disease caused by *Mycobacterium ulcerans*, has been endemic in the Central African Republic since 2008 through confirmation of 4 cases by PCR.

Action taken

The Cotonou Conference has galvanized the campaign to control Buruli ulcer in the Central African Republic. The following action has been taken:

1. Official status accorded to Buruli ulcer campaign;
2. Advocacy meeting with the political and administrative authorities and community leaders in 4 prefectures;
3. Disease surveillance.

Results

1. Implementation of a national programme to control neglected tropical diseases including Buruli ulcer;
2. Development of a plan of operations for 2009;
3. Raising awareness among the political and administrative authorities (prefects and mayors), chief medical officers of prefectures, directors of health districts and communities (community intermediaries, students, teachers) within prefectures;
4. Training: a training session for 30 health workers and 5 laboratory technicians per prefecture;
5. Fact-finding mission to Lobaye prefecture: 8 suspected cases, 2 samples taken, awaiting results;
6. Management of 2 cases using a combination of rifampicin and streptomycin under the currently applicable protocol with monitoring of treatment (2nd, 4th, 5th, 6th and 8th weeks).

Challenges

1. Estimation of the extent of the disease in the Central African Republic;
2. Early case detection;
3. Comprehensive case management.

Solutions

Given the favourable circumstances such as the willingness and commitment of the Government and the readiness of partners and local NGOs to support the campaign against neglected tropical diseases, we hope to continue organizing advocacy meetings in order to promote Buruli ulcer control efforts.

In addition, we desire strong and continuous support from financial and technical partners with a view to mobilizing financial resources for BU mapping throughout the Central African Republic and providing logistical support to coordinate implementation of the planned activities in a timely manner.

Conclusion

Efforts to control Buruli ulcer in the Central African Republic are still in their infancy.

The commitment of the Government and partners will expedite efforts to manage the disease more effectively.

Buruli ulcer control in Cameroon in 2009

Authors: Earnest Njih Tabah¹, Alphonse Um Boock², Sarah Eyangoh³

¹National Committee for leprosy, Buruli ulcer, yaws and leishmaniasis control; ²Regional Bureau for Africa of ALES; ³Centre Pasteur du Cameroon

Introduction

Cameroon is one of the countries in Africa endemic for Buruli ulcer. There are 6 confirmed endemic areas in the country and a number of other suspected areas. Control activities against Buruli ulcer started in the country in 2002 with the creation of the National Control Programme in the Ministry of Public Health. The national control programme is being supported by a number of partners namely the WHO, ALES, MSF-CH and Centre Pasteur du Cameroon.

I. Implementation

The Buruli ulcer control activities in Cameroon for 2009 included national level and operational level activities. The national level activities were mainly carried out by the office of the national control programme, with the technical support of partners like ALES. The activities included: strategic planning and orientation, coordination and follow-up of activities of the operational level, organization of case confirmation, acquisition and supply of drugs and consumables to treatment centers, training of operational level staff and participation in research activities.

The operational level activities based for the most part at the diagnostic and treatment centers included case finding in the communities, antibiotic treatment, wound dressing and surgery, prevention of disability in, rehabilitative and psycho-social care. This level also carried out community sensitization, data compilation and reporting.

II. Results

Training: 24 health personnel (8MDs, 11 nurses, and 6 lab technicians) from 5 BU diagnostic and treatment centres (BU DTC), the East Region, and the National control programme were trained on the fine needle aspiration technique. Ten (10) nurses from 5 BU DTC received a level-2 training on prevention of disability in BU patients. Thirteen (13) nurses and 63 community relays were trained in the Atock health area, a new endemic area in the East region, in view of establishing a new BU DTC there. Twelve (12) chiefs of health centers and nurses from 7 health centers in the Ayos health district were trained on management of early forms of BU, in view of decentralizing treatment to the health centers of the district. Three MDs and 5 nurses from 5 BU DTC were trained on some aspects of reconstructive surgery. Finally, 2 national level trainers attended a training of trainers' workshop on FNA technique in Benin.

Case finding, confirmation and management:

Some 429 patients with ulcers were consulted at the 5 BU DTC in 2009. Three hundred and twenty three (323) were confirmed as clinical cases of Buruli ulcer, bringing the cumulative total to 2730 since 2001. Although most of the cases came from the known endemic areas of Akonolinga, Ayos, Bankim, Ngoantet (Mbalmayo), Mbonge and Atock (Abong Mbang) 18 other health districts notified cases. Males constituted 55% of the cases while 53% were less than 15 years of age. Majority (81%) of the lesions were ulcers. The lesions were mostly found on the lower limbs (68%) and the upper limbs (26%), and were of categories I (44%) and II (37%) mainly, with category III lesions constituting only 19%. Limitation of joint movement at diagnosis was more present in lesions of category III and II.

Of the 429 suspected patients, specimens were collected from 361 (84%) for laboratory confirmation. 34.1% and 24.7% of the patients were positive for PCR and Ziehl Neelsen stain respectively.

Two hundred and eighty-nine (289) of the patients completed the streptomycin-Rifampicin treatment while 17 of them underwent heat treatment, within the framework of the 2nd phase of the heat treatment trial. Seventeen others either abandoned their treatment or did not begin treatment at all. Five (5) patients healed with limitation of joint movement.

The major reasons for refusal or abandonment of treatment were related to worries about leaving farm work and other personal activities for the period of treatment or lack of family support while at treatment center.

Functional rehabilitation

Within the framework of the Swiss Surgical Mission, 33 victims of BU who healed with disabilities benefited from plastic/corrective surgery in 2009. This brought to 105 the number of victims who have benefited from the project since 2007.

External evaluation

The Buruli ulcer control programme in Cameroon received two separation missions of external evaluation in 2009. The first one was by Dr Roch Christian Johnson from Benin, who evaluated the whole control programme. The second was by Dieter Imhof who evaluated the Ngoantet – Mbalmayo Buruli ulcer project.

III. Challenges

- Weak institutional basis of the control programme
- Insufficient allocation of government resources (risk of sustainability)
- Emergence of new suspected endemic areas
- Refusal/ abandonment of treatment by some patients
- Irregular supply of Streptomycin –Rifampicin antibiotics by the WHO, with risk of stock outs
- Insufficient laboratory confirmation of cases

IV. Perspectives

- Advocacy to the high authorities of the Ministry of Public Health
- Need for survey and confirmation of new suspected endemic areas
- Improve on the laboratory confirmation chain
- Speed up decentralization of treatment in endemic districts with BU DTC
- Develop new strategies to curb refusal/abandonment of treatment
- Encourage and intensify operational research activities

Buruli ulcer: the epidemiological situation in 2009 in the Republic of the Congo

Author : Dr Damas Obvala

Coordinator, national leprosy/Buruli ulcer control programme

Buruli ulcer ranks second to leprosy among endemic dermatological diseases that present a public health problem in our country.

The following six departments are affected: Kouilou, with epicentres in Kakamoéka district and Pointe Noire; Niari; Bouenza; Pool (in Kindamba district) and Cuvette (Mosaka district).

Activities carried out in 2009 include activities to improve community awareness, supervision of health workers responsible for case-management facilities, case detection and drug and surgical treatment of patients, training for the programme's health workers and managers at all levels and systematically taking samples from cases for confirmation by the specialized laboratory in Angers (France).

The results achieved in 2009 include training 68 health workers from the disease-endemic departments of Bouenza, Niari and Pool; training for 130 community intermediaries from the endemic areas of Kouilou, Niari, Bouenza, Pool and Mossaka; five epidemiological surveys in Cuvette Ouest, Likouala and Sangha departments and in Mossaka and Kindamba districts resulting in the detection of 10 confirmed cases of Buruli ulcer; three supervisory rounds involving 67 health workers responsible for case management in endemic departments; case detection and treatment for a total of 137 new cases; collection of 76 samples for confirmation by PCR and systematic collection of photographs of each patient to improve case documentation.

Of the aggregate total of 1186 cases notified since 2004 and including 2009, **147 new cases** of Buruli ulcer were treated at the different centres; 59 (43%) in Kouilou and Pointe noire departments; 56 (40.8%) in Niari; 22 (8.7%) in Bouenza; 4 (2.9%) in Cuvette (Mossaka district) and 6 (4.3%) in Pool (Kindamba district).

Ulcerative lesions were the most common (91.1%), followed by nodular and oedematous (4.7%) lesions respectively, with children accounting for 27.2% of cases.

The majority of patients were male (63.2%); 75.4% of cases were in categories II and III; 71.1% of lesions were on the lower limbs and joint movement was severely affected in 44% of cases upon detection. A total of 61.2% of cases were cured with no sequelae.

Twenty-five new communities notified cases during 2009 and 7 new districts were affected by the disease.

The following epidemiological indicators were recorded for Buruli ulcer during 2009: new case detection rate - 10.55%; percentage of cases confirmed - 61.5%; proportion of ulcers among new cases - 89.49%.

Case confirmation by PCR was an important activity during the year; samples were taken for PCR from 43.6% of the 147 new cases, with 46.8% proving positive; swabs were the commonest type of sample.

Treatment by surgery is still problematic in Niari department and physiotherapy/rehabilitation still limited. The main constraint is lack of funds. Improved community awareness and further epidemiological surveys in departments not yet surveyed and in which Buruli ulcer is suspected, together with supervisory training activities are the main lines of activity planned for 2010.

Contribution to improving Buruli ulcer control in Songololo Territory, Democratic Republic of the Congo

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1. Introduction, objectives and methods

Songololo Territory is one of the most active foci of Buruli ulcer (BU) in the Democratic Republic of the Congo. The functional disabilities caused by BU have significant socioeconomic consequences for patients and their families. In 95% of the 64 patients admitted to the Kimpese General Referral Hospital/Evangelical Medical Institute in connection with BU during the period 2002-2004, the disease had been detected at the ulcerative stage; 36% of cases already presented with functional limitation at the time of diagnosis; and 23% had developed permanent disabilities. The average hospital stay was 89 days. Furthermore, an abnormally high fatality rate (19%) was observed. After more than three years of control measures undertaken by the Kimpese Evangelical Medical Institute BU project, covering the Kimpese and the Nsona Mpangu health areas, the morbidity and disability rates for BU remain high. Of 190 patients admitted to the hospital, 86% were diagnosed at the ulcerative stage and 20% made a recovery but with permanent disabilities. The object of this paper is to review the current state of knowledge on BU control and identify the progress, problems and limitations of the Buruli ulcer control project in Songololo, in order to suggest ways to enhance the interaction between the vertical control programme and core health services.

A combination of three methods has been used:

4. A review of the available literature on BU control, using vertical analysis as a conceptual framework;
5. Analysis of quantitative data from inpatients at the Kimpese General Referral Hospital/Evangelical Medical Institute in the period 2002-2007, using the conceptual framework for the evaluation of health programmes proposed by Habicht et al.;
6. Analysis of data from the BU prevalence survey conducted between June and August 2008 in Songololo Territory.

II. Results

The analysis reveals that options for improving BU control are currently limited. The mode of transmission of *M. ulcerans* from the environment to humans is still not entirely understood. There is neither a specific vaccine for BU, nor a rapid diagnostic test that could detect the infection in asymptomatic patients. The current control strategy focuses on early detection and treatment through a combination of antibiotics, with or without surgery.

Data from patients treated at the Kimpese General Referral Hospital/Evangelical Medical Institute show that the average number of annual admissions has tripled, from 21 cases in the period 2002-2004 to 63 cases in the period 2005-2007. The proportion of female patients increased from 30% in the former period to 49% in the latter ($p=0.005$). In the latter period, there was a significant reduction in the recurrence rate ($p<0.001$), ulcerative forms at case detection ($p=0.041$), mixed ulcerative forms ($p=0.0038$), and the fatality rate ($p=0.0001$). The proportion of patients cured without complications increased markedly in comparison with the period before the project. ($p=0.001$). Just over half of patients (56.3%) had benefited from specific antibiotic therapy. However, no significant difference was observed between the two periods with regard to the number of confirmed cases ($p=0.183$), cases of osteomyelitis ($p=0.302$), cases of restricted joint movement at the time of diagnosis ($p=0.119$), cases of recovery with restricted joint movement in all patients (however they were discharged from hospital) ($p=0.0496$), and

even the number of patients deemed to be cured ($p=0.119$) or referred for surgery ($p=0.052$). The average duration of hospitalization (almost 90 days) was almost the same in both periods.

According to the prevalence survey, of 259 patients with progressive lesions (a prevalence of 109 per 100 000), 74% are ulcerated, 48.8% are category I, 31.5% are category II, and 19.7% are category III. Functional limitation is found in 23.9% of cases at case detection and confirmed by laboratory tests in 27.8% of patients.

We note, however, that since the beginning of this year, 25 patients have already been admitted to the Kimpese General Referral Hospital/Evangelical Medical Institute. This shows that the numbers admitted to hospital are just the tip of the iceberg.

III. Challenges

1. Increase geographical and therapeutic coverage of the population at risk;
2. Boost early detection of BU cases;
3. Reduce the socioeconomic impact of BU;
4. Increase laboratory confirmation of BU cases;
5. Boost community involvement.

IV. Proposed solutions

1. Decentralization and integration of control activities at local health facilities in endemic foci. To reduce the BU burden, which is chiefly attributable to late diagnosis, and to increase coverage of the at-risk population, we believe that the most effective method of coordinating BU control in Songololo Territory would be to pursue a vertical programme - either centralized or decentralized - and to incorporate control measures into existing multidisciplinary facilities in the Kimpese and Nsone Mpangu health areas. Decentralization and integration of control activities will facilitate access to case detection and offer better treatment to BU patients in the most peripheral areas of the health system.
2. Establishment of an operational BU case confirmation network to monitor the decentralization of specific antibiotic treatment. At the periphery, the confirmation loop should be integrated into the existing networks of the tuberculosis programme.
3. Intensification of information, education and communication aimed at the general public via local radio, schools, churches and community-based organizations.
4. Development of community-based activities to improve early BU case detection, in close collaboration with district health management teams, community leaders, village volunteers and existing community-based organizations.

National Buruli ulcer control programme- 2009, Ghana

Authors: Edwin Ampadu¹, William Opare²

¹National programme manager, ² Technical officer to national programme

Introduction

Ghana continues to dominate in Buruli ulcer case load within the West African Buruli ulcer endemic countries. Six out of the ten regions of the country report on the disease. This is in the forest belt. The national Buruli ulcer programme, supported by Government of Ghana, collaborates with some local and external NGOs in implementing programme activities. In 2009, NGOs like the World Vision international **WVI**; Health Foundation Ghana **HFG**; Nestle Ghana Ltd, and **ANESVAD**, foundation, Spain supported the national programme implementation.

Implementations

Major activities carried out in 2009, were mainly at two levels; the national and districts levels.

At the National level mainly mobilizing funds, ensuring availability of essential programme logistics and also linking districts to interested NGOs to support some programme activities.

The main funding support to the national level programme comes from the ministry of health. Activities carried out included advocacy at the national and ministry's headquarters levels, lobbying for funds, facilitating training of staff and community volunteers, coordination and follow up to the districts and regional levels.

Occasionally, national programme was called to offer technical advice to management of Buruli ulcer. Most treatment centres were linked to the **WHO** recommended laboratories for case confirmation. Antibiotics were provided to all treatment centres and mobilizing funds to support districts and regional programme activities. Data validation and compilation were carried out every quarter both at the national level and at the major treatment centres/districts.

The national programme supported Togo, Benin, and Nigeria with antibiotics.

At the regional and districts levels, the **WVI** and **HFG** assisted 4 districts in organizing district case detection, advocacy works, community health education, case management and awareness on the disease. Other activities also included Surgery and antibiotics treatment, prevention of disabilities and improving wound care.

Follow ups and other coordination made in the districts

Results

Funding support provided to six regions to carry out surveillance and documentation training for 38 endemic districts and treatment centres. National programme provided technical support to early case detection and specimen collection techniques; *swab, fine needle aspiration and specimen transportation*.

Antibiotics were provided to all the six regions for further supply to the districts treatment centres.

Antibiotics use coverage increased from 29 treatment centres last year to 49 in 2009. This increase is best seen in Brong Ahafo region.

Improving wound care can be seen in some of the BU treatment facilities.

Promoting improved wound care is needed.

Buruli ulcer , Ghana 2009			
Indicators	Years		
	2007	2008	2009
Total new cases	626	986	853
%PCR confirmed	28.3	51.6	57
% Patient completing antibiotics			58
% Patient < 15yrs.	62.1	36.5	37.9
% Female cases	54%	48	54.6
% Cases with joint limitations	3.9	6.9	28.3
% Ulcerations	62,8	68.4	59.7
% Cat I and II	32.1	49.4	60.1

Laboratory confirmation was **57%** of total cases detected. There is high awareness of case confirmation practices both at the recommended laboratories and at the treatment centres. All treatment centres now communicate with the 3 reference laboratories for PCR confirmation. Specimen containers recommended by the laboratory were procured for distribution.

Training – **67** health workers and other community staff from 6 Buruli ulcer districts in Gt. Accra, Eastern, Ashanti and Brong Ahafo regions were trained in case detection, and case management. [6 disease control officer, **18** clinical nurses, **2** doctors, **6** laboratory persons and **12** public health staff community teachers] were trained in diagnostics, case management and use of the BU reporting forms.

Case findings and management - A national total of 853 cases were reported. These were from 49 treatment centres. Category of cases : Cat I **29.7%** Cat II **30.4%** Cat III **36.8%**

Additional surveillance forms were developed and printed and put to use to assist the programme implementation in the districts. They include:

- **BU referral and transfer forms BU09.** this is to allow better referrals, and to assist clinician in continuation of care
- **Laboratory examination request forms BU04** – to ensure that case management are backed with laboratory confirmations
- **Logistics Order form BU06b.** a system of tracking the utilization of logistics sent and to justify replacement
- **Quarterly Treatment outcomes report form BU06a** For use as case management assessment
- **District Buruli ulcer register** – as a registry system for updating BU cases in the area- DHA based.

External visit; The ANESVAD foundation, Spain paid a working visit to Ghana. The visit gave the opportunity to assess their support and to strengthen better collaboration in Buruli ulcer control. A team also from the America wound care association paid a working visit to Ghana to advocate for improved wound care and lymphoedema control practices. Lectures and training on modern wound care practices were provided and over 25 health facilities benefited from the training. The **ALM** and **MAP** international also visited Ghana to strengthen the prevention of disabilities associated with the disease.

Advocacy work; the national programme collaborated with local NGOs, based both in the country and outside the country. The programme collaborated with an NGO **Bishop T foundation** to launch a programme to support Buruli ulcer victims in Ga West. The NGO is based in the UK.

Nestle Ghana Ltd also supported some surgical outreach works in Ashanti – Amansie East and Central region.

Challenges

Buruli ulcer continues to be low on Government agenda may be as a result of low number of cases generally reported though debilitating and also because it is low to nil case fatality as compared with other diseases.

The recommended laboratories to support with PCR investigations are not all accessible to some of the treatment centres, geographically.

High attrition of health workers in the remote treatment centres

Regional and district monitoring always waiting for further direction from the national programme before action is taken.

Prevention of disabilities related to the disease still a low priority even to the treatment centres and staff not much trained.

Probable solution

Treatment centres be allowed to use Z N [microscopy] to confirm and when is negative, PCR requested.

Encouraging more NGOs to participate in the local control programme

Government to increase Funding support Buruli ulcer control procurement of surgical dressings, antibiotics, support surgical outreaches

Regions and districts must show leadership drive in Buruli ulcer control as in other disease control activities despite funding limitations

Improve programme transport mobility to strengthen monitoring activities and also local outreach works to the regions and districts

Review national programme implementation to forge ahead new direction

Conclusion

With the current trend in case management, soon the disease can be demystified. This is possible and all stakeholders need to support programmes to achieve that.

Funding is urgently needed to improve on antibiotics treatment and to further decentralize treatment to make it more accessible to the remote communities.

Buruli ulcer control: Nigerian situation

Author : Dr Agbochenu S. Aboje

(BU focal person)

Buruli ulcer is being recognized as a public health problem in Nigeria following the BU assessment that was carried out in 5 states in 2006 and the sensitization workshops for the TBL control officers from the 36 states and Abuja. Since then increasing number of cases are being reported, mainly from the southern states (25 cases from 2 states in 2009).

In 2009, BU experts from WHO Geneva, WHO Afro and Benin Republic were in Ogun State for field assessments and participated in the training of General Health Workers (GHWs). This has increased the number of registered BU cases in the state (12 in 2009, 4 of which were confirmed by PCR and 3 others by AFB only). After the field assessment in Ogun state, sensitization and health education campaign in endemic communities in Ogoja, Cross River State yielded 13 suspected cases (5 confirmed by PCR). Similarly, data from other states in the south show an increase in the number of suspected cases.

WHO donated Rifampicin and Streptomycin in December, 2009 and have been distributed to various states that are reporting cases. The usage of these drugs and the outcome of the cases treated with the drugs are being monitored and will be reported at the meeting.

Cross-border collaboration between Benin Republic and Nigeria has impacted much on our programme. Cases are being referred across the border and treated. Currently, all PCR tests are being done in Benin Republic where some general Health workers and lab personnel have been trained.

The focus for our activities in 2010 is to expand awareness creation and sensitization activities to the Northern States with special attention to NOMA Hospital in Sokoto state where various types of ulcer from neighboring villages are being treated. There will be a need to collaborate with Nigeria Institute of Medical Research (NIMR) and TB and Leprosy International Training Centre Zaria so that their laboratories can be used for confirmation of suspected cases using PCR technique.

Nigeria presentation at the Global Buruli Ulcer meeting will focus on cases so far treated, outcome of treatment, challenges of diagnosis and the need to collaborate with existing centres in Nigeria for PCR technique. Awareness of the disease among Medical and Health personnel is still very low. A field mission for awareness creation, especially among Health workers in the rural communities will go a long way to increase case finding.

Buruli ulcer control in Togo in 2009

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Introduction

The Yamoussoukro Conference on Buruli ulcer in 1998 marked the beginning of a stronger commitment by the Togolese Government to Buruli ulcer control. The National Buruli Ulcer Control Programme (PNLUB) was set up and provided with a coordinating mechanism - actually in name only, because the campaign really got under way only after DAHW became involved in 2006. Since early 2008 the human and material resources of the programme have been strengthened with help from DAHW, which has built and equipped the referral centre. In its efforts to control the disease, Togo has also benefited from technical and financial assistance from WHO and Handicap International.

I. Implementation

The National Buruli Ulcer Control Programme aims to reduce Buruli ulcer morbidity and related disability in Togo. Unfortunately only three development partners (WHO, DAHW and Handicap International) are involved in this campaign; the Government's contribution is negligible by comparison. Following negotiations, the Government has agreed to allocate 20 million CFA francs to the PNLUB for 2010.

At the operational level, BU control activities have focused mainly on the national referral centre supported by two treatment pilot centres (one run by the Government and the other by a religious denomination). The number of BU cases recorded in 2009 was 52, of which 28 were confirmed by PCR. The majority of the cases could not be authenticated or confirmed, given that case confirmation only began in 2006.

II. Results for 2009

Training: 30 community health workers were trained in one session; 14 health post nurses were trained in two sessions and 5 leprosy/tuberculosis/Buruli ulcer monitors were trained in one session;

Case detection, confirmation and treatment: There were 52 suspected cases of BU in 2009 of which 28 were confirmed (53.8%). 40 of these cases (76.9%) were from the endemic area (Maritime region), and 8 cases or 15.4% from the Central region; two of the other national regions recorded just 1 case.

The proportion of cases was split evenly between the sexes (26 cases or 50% each); among confirmed cases, 20 were ulcerative (71.4%) and 4 presented with nodules (14.3%). 2 cases (7.1%) presented with oedema and 2 with plaques (7.1%). 4 cases (14.3%) were category I; 8 (28.6%) were category II; 9 (32.1%) were category III and in 7 cases (25.0%) the category was undetermined.

Functional rehabilitation: 17 patients were cured without sequelae; 4 patients were cured with sequelae; 17 patients were admitted to hospital in 2009 and 5904 physiotherapy sessions were conducted by the national referral centre.

Outside visits: the Togolese National Buruli Ulcer Control Programme hosted three outside visits in 2009: the first from 2 staff of Handicap International; the second from Dr Jorg, who performed a number

of surgical interventions; and the third from Dr Asiedu and Dr Tiendrebeogo of WHO, who made a number of recommendations to the Government. To date these have not been followed up.

III. Difficulties

- The principal difficulty faced by the PNLUB in carrying out its activities is the shortage of human, material and financial resources.
- Very limited input from Government in all activities undertaken by PNLUB.
- The frequent turnover of coordinators; between 2006 and 2009 the programme has had 4 coordinators.
- Late case detection of Buruli ulcer.
- Poor community awareness-raising.

IV. Future action

To step up its efforts to control Buruli ulcer in Togo, PNLUB intends:

- To carry out detailed mapping of BU cases;
- To improve decentralized treatment of cases;
- To train community intermediaries and treatment providers from other endemic areas in early detection and case management;
- To raise public awareness of Buruli ulcer in other endemic areas;
- To combine leprosy, yaws and Buruli ulcer in a single programme;
- To promote and intensify operational research activities;
- To participate in and actively contribute to implementation of the BuruliVac programme.

V. Conclusion

The campaign to control Buruli ulcer has made headway, especially in the Maritime region of Togo, thanks to the project to bolster the campaign against Buruli ulcer in this region. Significant challenges must be overcome, however, before the campaign can be extended to all endemic areas in the country. The active involvement and input of the Government is required alongside that of development partners (WHO, DAHW and Handicap International).

Surveillance and data management: Adjumani Hospital, Uganda

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Background

Adjumani and Moyo districts are the two districts in Uganda which are currently endemic of Buruli Ulcer disease (BUD). The numbers of cases continue to fluctuate annually as well as within the year. We report on cases diagnosed between June and December 2009.

What was done

Adjumani hospital is a major hospital close to the Albert Nile water expanse and receives cases referred from both districts of Moyo and Adjumani. Dr Dramentu has specialized in surgical management of BUD and operates on all the cases. During the months June-December 2009 three cases were diagnosed (all the three diagnosed in September). These were all ulcerative forms and one was recurrent. All three were confirmed on histology and Ziehl Nelsen stain. The lesions were on left lower limbs in all the three cases. They underwent wide excision of the ulcers with skin grafting.

Achievement/Outcome

One patient died of septicemia and two improved and discharged

Challenges

The few numbers diagnosed in this region is probably due to underreporting as a result of poor sensitization of the communities about the disease and its management.

Solutions

Institute grass root sensitization on the current management of the disease particularly in communities along the banks of the river Nile.

Conclusion

Adjumani and Moyo districts continue to register cases of BUD and will need sensitization workshops right from communities up to health workers in Adjumani hospital.

SURVEILLANCE

Buruli ulcer in Côte d'Ivoire: a new disease focus identified in Adiopodoumé, Yopougon commune (Abidjan, Km 17)

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Introduction

In Côte d'Ivoire, *Mycobacterium ulcerans* or «Buruli ulcer» infection is the second human mycobacterial disease after tuberculosis. After the first cases were discovered in 1978 in the region of Daloa, there was a long period with no further developments. After 1989, a large number of cases were reported, justifying a national survey in 1997 in which 10 382 cases were identified. Since then, the geographical area affected by the disease has expanded. From two endemic foci initially, Buruli ulcer now affects 33 health districts. Previously, the region of Abidjan was one of those in which no cases had been recorded. However, in 2008, there were confirmed cases in Bonoua, a town 35 Km east of Abidjan. In December 2009, suspected lesions were identified on inhabitants of Adiopodoumé, a village in Yopougon commune. A prospective and active case-detection survey found suspected cases that were confirmed by the laboratory at the Institut Pasteur in Côte d'Ivoire. This presentation describes the epidemiological, sociological, clinical, microbiological and ecosystem characteristics of this new disease focus in Abidjan.

Methods and patients

An investigation was carried out throughout the village of Adiopodoumé. Each patient with a suspected Buruli ulcer lesion answered a questionnaire. Skin exudates were collected with the patients' consent and placed in a medium composed of Middlebrook 7H9 and 0.75% cetylpyridinium chloride (CPC). The exudates were preserved at laboratory temperature for 24 hours and cultivated on Lowenstein-Jensen medium. After centrifuging, the plug was stained with Ziehl-Neelsen and PCR targeting the IS2404 insertion sequence was carried out.

Results

Epidemiological characteristics: 13 patients were identified: one 14-year-old schoolchild and 13 adults aged from 20 to 60. A total of 77% of the subjects were Ivorian and 23% from ECOWAS countries. **Sociological characteristics:** 85% of the subjects relied on traditional treatments, either exclusively or before attending a medical facility. In all, 92 % of them said they had developed the disease naturally, and had no idea of its etiology. **Clinical features:** 77% of the lesions were ulcers, with a single oedema (7%) while 26% presented sequelae such permanent scars and joint contractures. A total of 85% of lesions were located on the lower limbs. In two cases (15%), Buruli ulcer was suspected by health workers. None of the subjects had received specific medical treatment in accordance with the WHO protocol. **Microbiological characteristics:** a sample of exudate was taken from 85% of subjects and in 77% of cases Buruli ulcer was confirmed by PCR targeting IS2404. **Ecosystem characteristics:** the village is surrounded by a swamp, a river and a lake and several sites are flooded during the rainy season. A total of 92% of the subjects had been in contact with one of these at a period unspecified.

Conclusion

After a long period without Buruli ulcer, Abidjan has recently reported the first residents with the disease. Thirteen patients with suspect skin lesions were registered in December 2009. Analysis of the exudates collected detected the presence of *Mycobacterium ulcerans*, thus providing confirmation of cases of Buruli ulcer in Abidjan.

Keywords: Buruli ulcer; *Mycobacterium ulcerans*; Adiopodoumé (Abidjan Km 17); Côte d'Ivoire.

The results of one year of decentralization of Buruli ulcer (BU) control activities in Songololo Region, Democratic Republic of the Congo (DRC)

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Introduction

The BU control programme in Songololo region (the most endemic area in the DRC) is based at the General Referral Hospital of the Kimpese Evangelical Medical Institute. Starting in September 2009, after conducting the area prevalence study, the specialist team at the General Referral Hospital decentralized its activities to the 2 health areas in the region (Nsona-Mpangu and Kimpese).

Implementation of decentralization

56 health workers (tenured nurses and their assistants), 4 front-line physicians and more than 150 community intermediaries have undergone basic BU training. Local health facilities have been given the necessary tools to treat patients in the community, namely 60 bicycles, 2 motorbikes, reporting forms BU01, BU02 and BU06, medicines, equipment for collecting samples, transport media and other consumables, etc. BU control activities have thus been incorporated into community health services. The specialist team at the General Referral Hospital continues to treat complex cases, in addition to carrying out supervisory duties and providing expertise where necessary.

Results

1. Case detection in 2009

172 new cases were detected in 2009, of which 97 (56.4%) were from Nsona Mpangu health area, 69 (40.1%) from Kimpese health area and 6 from outside Songololo region. Among the detected cases, 34 (19.8%) were admitted to hospital at the Kimpese Evangelical Medical Institute and 138 (80.2%) were treated at peripheral facilities. Women accounted for 56.4% of the total number of patients; patients under 15 accounted for 48% of the total.

2. Clinical presentation of detected cases

Most new cases (80.2%) were category I and II. Ulcers accounted for 74% of cases. 48 patients (27.9%) presented with limitations of joint movement at the time of diagnosis.

3. Confirmation of diagnosis

Of 42 specimens analysed at the Antwerp Institute of Tropical Medicine, 28 (66.6%) were PCR positive. Of the 34 patients admitted to hospital at the Kimpese Evangelical Medical Institute, 25 (73.5%) lab tested positive, 23 by Ziehl Neelsen stain and 2 by histopathology. However, among samples collected in the community, only 18.9% of Ziehl Neelsen stain results were positive.

4. Treatment with rifampicin and streptomycin

A total of 138 patients (80.2%) in both health areas have been treated with specific antibiotics.

Challenges

- Case detection should be expanded beyond highly endemic villages.
- PCR is not always feasible at the national laboratory.
- Weakness of case confirmation at the community level.
- Weakness of POD practice.

Proposed solutions

- Intensify supervision and resume awareness-raising campaigns.
- Advocacy vis-à-vis the national and provincial governments and partners.
- Re-organize the system of case confirmation in the community.
- For POD, see 2nd text.

Conclusion

The decentralization of BU control activities has been effective in our programme; since implementation in September 2008, we have been able to increase the number of cases detected in peripheral areas and treated in the community, thereby bringing about a significant decline in the number of cases admitted to the Kimpese Evangelical Medical Institute every year. It is a promising initiative which is worth pursuing in the future, while at the same time it offers a remedy for current problems.

Buruli ulcer in the WHO African Region: An Epidemic or Endemic disease?

Author : Dr Alexandre Tiendrebeogo

Background

Africa is the most affected continent by Buruli ulcer (BU). Considering the trend of the disease at Regional and National levels could lead to consider the disease as an endemic. However, with the occurrence of outbreaks of BU cases in some foci or villages, an epidemic start of BU could also be described. Analysing the trends of the disease over years in the WHO African Region could help in better understanding the epidemiology of the disease and lead to preventive measures and control activities.

Methodology

Compilation of annual reports from BU endemic countries in the WHO African region enables to build graphs of BU trends in the Region and in major endemic countries. Annual detections of BU cases in some high endemic villages and foci were also collected and analysed by graphs of trends to decide if they had an epidemic or endemic evolution.

Results

Trends of BU at Regional, sub regional and National levels appeared to describe an endemic trend, even if some spikes of increased annual cases, due to active case search during National surveys, were observed in Cote d'Ivoire, Ghana and DR Congo. In some high endemic foci (Benin, Gabon), BU started by a sudden increase of new cases imitating an epidemic outbreak. Even in these villages, in the long term, the disease returns to endemic trends with a curve in plateau. Epidemiological studies complemented with ecological ones during the epidemic phase of the disease in foci or villages could help in clarifying the epidemiology of the disease, mainly the existence of a reservoir or a vector.

Conclusion

BU is an endemic disease which could start as an epidemic outbreak in villages or high endemic foci. Country programme managers should collaborate with epidemiologists and ecologists to investigate epidemic phase of the disease in new endemic foci.

RESEARCH

***M. ulcerans* in the environment and transmission to humans**

Risk factors for Buruli ulcer in Bankim, a newly endemic area in Cameroon

Presenter: Dr Sara Eyangoh

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In Cameroon, only one endemic area had been described since 1969, in the Nyong River basin between Ayos and Akonolinga in the central region. Five years ago, clinically suspected BU cases were reported for the first time in the district of Bankim, a rugged land in north-western Cameroon. Interestingly, a dam was built in this region in 1989, creating an artificial lake of 3.2 billion m³ capacity. Large amounts of farmland were flooded and several villages relocated. Since 2007, around 75 cases of BU were reported each year from Bankim, for a population close to 70000 inhabitants.

In 2007, a case-control study on BU in Akonolinga suggested a protective role of the use of bednets. In order to verify and further investigate this result, a case-control study was conducted in Bankim from the 1st June to 17 August 2009. This study also aimed at investigating other risk factors for BU in a savanna setting. Each of the 79 included cases was matched with two controls of the same sex, age and village. Data analysis was performed using conditional logistic regression.

Multivariate analysis confirmed that using a bednet everyday was a protective factor against BU (Odds-Ratio(OR)=0.4 ; 95% Confidence Interval(95%CI)=[0.2-0.9]). Few people had good quality bednets, and thus investigations on impregnation or holes remained inconclusive. Taking proper care of wounds was a strong protective factor (OR [95%CI]=0.1 [0.04-0.4]), providing confirmation for another association identified in the Akonolinga study. Surprisingly, growing cassava was a protective factor (OR[95%CI]=0.4 [0.2-0.9]). We hypothesize that this protection could result from a lower exposure to disease on the savanna farmland where Bankim farmers usually plant cassava or from a diversified nutrition of farmers and their families.

Increased risks were associated with Contact not only with the dam swampy area but also with the other River in region and water bodies neighboring houses and environment exposures such as not wearing shoes during washing clothes or household activities (OR[95%CI]=7.7 [1.4-42]). Reporting skin lesions due to itching after insect bites was significantly and independantly associated with disease (OR[95%CI]=2.7 [1.3-5.5]), but this association might result from memory bias.

In conclusion, this study shows for the second time that using a bednet is associated to a significant protection against BU. This finding advocates for further studies on transmission of BU focus on dwellings and neighboring water bodies. Communication regarding protection methods should now be provided to local population.

Biological diversity and the possible involvement of aquatic Hemiptera in the transmission of *Mycobacterium ulcerans*, an etiological agent of Buruli ulcer in Côte d'Ivoire (West Africa)

Presenter: Professor Julien Doannio

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A "neglected" tropical disease, Buruli ulcer was declared an emerging disease by WHO in 1998. Caused by *Mycobacterium ulcerans*, a mycobacterium living in a hydrotelluric environment, Buruli ulcer has in recent years become the third most prominent mycobacterium after leprosy and tuberculosis. It is spreading at an alarming rate in West Africa and especially in Côte d'Ivoire, which is the most severely affected country in Africa and worldwide with 25 000 cases between 1978 and 2007 and another 2000 cases detected each year. The disease is particularly frequent in precisely delimited zones; it spreads very rapidly in marshy and grassy areas near watercourses. Understanding the epidemiology of the disease is key to controlling it. While it has been convincingly demonstrated that person-to-person transmission is impossible, people are apparently infected through contact with the aquatic environment via a mode of transmission that is still poorly understood. Recent studies suggest that aquatic insects, specifically water bugs, might play a role in the transmission of *M. ulcerans* to humans after accidentally biting them. A study of the specific diversity, biology and ecology of water bugs and their role in the transmission of *M. ulcerans* to humans was conducted in Côte d'Ivoire in 2008. Specimen water bugs were collected at monthly intervals from various aquatic environments in Dabou and Tiassalé regions, and subsequently in Bouaké region, which are Buruli ulcer endemic areas. The bugs were identified by family, genus and occasionally species using a binocular microscope and the *Illustrated Catalogue of Aquatic Insects of Côte d'Ivoire*. Their distribution, frequency and the monthly variations in their ecodistribution at various locations were correlated with human activities. Monospecific batches of water bugs were regularly constituted to identify the molecular signatures of *M. ulcerans* using PCR. Eighteen (18) species of water bugs belonging to 8 families were inventoried. The most abundant species, present at all the sites with significant numerical frequency, belong to the genera *Diplonychus*, *Micronecta*, *Naucoris* and *Ranatra*. Observed human activities were extremely diverse (bathing, fetching drinking water, fording, fishing, washing clothes and cooking utensils). 289 monospecific batches of water bugs were PCR tested and 26 specimens (about 9%) tested positive. These belong to the families Belostomatidae (57.89%), Naucoridae (10.53%), Nepidae (5.26%), Ranatridae (10.53%), and Notonectidae (10.53%). The specific diversity of water bugs, their distribution and the presence of *M. ulcerans* in certain species that accidentally bite humans in the water suggest involvement in the transmission of Buruli ulcer that is closely linked with human activities connected with bodies of water. In addition, species of the genus *Diplonychus*, attracted by light sources, are able to fly considerable distances and could bite people either outside or inside their homes.

Key Words : Biological diversity - Possible involvement - Aquatic hemiptera - Transmission of *Mycobacterium ulcerans* - Buruli ulcer - Côte d'Ivoire - West Africa

A Matter of Scale: Demography, Micro-geography, *M. ulcerans*, and Buruli ulcer in the hamlets of a Buruli Ulcer endemic village in Benin

Presenter: Professor Pamela Small

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The distribution of Buruli ulcer and *Mycobacterium ulcerans* in the environment has been a main focus of recent research on the transmission of Buruli ulcer. We have begun small scale mapping and population studies of Buruli Ulcer and *Mycobacterium ulcerans* at the hamlet level in rural arrondissements in Benin where Buruli ulcer has high prevalence. Hand-drawn maps were constructed in the field and a GPS unit was used to gather spatial data for computer analysis and mapping. In the study area, villages are comprised of numerous small hamlets. Whereas some hamlets within a village are geographically discrete, in other cases hamlet designation is primarily based on social criteria and two hamlets may be contiguous. We have conducted initial mapping and population data on three small hamlets within a single village. The hamlets studied share the following characteristics: homogeneity with respect to ethnic composition, a shared village plan in which all residences are adjacent to agricultural areas, house construction based on ferruginous clay and similar domestic agriculture within the village compound. None of these hamlets are located on the river. Each hamlet has a single closed well. Secondary water sources include cisterns and water jugs for collection of rain water from roof run-off. All three hamlets are within a few kilometers of each other.

It was surprising to find that the prevalence of Buruli ulcer differs between hamlets from 26/1000 to 141/1000 over a three year period. When cases were mapped to individual houses within a hamlet, spatial clustering was not observed consistent with other data suggesting that transmission does not occur within the residence. Preliminary information on social structure suggests that the most common household unit is a mother and her children. In every hamlet multiple cases of BU were identified in at least one residence.

Environmental samples were screened for the presence of *M. ulcerans* using ER and IS2404 qPCR. Results from these studies show a strong correlation between BU prevalence and the % *M. ulcerans* positive samples/site. Although country-wide data in Benin does not support seasonal variation in Buruli ulcer, data collected at the village and hamlet level show an unequal distribution of cases across the year. Studies are underway to create agricultural land use maps for each land owner/leasee in order to determine whether type or location of agricultural activities is associated with *M. ulcerans* infection.

This work has led to the development of methodology for small scale mapping of Buruli ulcer and *M. ulcerans* which may reveal factors important for transmission that are not obvious from large scale analysis.

Presence and Abundance of *Mycobacterium ulcerans* in 25 endemic and nonendemic villages in Benin

Presenter: Dr Heather Williamson

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A particularly interesting aspect of Buruli ulcer epidemiology is the fact that it has been reported that endemic and nonendemic villages may be separated by only a few kilometers. Factors contributing to this phenomenon have been proposed such as unique environmental conditions that may affect bacterial presence and abundance, poor disease reporting, and behavioral factors that play a role in increased contact with a contaminated environment. In order to document the relationship of *Mycobacterium ulcerans* in the environment with Buruli ulcer, we conducted a study to identify *M. ulcerans* in environmental sources in 25 sites in Benin where a village-based active case surveillance program has been in place for over five years. In this study we compared the presence and abundance of *M. ulcerans* in non-endemic villages along the Mono River basin, and endemic villages along the Ouémé, and Couffo basins. Environmental samples were also taken in 4 villages along a ridge at 100 M adjacent to endemic sites on the Ouémé. Standard sampling included 3 water samples per water source, 3 samples from dominant macrophytes, and random samples from soil, and biofilm accumulation. Samples were assayed for the presence and quantity of *M. ulcerans* and other mycolactone producing mycobacteria (MPM) using serial analysis of IS2404-PCR followed by ER-PCR. Results from this study show a positive association between bacterial distribution and abundance with BU prevalence. The most accurate predictor of BU prevalence was ER-PCR. ER-PCR accurately predicted endemicity in 17/22 (77%) villages where case data was available. Although IS2404-PCR positive samples were identified in all BU endemic sites, IS2404 positive results were also obtained from all but one non-endemic site where BU had never been reported. Thus the overall correlation between IS2404 and endemicity, although positive, was less than that for ER-PCR. There was also a strong relationship between % ER-PCR positive samples per site and BU prevalence. The mean number of bacteria per water filtrate was 1679.9 genome units (GUs)/mL. The highest number of MU GUs was detected from soil followed by biofilm and macrophyte samples. Results of this study advocate the use of active case surveillance when linking environmental factors and Buruli ulcer.

The role of terrestrial small mammals as reservoir of *Mycobacterium ulcerans* in Benin

Presenter: Dr Lies Durnez

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Mycobacterium ulcerans, which is considered an environmental pathogen, is the causative agent of Buruli ulcer (BU), a skin disease associated with aquatic ecosystems. In line with recent findings in Australia supporting a role of mammals in the ecology of *M. ulcerans*, we hypothesize that terrestrial small mammals are part of the reservoir of *M. ulcerans* in Benin.

Rodents and shrews were trapped in BU high and low endemic villages in Benin. Organ samples were analyzed for the presence of *M. ulcerans* and other mycobacteria by using culture and PCR.

In total 565 animals were collected (343 rodents and 222 shrews). Mycobacteria were detected in 12.0% of the animals, but no *M. ulcerans* was detected. Relative animal species abundance differed significantly with more shrews caught near water bodies in BU high as compared to low endemic areas. More mycobacteria were detected in shrews as compared to rodents and a contrasting seasonal difference was observed in the prevalence of mycobacteria in rodents and shrews.

The present study is the first aiming at detecting *M. ulcerans* in wild animals in West-Africa. Although *M. ulcerans* was not found, more research should be carried out on a higher number of specimens and species of (small) mammals.

Données scientifiques sur la colonisation de l'appareil digestif de mammifères par *Mycobacterium ulcerans*

Presenter: Dr Janet Fyfe

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On a détecté l'ADN de *Mycobacterium ulcerans* (Mu) par PCR en temps réel dans les déjections d'opossums, appartenant aux espèces phalangers à queue annelée et phalangers-renards, et de rats noirs vivant dans les régions de l'État de Victoria (Australie), où l'on a observé dans le passé et actuellement des cas humains d'ulcère de Buruli. On a aussi obtenu à l'occasion des échantillons fécaux Mu-positifs d'autres espèces de marsupiaux, comme des phalangers-renards des montagnes, des koalas et un potoroo à longs pieds. Point intéressant, à l'exception du potoroo, toutes ces espèces sauvages sont principalement arboricoles et n'ont que peu ou pas de contacts avec des nappes d'eau.

En 2008-2009, 42 phalangers à queue annelée et 21 phalangers-renards ont été capturés à Point Lonsdale, ville d'endémie pour l'ulcère de Buruli. On a examiné tous les animaux pour voir s'ils avaient des lésions et, si c'était le cas, elles ont été écouvillonnées et des échantillons sanguins et fécaux ont été prélevés. Chez les phalangers à queue annelée, 16 (38%) présentaient au moins une lésion cutanée à Mu (confirmée par culture et/ou PCR en temps réel ciblant IS2404) ou avaient des déjections IS2404-positives. Le plus souvent, l'estimation de la charge bactérienne en Mu dans les échantillons fécaux des différents animaux a varié d'un facteur allant jusqu'à 10⁶, entre 10⁷ Mu/gramme et <10 organismes/gramme. Les animaux porteurs de lésions multiples avaient les plus fortes concentrations fécales en Mu. Six phalangers à queue annelé Mu-positifs ont été tués et autopsiés pendant l'étude et, dans chaque cas, les estimations du nombre de Mu dans le contenu des différents compartiments de l'appareil digestif ont été remarquablement semblables et bien plus élevées que celles pour l'épithélium intestinal. Mu a été mis en culture à partir du foie et de la rate d'un de ces animaux et il a été détecté par PCR dans les organes de deux autres opossums. La probabilité de lésions a été moins élevée pour les phalangers-renards (1 animal/21 ayant une lésion à une patte), mais on a détecté un faible portage fécal (jusqu'à 10³ Mu/gramme) chez 4 autres animaux.

Sur le même site, 50 rats ont été tués et autopsiés à la suite de leur capture dans des pièges à opossums. L'ADN de Mu a été détecté dans le contenu intestinal (mais pas dans les organes) de 30 % de ces animaux. Seul un de ces rats présentait une lésion cutanée Mu-positive.

À partir de cette étude, on a conclu qu'il était possible de détecter Mu dans les voies digestives d'opossums et de rats en bonne santé, vivant sur ce site d'endémie, mais que la charge bactérienne est plus forte chez les animaux porteurs de lésions.

Nous avons émis l'hypothèse que Mu pourrait s'introduire dans les voies digestives des mammifères par l'ingestion d'aliments contaminés, puis se multiplier et/ou persister dans cet environnement. Pour tester cette hypothèse, nous avons administré par gavage gastrique une dose unique contenant environ 10⁹ cellules viables de Mu à 17 souris C57BL/6 femelles adultes. Nous avons contrôlé chaque semaine la présence de Mu dans les boulettes fécales par PCR-IS2404. Six semaines après l'inoculation, Mu n'était pas détectable dans les matières fécales des souris. En revanche, 7 semaines après l'inoculation, deux souris ont donné des échantillons fécaux positifs pour IS2404 et 12 semaines après l'inoculation (à la fin de l'expérience) 5 souris/17 ont donné des échantillons fécaux positifs pour IS2404, ce qui semble indiquer une colonisation productive de l'appareil digestif des souris par Mu.

Systemic lesions associated with *Mycobacterium ulcerans* in naturally infected Australian marsupials

Presenter: Dr Christina McCowan

Authors: Christina McCowan, Janet Fyfe, Carolyn O'Brien, Kath Handasyde, Caroline Lavender, John Hayman, Paul Johnson

In humans, *Mycobacterium ulcerans* causes cutaneous disease, with dissemination sometimes occurring to bones. The organism grows preferentially at low temperatures and oxygen tensions, and this has been proposed to regulate the localisation of lesions. In the past three decades, however, there have been reports of granulomatous lesions in viscera of two koalas and a long footed potoroo with ulcerative skin lesions. *M. ulcerans* was cultured from the skin lesion, liver and spleen from the potoroo.

We autopsied 6 ringtail possums, 5 with skin lesions and 1 without, from the currently *M. ulcerans* endemic town of Point Lonsdale, in Victoria. All 6 were positive for *M. ulcerans* in the faeces by PCR. For comparison, tissues were collected from PCR negative ringtail possums from urban Melbourne, submitted to the University of Melbourne Veterinary School for euthanasia or disposal following trauma. No animals had osteomyelitis. All the Point Lonsdale animals had granulomatous hepatitis, ranging from very mild, with a few lymphohistiocytic clusters and no demonstrable mycobacteria in one animal without skin lesions, to moderately severe, with numerous foci of histiocytic accumulation, in all the animals with skin lesions. These latter also demonstrated granulomata in lung and small numbers of acid fast bacilli were found in both liver and lung lesions. *M. ulcerans* was cultured from liver, lung and spleen of one of these animals.

Further investigation is required to demonstrate the route of infection of viscera, whether from intestine or skin, and to document the frequency with which visceral lesions occur. Further study also will be important to identify factors which permit survival of *M. ulcerans* in the warm, well oxygenated environment of the liver, lung and spleen.

The role of climate-environmental-human interactions for predicting Buruli ulcer emergence in Victoria, Australia

Presenter: Dr Eric Benbow

Authors: M. Eric Benbow, Jenni van Ravensway, Anastasios Tsonis, Lindsay P Campbell, Paul DR Johnson, Janet Fyfe, Caroline Lavender, John Hayman, Qiongxia Song, Lijian Yang, Steven Pierce, Shuai Chen, Jiaguo Qi

Buruli ulcer disease (BU) occurs predominately in the coastal regions of West Africa; however, disease incidence has substantially increased in southern Australia over the past 30 years, particularly in Victoria, Australia. The disease first emerged in the Bairnsdale region around 1940 and moved westward with an outbreak on Phillip Island in the early 1990's, followed by another at St. Leonard's in the late 1990's, while the most recent and severe outbreak began in the early 2000's in Pt. Lonsdale. Why these outbreaks occur in such focally ephemeral patterns remain an area of active research, and was the premise of this study.

To date studies addressing factors involved in BU outbreaks of Victoria have been relatively focused on each area independently, and there have been no studies that have tested climate or landscape drivers of disease outbreaks in Australia, although these variables are known to be associated with many other diseases. The most recent studies of the Pt. Lonsdale emergence have suggested a strong potential role of mosquitoes in the transmission cycle in this region; however, the exact role of mosquitoes is not known. Indeed, larval mosquito habitat is a product of precipitation patterns and landscape topography, factors that have been associated with BU prevalence in West Africa, suggesting a set of possible common variables of BU transmission that transcends geographic regions. Previous studies and recent unpublished evidence evaluating environmental drivers of BU emergence suggest that flooding is often associated with increased risk of BU outbreaks. Heavy precipitation and associated flooding often generates larval mosquito habitat, but the extent and quality of such habitat for mosquito populations depends on local topography and other weather (e.g., temperature) conditions.

Flooding in low-lying areas during increased precipitation offers two broad transmission hypotheses related to weather-topography drivers of disease emergence: 1) increased extent and duration of inundated flooded areas act as 'incubation habitats' of *M. ulcerans* (or its reservoir[s]), providing increased potential of direct human contact associated with flooded waters/soil/vegetation, or exposure to recently inundated household gardens or other habitats of soil-related human activities; and, 2) those same inundated areas are increased habitat for larval mosquitoes, and thus biting adults, contaminated with *M. ulcerans* upon emergence and serving as mechanical vectors. In both hypotheses, it is assumed that *M. ulcerans* is already in the flooded habitat, or is brought in from higher elevations with the floodwaters, effectively 'coating' the ground and vegetation surfaces of flooded areas. Vertebrate reservoirs could act as initial or additional modes of *M. ulcerans* contamination and movement in the low-lying environment that contribute to 'incubation habitats'. To test these hypotheses, a first step is to evaluate the interactive role of weather and topography as they relate to BU emergence.

The objectives of this study were two-fold: 1) determine whether BU incidence in Victoria, Australia is random, and if not, investigate potential climate, landscape, and social/occupational factors that may be associated with increased incidence; and, 2) develop transmission scenarios that can be examined in future case studies. Examining the spatio-temporal pattern of BU incidence allowed us to determine if the disease network was being driven by external forces or if the pattern of cases was occurring randomly. Identification of the random vs. non-random nature network of disease incidence, which can be accomplished through network analysis, is often an initial step in transmission modeling to help understand whether deterministic factors are responsible for the pattern of disease cases. If the pattern of disease incidence is non-random, the second step is to statistically test variables most likely to be

associated with the disease; in the case of BU, the environmental (precipitation, landscape, temperature) factors that contribute to flooding, which has been consistently reported as a risk factor in many studies.

To test whether there was a spatial pattern of Victoria BU incidence, we employed a network analysis of the geographic locations of BU cases over time from 1980 – 2008 that include 302 cases (from 1 – 97 cases in any town). With this analysis, we were able to compare the connectivity of the BU network with a series of 100 randomly generated surrogate networks to test whether the BU network was significantly different from a random network. The analysis revealed a clustering coefficient of 0.32 for the BU network, while the average clustering coefficient of the surrogates was 0.23, resulting in the rejection of the null hypothesis, ' H_0 = the BU network is random' at a 99% significance level. Our results also indicated that the BU network possesses small-world properties making the network stable and efficient in transferring information, or in this case BU. Thus, our results clearly suggest that the BU network is not random, indicating that deterministic forces influence the occurrence of this disease.

We also tested a series of climate, landscape, and occupational variables individually in relation to BU incidence. In order to evaluate if there was a weather association with BU, we included climate (e.g., parameters of rainfall and temperature) covariates from 4 to 24 months prior to each case observation in our initial set of independent variables. We chose to examine climate conditions up to two years prior due to the unknown lag times regarding pathogen development in the environment and human exposure to the pathogen and/or potential vector/reservoirs.

In order to assess potential flooding conditions and optimal conditions for mosquito abundance, we analyzed Victoria BU data with monthly precipitation and temperature data from 1980-2008. Exploratory statistical analysis of all predictor variables was performed using Generalized Linear and Additive Models (GLM and GAM, respectively). A GLM was used initially to identify significant variables, and then a series of GAM models were fitted to these variables to sequentially reduce them. The GAM results suggested multiple outdoor occupations increased risk for BU incidence (e.g., employees of parks, gardens and construction), which would likely involve frequent contact with surface soils, a habitat for *M. ulcerans*. However, there was a lack of significance of water related occupations (fishing, water and sewer industries).

Further, we found positive associations of BU with maximum wetness index and percentage of water cover within endemic towns, both indicators of areas with frequent flooding potential and results similar to published landscape findings from West Africa. In addition, our modeling revealed that two climate variables at two different lag periods significantly predicted disease: increased precipitation and increased minimum temperature 13 and 12 months prior to outbreaks, respectively. This combination indicates that increases in precipitation and temperature are environmental conditions important to cases that are reported about a year later.

In summary, our results indicate that BU emergence is not random in Victoria, Australia and that climate is an important driver of emergence. Populations of people working outdoors and in ground-related activities are at higher risk of disease. Importantly, there is a one year lag time between the climate event and case reporting, suggesting several unknown lag events that may be due to variability in environmental habitat or reservoir incubation conditions, the timing and extent of specific human activities associated with transmission and human incubation time. These data also suggest that this long lag time between an environmental factor (rainfall and temperature) and case reporting will make it difficult to identify sources of contact with *M. ulcerans* and human populations. Because our results indicate that weather is an important correlate with disease, similar conditions may be important for disease emergence in West Africa; however, identifying such patterns could be more difficult considering gross differences in socio-cultural activities, disease surveillance and reporting and health seeking behavioral differences.

Benthic macroinvertebrate metrics associated with *Mycobacterium ulcerans* in Ghana, West Africa: can invertebrate communities indicate the probability of pathogen occurrence?

Presenter: Mr Ryan Kimbirauskas

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Several studies in West Africa have associated Buruli ulcer with disturbed aquatic environments, while others have implicated aquatic insects, particularly biting Hemiptera, as reservoirs of *Mycobacterium ulcerans* and vectors of Buruli ulcer disease. The purpose of our study was to: 1) characterize and compare overall macroinvertebrate communities from aquatic environments in Ghana; 2) identify macroinvertebrate community metrics (MCMs) associated with the presence and absence of *M. ulcerans* in aquatic environments; and 3) identify potential relationships between specific macroinvertebrates and pathogen presence. This study was part of a multi-year survey in Ghana investigating the ecology of *M. ulcerans* in aquatic habitats, which is to date the largest systematic and standardized study investigating *M. ulcerans* in the environment. During this survey, standardized macroinvertebrate collections were taken from 98 water bodies in Ghana, of which more than 73,000 individual macroinvertebrates were identified and used for analyses. In addition, macrophyte communities and water column seston (suspended material in the water column) were assayed for *M. ulcerans* DNA, and a tiered PCR method (ER positivity followed by VNTR) was used to detect pathogen presence at each site. We found that after accounting for lentic (standing water) and lotic (flowing water) water body flow types, there were significant differences in specific MCMs in relation to the presence and absence of environmental *M. ulcerans*. In lentic habitats, taxa diversity and evenness, as well as percent Diptera, were significantly greater in water bodies where *M. ulcerans* was detected, suggesting eutrophication and other land-related disturbances at those sites. In lotic habitats, sites where *M. ulcerans* was detected had significantly lower ratios of scraping to collector-filtering macroinvertebrates, indicating an unbalanced community likely responding to an overabundance of filamentous algae, which is another indicator of eutrophication. In lotic sites where *M. ulcerans* was detected there was a significant increase in percent piercing-predators, which included biting Hemiptera; however, overall numbers of piercing-predators were low and on average constituted less than 1% of total organisms per site. Indicator Species Analysis (ISA) revealed several individual taxa in both aquatic habitats to be associated with the presence of *M. ulcerans*, however only in lotic environments were there indicator taxa associated with the absence of the pathogen. Water striders (Gerridae) were identified as an indicator of *M. ulcerans* presence in lotic, but not lentic habitats, and no biting Hemiptera (Naucoridae, Belostomatidae) were identified as indicators of pathogen presence or absence using ISA procedures. Results from this large survey of aquatic environments in Ghana suggest that macroinvertebrate communities and individual taxa may be useful sentinels for initial identification of pathogen presence or habitat conditions associated with disease agent transmission.

Spatial variation of *Mycobacterium ulcerans* within an endemic water body

Presenter: Dr Mollie McIntosh

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Numerous studies have associated Buruli ulcer disease with disturbed aquatic habitats; however, the natural reservoir, distribution and mode of transmission of the bacterial pathogen, *Mycobacterium ulcerans*, remain unknown. Over the past ten years, *M. ulcerans* has been identified from numerous environmental samples collected within aquatic habitats, including water filtrates, plant and plant biofilms, invertebrates, amphibians, fish and artificial substrates (e.g., glass slides). However, noted variability in the identification of *M. ulcerans* among samples from the same substrate, among samples within the same aquatic habitat and over time suggest that *M. ulcerans* may be naturally patchy in aquatic environments. Yet, this variation could also be an artifact of the study design, with non-standardized sampling and a lack of, or low, replication. As a result, the main objective of this study was to understand the distribution of *M. ulcerans* populations within a single endemic water body. This information, in addition to corresponding abiotic and biotic data (physical, chemical and biological parameters) will provide needed information on (1) the natural spatial distribution and variation of *M. ulcerans* within a water body and (2) conditions necessary to establish and sustain effectively detectable *M. ulcerans* populations.

This study was conducted in a single lentic water body located within an endemic village in the Ga West District of Ghana, Africa, during August 2009. A three-dimensional grid was established to cover a portion of the water body, including the inundated water and transition (riparian) zones. At selected grid points, replicate samples of natural substrates were collected to test for the presence and abundance of *M. ulcerans*, including water filtrates (from both open and vegetated water zones), biofilm from three locations on emergent plants (exposed dry stem 10cm above the water line, the stem 5cm above and below the waterline, and the submerged stem 10 cm below the waterline), invertebrates, transition zone soil and plants. In addition, artificial substrates (wood poles) were placed in both water zones at selected grid points for a 7-day incubation period; each pole had two perpendicular wood branches at multiple depths (high, low) with glass slides attached to the top and bottom of each branch (shade, exposed). Detection and enumeration of *M. ulcerans*, from both natural and artificial environmental samples were conducted using a quantitative PCR technique targeting the enoyl reductase gene. In addition, numerous abiotic variables (water chemistry, depth, light) were measured at multiple depths (surface, 25%, 50% and 75% of total depth) at each grid point to associate *M. ulcerans* populations with microhabitat environmental conditions.

Preliminary results (including water filtrate, plant biofilm and artificial substrates only) indicated that 20% of all samples (n=622) were identified as *M. ulcerans* positive. A majority of these positives were found on artificial glass slide substrates (11.4%), followed by natural plant biofilms (4.5%) and water filtrates (3.7%). The spatial distribution of *M. ulcerans* appeared non-specific, with positive samples located on all substrate types, in both open and vegetated water zones, and at multiple depths within and above the water column. However, the extent of distribution was dependent on substrate type, with *M. ulcerans* presence highly variable and non-uniform on natural substrates compared to more consistent and uniform presence on artificial glass substrates. This could be due to the horizontal distribution of the glass slides within the water column or to the introduction of new uncolonized substrates into the aquatic environment. The abundance of *M. ulcerans* also varied spatially throughout the water body, with highest

abundance consistently observed in the vegetation zone. Of the positive vegetation zone samples, artificial substrates had the highest overall mean abundance of *M. ulcerans* (20,353 +/- 11,388 GFUs/ml), followed by plant biofilms (8,080 +/- 1,981 GFUs/ml) and water filtrates (311 +/- 167 GFUs/ml). The observed vertical distribution of *M. ulcerans* within the water column ranged from 8 to 80 cm in depth and was also identified above the waterline on emerged dry plant stems. On artificial substrates, higher abundance of *M. ulcerans* was found near the water surface whether exposed or shaded from light; however, this result was only observed in the vegetation zone compared to the open water. The horizontal and vertical distribution of *M. ulcerans*, specifically with more presence within the vegetation zone and near the water surface, was significantly associated with certain abiotic variables, including shallower depths, higher dissolved oxygen levels and higher temperatures. The results of this study provide valuable information regarding the distribution of *M. ulcerans* within a single water body, however, it should be noted that natural (season) and human-induced (development, pollution) changes to the environment could alter the spatial distribution of the bacteria. This study also provides insight into the amount of replication and standardized methods needed to detect *M. ulcerans* in an aquatic environment, which could have important implications for future environmental research studies and for the management of the Buruli ulcer disease.

Diatom-indicated characteristics of aquatic plant biofilms associated with *Mycobacterium ulcerans*

Presenter: Dr Stephanie Miller

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Recent observations of *Mycobacterium ulcerans* on aquatic plant surfaces have suggested that biofilms might be a key environmental niche for *M. ulcerans*, but specific habitat requirements necessary for *M. ulcerans* persistence in biofilms are not known. Diatoms are a diverse group of eukaryotic algae common in aquatic biofilms, and are used worldwide as biological indicators of ecological conditions due to their sensitive, species-specific response to a wide range of environmental parameters. We hypothesized that diatoms could be used to characterize the microhabitat of plant biofilms suitable for *M. ulcerans* colonization. As part of a large, standardized survey of aquatic habitats, we collected biofilms of 150 aquatic plants from 80 waterbodies in the Volta, Ashanti, and Accra regions of Ghana, Africa. PCR-based methods were used to assess the presence of *M. ulcerans* on each plant via amplification of the enoyl reductase (ER) domain. Diatom species in each biofilm were identified, and habitat preferences of each species were used to characterize the microhabitat conditions in each biofilm. Specifically, diatoms were used to indicate the pH, salinity, nitrogen uptake metabolism, oxygen requirements, trophic state, sediment load, and drying exposure of microhabitats in which *M. ulcerans* was observed. Diatom assemblages were different between lotic (flowing water) and lentic (still water) habitats, so we hypothesized that factors affecting *M. ulcerans* presence would be different between lotic and lentic habitats, and compared them separately. *M. ulcerans* DNA (ER) was identified on 43% of lotic plants and 34% of lentic plants. Diatom assemblages were different between ER+ and ER- plants in both lotic and lentic habitats, suggesting drivers of diatom assemblages and *M. ulcerans* (ER) presence were similar. In lotic habitats, classification tree analysis of diatom-inferred microhabitat conditions showed *M. ulcerans* was more likely to occur in low salinity biofilms, or in mildly saline biofilms with relatively higher pH in the Ashanti and Accra regions. In lentic habitats, *M. ulcerans* was more likely to be present in biofilms in the Ashanti and Accra regions containing diatoms tolerant of sedimentation, or in lower sediment, eutrophic conditions. Our data indicate the presence of *M. ulcerans* in aquatic plant biofilms may be driven by environmental factors, but relationships are complex and differ between lotic and lentic habitats. Specifically, these data suggest that water chemistry may be an important predictor of *M. ulcerans* in lotic habitats, but that landscape disturbance-related factors may be key parameters driving *M. ulcerans* presence in lentic habitats. Identifying habitat requirements of *M. ulcerans* and environmental changes that can promote its emergence may help identify specific areas of risk that, with further understanding and communication, could be used to reduce transmission of Buruli ulcer disease.

RESEARCH

***M. ulcerans* pathogenesis and host response**

Experimental infection of Medaka (*Oryzias latipes*) with *Mycobacterium ulcerans*: A model for transmission, pathogenesis and toxicity to fish.

Presenter: Dr Lydia Mosi

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Mycobacterium ulcerans causes Buruli ulcer in humans. This pathogen synthesizes the mycolactone toxin which has been implicated in the progressive necrosis of the ulcerative lesions of the epithelium. A molecular analysis of *M. ulcerans* reveals that this environmental mycobacterium is closely related to *M. marinum*, which causes disease in both fish and man. There are proposals of a potential association of *M. ulcerans* with fish stemming from diagnostic PCR assays for the insertion sequence IS2404. However, fish infections by *M. ulcerans* have not been well documented; moreover, the IS2404 is not unique to *M. ulcerans* and is found in other mycolactone producing mycobacteria which cause infections in fish and frogs. We have thus, employed two experimental approaches to test for *M. ulcerans* in fish. We show here that *M. ulcerans* with or without the toxin does not mount acute infections in Japanese Medaka (*Oryzias latipes*) even at high doses. Moreover, *M. ulcerans*-infected medaka did not exhibit any visible signs of infection nor disease and the bacteria does not appear to replicate over time. In contrast, similar high doses of the wild-type *M. marinum* or a mycolactone producing *M. marinum* (DL) strain are able to mount an acute disease with mortality in medaka. Although these results would suggest that *M. ulcerans* does not mount infections in fish we have evidence that CLC macrophages from goldfish are susceptible to mycolactones. This is the first experimental report that tests the hypothesis that fish act as a reservoir for *M. ulcerans* within the aquatic environment.

Laboratory study of the biology and behaviour of *Diplonychus sp* (Belostomatidae) and its vector competence in the transmission to humans of *Mycobacterium ulcerans*, the pathogen causing Buruli ulcer in Côte d'Ivoire (West Africa).

Presenter: Professor Julien Doannio

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Buruli ulcer is a skin infection caused by a mycobacterium occurring in the environment, *Mycobacterium ulcerans*. Ranked third in terms of number of mycobacterial infections after leprosy and tuberculosis, its epidemiology is the most poorly understood of the three. Humans are infected through the skin while performing daily living activities (e.g. rice cultivation, fish farming, fishing, laundry, fetching water, agricultural work) or leisure activities (e.g. bathing) in endemic areas. The disease is particularly prevalent in West Africa, where the number of cases is increasing annually (2442 cases in 2008). Côte d'Ivoire is the most severely affected country in the world with more than 25 000 cases since 1978. The numerous endemic foci for Buruli ulcer are scattered throughout the country. Over the past four years impressive progress has been made in researching the mode of transmission of *M. ulcerans* infection. Yet despite this, many questions remain unanswered. In Central and West Africa aquatic insects of the order Hemiptera appear to be involved in the transmission of Buruli ulcer. It is likely that aquatic Hemiptera play a role. Studies of the salivary glands of insects that have been experimentally infected and allowed to bite white mice demonstrate the probable role of water bugs as hosts or possible vectors of *M. ulcerans*. In addition, at least 10% of environmental biological specimens tested positive using PCR in Cameroon and Côte d'Ivoire. These pterygot insects are also able to fly from one body of water to another over varying distances, attracted by lights in houses near marshy areas. This method of displacement could account for the current progression of the disease in West Africa and particularly in Côte d'Ivoire, where it is spreading northwards from the south, west, east and centre of the country. Buruli ulcer is not a contagious disease. It is, however, terrifying and severely disabling. Patients must spend long periods in hospital, treatment is costly and burdensome, and can lead to social marginalization or even total exclusion; above all, the mode of transmission is still poorly understood. Local people must therefore be familiarized with preventive measures focusing on identification of the probable vectors of the disease in order to break the chain of transmission of *M. ulcerans*. This presupposes a sound knowledge of the biology, ecology and behaviour of these potential vectors. Accordingly, we have proceeded to laboratory farm the bug most commonly encountered in the environment that shows the highest rates of infection by *M. ulcerans* (more than 10%), namely *Diplonychus sp* of the family Belostomatidae. Adult specimens were collected in the vicinity of fishponds at an experimental station situated between Abidjan and Dabou (a non-endemic site) and subsequently farmed in the laboratory at the Côte d'Ivoire National Institute of Public Health. The parameters used to farm this water bug were standardized in the laboratory (water quality, depth, turbidity, pH, temperature, luminosity, suitable vegetable environment). The insects were fed regularly with mosquito larvae. The embryonic lifespan, the hatching time, the larval period and the number of larval stages prior to adulthood were studied. The adult lifespan was also estimated. Five successive generations of *Diplonychus sp* were obtained. From

egg to adulthood, the larval lifespan is 41 days on average, with deviations from the mean of 29 to 54 days. Hatching generally took place 7 days after egg laying. Five larval stages are distinguishable, separated by five metamorphoses. The lifespan of certain adults obtained in the laboratory varied between 16 and 150 days. The last original parental specimens survived for 11 months. Proficiency in the laboratory farming of *Diplonychus sp* could enable us to decode its genome and collect saliva samples, while also allowing us to perform experimental infections using human strains of *M. ulcerans* in order to confirm or rule out the involvement of this insect in the transmission of Buruli ulcer in Central and West Africa.

Key words: Laboratory study - Biology - Behaviour - *Diplonychus sp* - Belostomatidae - Vector competence - Transmission to humans - *Mycobacterium ulcerans* - Côte d'Ivoire – West Africa

BCG protection against experimental Buruli ulcer: role of mouse and *M. ulcerans* strains

Presenter: Dr Paul Converse

Authors: Paul J. Converse, Deepak V. Almeida, Eric L. Nuermberger, and Jacques H. Grosset
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Background

Vaccination with *Mycobacterium bovis* bacille Calmette-Guérin (BCG) is widely used to reduce the risk of childhood tuberculosis and has been reported to have efficacy against two other diseases, also caused by mycobacteria, *M. leprae* and *M. ulcerans* (*Mu*). Studies in experimental models have also shown some efficacy against *Mu*. In mice, most studies use the C57BL/6 strain that is known to develop good cell-mediated protective immunity. We hypothesized that there may be differences in vaccination efficacy between C57BL/6 and the less resistant BALB/c strain. We evaluated BCG vaccine efficacy against *M. ulcerans* using three different strains of this bacterium: initially, the Australian type strain, designated Mu1617, then, a Malaysian strain, Mu1615, and a recent Ghanaian isolate, Mu1059. The latter two strains both produce mycolactone while the Australian strain has lost that capacity. All 3 strains do, however, induce, severe swelling in the footpads of control unprotected mice, although the severity is not always seen with Mu1617. This report will mainly focus on the experiment involving Mu1615 and Mu1059.

Methods and materials

Mice were vaccinated with $\sim 5 \times 10^7$ BCG Pasteur or control diluent (sham). After 8 weeks mice were challenged with $\sim 3 \times 10^5$ *M. ulcerans*, i.e. Mu1615 or Mu1059 in the right hind footpad. CFU of both BCG and *Mu* were determined at intervals after infection. Time to footpad swelling was assessed weekly.

Results

BCG induced visible scars in 95.5% of BALB/c mice but only 43.4% of C57BL/6 mice. BCG persisted at higher levels in spleens of BALB/c than C57BL/6 mice. During the first 9 weeks, BCG CFU ranged from 0.5 to 1.0 \log_{10} higher in BALB/c spleens than in C57BL/6 spleens. At week 14 the difference was $\sim 1.5 \log_{10}$ and at week 25 there were nearly 3 \log_{10} BCG CFU in BALB/c spleens while none were detected in C57BL/6 spleens.

Two weeks after challenge with *Mu*, both C57BL/6 and BALB/c mice had $\sim 3 \log_{10}$ *Mu* CFU in their infected footpad regardless of vaccination. At 6 weeks, sham-vaccinated mice of either strain showed footpad swelling and $\geq 5 \log_{10}$ *Mu* (either 1615 or 1059) CFU. BALB/c mice vaccinated with BCG had $\sim 2 \log_{10}$ Mu1615 CFU and $\sim 1 \log_{10}$ Mu1059. BCG-vaccinated C57BL/6 mice at week 6 had $\sim 2 \log_{10}$ Mu1615 CFU but $\sim 4.5 \log_{10}$ Mu1059 CFU and most had developed footpad swelling. BCG vaccination significantly delayed swelling in BALB/c mice, regardless of *M. ulcerans* strain, 8 weeks for Mu 1615 and >20 weeks for Mu1059. Vaccination delayed footpad swelling in C57BL/6 mice against challenge with Mu1615 for 7 weeks, but conferred almost no protection against Mu1059. Possible correlates of the better protection of BALB/c mice included 1) the near universal development of BCG scars in these mice compared to the less frequent and smaller scars observed in C57BL/6 mice and 2) the induction of sustained cytokine, e.g., IL17, production as detected in the spleens of BALB/c mice whereas cytokine production was significantly reduced, e.g., IL17, or transient, e.g., Ifn γ , in the spleens of C57BL/6 mice.

Conclusions

These findings may help understand that the efficacy of BCG against *M. ulcerans*, in particular, and possibly mycobacteria in general, may vary due to differences in both host and pathogen.

Protective effect of Water bug saliva against *M. ulcerans* lesion development

Presenter: Dr Laurent Marsollier

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At this day, no specific vaccine against Buruli ulcer is available. BCG vaccination has first been suggested to have incomplete but significant short-term protective effects [9,10]. Subsequent studies have provided highly controversial results with some showing partial protection against *M. ulcerans* osteomyelitis (1, 2). The only study that did not solely rely on BCG scar for assessing BCG vaccination status (a procedure that has been shown to be unreliable when BCG is given under the age of three months which is the case in most African countries), but used vaccine booklet examination, did not find any significant protective effect of routine BCG vaccination against Buruli ulcer (3). Vaccination based on mycobacterial antigens (Hsp65, Ag85A) showed a short delay in lesion development and borderline impact on bacterial loads (4-7). These results demonstrated that the most efficient vaccine for Buruli ulcer does not solely rely on the Mycobacterium alone but is a combination of bacilli- and vector -based molecules as already shown for other microbial agents such as *Borrelia* and *Leishmania* (8-13). Recently, we have demonstrated that repeated water bug bites free of *M. ulcerans* could protect against *M. ulcerans* induced lesions (14).

To test whether vector salivary proteins can protect against *M. ulcerans* lesion, a mouse model was developed involving intradermal inoculation in the tail of 1000 bacilli together with *Water bug* salivary gland homogenate to mimic a potential natural transmission. Before *M. ulcerans* inoculation, mice were immunized by immunogenic salivary proteins purified by affinity chromatography.

We showed that salivary proteins confer protection against the *M. ulcerans* lesion development in mouse model. Moreover, two candidate proteins (isolated from salivary gland homogenate) that are able to bound *M. ulcerans* surface, were identified and detected in saliva fluid. These proteins were detected in several water bugs families.

This work reinforces the concept of using components of arthropod saliva in vaccine strategies against *M. ulcerans* lesions.

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Description of a specific strain of *Mycobacterium ulcerans* isolated in Côte d'Ivoire: Identification using MIRU-VNTR and sequencing

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Introduction

Buruli ulcer is an endemic skin disease in Africa. Côte d'Ivoire, where there are more than 2000 new cases each year, and where new foci are emerging in the vicinity of the large cities, is the country most affected. In order to study the genetic biodiversity of the strains circulating in the country, a study using MIRU VNTR for molecular typing of the Ivorian strains of *M. ulcerans* was carried out.

Material and Methodology

This preliminary study involved a total de 40 samples including 33 strains isolated at the Pasteur Institute's Tuberculosis and Atypical Mycobacterial Unit in Côte d'Ivoire and 07 taken from the DNA of reference strains. The 33 Ivorian strains were isolated from patients living in the different regions of Côte d'Ivoire in which the disease is endemic.

The strains were confirmed as *M. ulcerans* by conventional PCR and by real-time PCR targeting the IS2404 and IS2606 insertion sequences and the ketoreductase (KR) gene, involved in synthesis of the mycolactone produced by the bacterium. Molecular typing was first of all performed using PCR amplification of loci 1, 2, 5, 6, 9, 33 and Mul_0583, after which the PCR products were sequenced and analysed using CLC viewer 5.1.2 and Geneious Biomatters 3.8.4. software.

Results

Of the 33 Ivorian strains analysed, only 17 produced amplification products on all the loci targeted. All 17 strains presented the characteristic (3113) repetition profile of African strains on the following loci: 1, 6, 9, and 33.

As a whole, analysis of the amplified product sequences on the 7 loci studied did not make it possible to observe significant variations among the strains. This demonstrates a high level of preservation of sequences at these loci in the Ivorian strains. However, at locus 1, one strain did show a large deleted region in zones which are as a rule preserved in Ivorian and African strains, together with numerous mutations in neighbouring regions.

Conclusions

Using these 7 genetic markers, this preliminary study shows that the Ivorian strains are monomorph. However, at locus 1, one strain presented differences from the other strains. At this locus, it was 97 % identical with the *Stenotrophomonas maltophilia* strain. This could be a new strain of *M. ulcerans*. Use of new sets of markers or SNP typing might make it possible to detect the existence of a new strain or genetic differences between Ivorian *M. ulcerans* strains.

Keywords: Buruli ulcer - *Mycobacterium ulcerans*- molecular typing - MIRU VNTR- Côte d'Ivoire.

Comparison of murine and human histopathology after chemotherapy

Presenter: Professor Masamichi Goto

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Chemotherapy for Buruli ulcer is widely applied today, but there are only small number of detailed histopathological studies after the chemotherapy. Here we report the histopathological observation of mouse footpad after drug administration, in comparison with those of human skin lesions after chemotherapy.

Animal experiment was conducted as follows; *M. ulcerans* African strain 97-107 (CFU=1.3X10⁶/ml, 25 microliter) was inoculated to bilateral footpads of female BALB/c mice. After day 33, 5 mg/kg/day of rifalazil (RLZ, an antibiotic structurally related to rifampin) was orally administered for 15 weeks, and histopathological evaluation was performed periodically. In untreated mice, extensive subcutaneous edema and fibrin exudate were observed at day 40. A few neutrophils and monocytes were scattered in the edema, but granuloma formation was not observed. Clusters of solid acid-fast bacilli (AFB) were present in the monocytes and in the stroma. In the RLZ-administered mice, fragmentation of AFB started at day 40, and edema and fibrin gradually subsided. Degenerated AFB were phagocytosed in the monocytes. Epithelioid cell granuloma without necrosis was first observed at day 54, which became smaller at day 98.

Skin biopsies were taken from 5 Ghanaian patients with proven *M. ulcerans* infection just before treatment (week 0) and 6 weeks after starting daily streptomycin (15 mg/kg) - rifampicin (10 mg/kg) according to WHO guidelines. Samples were obtained from patients with nodule (1), ulcers (3) and edema (1). At week 0, most cases showed necrosis and mild neutrophilic infiltration, but at week 6 the majority of specimens showed fibrosis with lymphocytic infiltration and/or epithelioid cell granuloma formation. In one nodular lesion, CD3+ T lymphocytes and CD20+ B lymphocytes were diffusely infiltrated in the pretreatment lesion, which increased after the treatment. One ulcerative lesion showed infiltration of CD3+ cells and CD20+ cells only after the treatment. CD68+ cells were observed both in pretreatment and after the treatment, however, the cytoplasm of these cells were larger and often multinucleated in the latter condition.

In this comparative study, bacterial generation and phagocytosis were clearly correlated with granuloma formation in the animal model. In contrast, human lesions did not show obvious concordance, but increase or appearance of T cells and B cells after the treatment may reflect the emergence of host immunity.

Study of the impact of cell death on the immune response during experimental *Mycobacterium ulcerans* infection

Presenter: Professor Jorge Pedrosa

Infection with *Mycobacterium ulcerans* has been associated with impaired adaptive immunity despite the evidence that the protective immune response in Buruli ulcer is mediated by T helper 1 (Th1) cells. To further characterize the development of this response during progressive infection with a virulent *M. ulcerans* strain, we studied the T cell dynamics in both the draining lymph node (DLN) and the footpad of infected mice.

We found that early during experimental *M. ulcerans* infection, mycobacteria-specific Th1 cellular responses developed in the DLN, followed by the migration of the T cells to the active focus of infection in the footpad. However, the progression of the infectious process was associated with increased bacterial proliferation and footpad tissue destruction, which led to the depletion of recruited T cells. In addition, the dissemination of *M. ulcerans* to the DLN was accompanied by massive apoptotic cytopathology, and ultimately to the destruction of the node, further contributing to the depletion of T cells and abrogating IFN- γ expression at later stages of infection.

Our data show that the failure to control *M. ulcerans* infection is associated with the impairment of the maintenance of an adaptive immune response, due to extensive apoptotic cell death in the DLN. Our results suggest that limiting the spread of *M. ulcerans* during the initial phase of the infection would allow for a sustained protective cell-mediated response with the control of this devastating disease.

Modulation of T lymphocyte trafficking by mycolactone

Presenter: Laure Genin-Macé

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We have explored the effects of mycolactone on the migratory properties of lymphocytes in a mouse model of subcutaneous injection. Administration of mycolactone led to a visible and dose-dependent reduction of the size of spleen and draining lymph nodes after 24 h, without inducing cell mortality within these organs. Intriguingly, a concomitant increase in T cell counts was observed in peripheral blood, leading us to hypothesize that mycolactone may alter the capacity of blood T cell to reach lymphoid organs. Amongst the molecules playing an essential role in T cell homing to lymph nodes is the CD62L selectin. The surface expression of CD62L was dose-dependently suppressed in blood and lymph node T cells of mice injected with mycolactone. *In vitro*, non-cytotoxic doses of mycolactone induced a significant suppression of CD62L expression by human peripheral blood-derived CD4⁺ T cells. Mycolactone did not promote the proteolytic cleavage of CD62L from the cell surface, neither its intracellular degradation. It reduced the cellular amount of CD62L mRNAs by 40% after only 4h of treatment. Together, these results suggest that mycolactone impairs T lymphocyte transmigration to lymph nodes by altering the expression of L-selectin at the transcriptional level.

High throughput screening for identification of mycolactone targets : Relations between *M. ulcerans* and nervous system

Presenter: Ms Estelle Marion

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Buruli ulcer is an infectious disease transmitted by arthropod vectors harboring *Mycobacterium ulcerans*, a mycobacterium which belong to the same family of bacteria causing tuberculosis and leprosy. The infection causes painless swelling and severe skin lesions. One key feature of *M. ulcerans* bacterium is its ability to secrete a necrotic toxin, the mycolactone within small lipophilic vesicles, which are critical for the bacterial induced cytotoxicity. The biological knowledge as well as the preventive and therapeutic means for this invalidating disease is still very limited.

Our first approach was to investigate whether the mycolactone toxin could be involved in the neutralization of pain by acting directly on the peripheral nervous system without causing destruction of nervous fibers. By use of live time fluorescence microscopy and appropriate markers, we showed that the addition of toxin at sub-toxic dose provokes modification of ionic currents of neuron cells.

Based on this ability of the toxin, a molecular high throughput methodology was developed for the screening of a genome wide siRNA library and small molecules inhibitors to enable the search of the cellular targets for the toxin. The cell-based assay relies on automated confocal microscopy on macrophages coupled with dedicated image analysis. These two screening allowed us to identify a putative toxin target, and a toxin inhibitor. A binding assay confirmed that the putative target is a receptor of the toxin. Together these results allowed us to build a potential signaling pathway activated by the mycolactone and implicated in ionic channel activities.

The second approach was to confirm this model in the mouse model of *M. ulcerans* infection and its role in the hypoesthesia of the lesions. Toxin inhibitor, daily administered to mice, which were experimentally infected by *M. ulcerans*, conducted to the absence of the hypoesthesia of the lesions. Furthermore, a histological study of neuronal fibers did not show a destruction of neuronal cells. Moreover, in vitro studies have showed that *M. ulcerans* are able to colonize neuronal cells. Then, these results suggested that the hypoesthesia of the *M. ulcerans* lesions could be caused by a non-destructive process of nervous cells.

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Let there be light - a novel approach to the assisted healing of Buruli Ulcers

Presenter: Professor Sven Britton

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We have explored the notion that mycolactone in order to preserve its toxicity has to be kept in relative darkness.

In previous experiments presented at the WHO BU initiative meeting in Cotonou in 2009, we have shown that mycolactone is toxic to keratinocytes in a dose dependent fashion in vitro and that this toxicity is at least partly dependent on the release of free radicals.

However and as we now report, if mycolactone is exposed to UVA light corresponding to less than 30 minutes of sunlight, its toxicity towards keratinocytes- the key cell in wound healing - is completely abolished. We believe that this unequivocal finding may be used therapeutically by exposing the open wound to sunlight e.g twice daily for 30 minutes. In addition to detoxify mycolactone we believe sunlight will increase the local synthesis of

Vitamin D which is essential for the synthesis of cathelicidin (LL37), a peptide essential in wound healing. Most likely the area of the wound still covered by skin is the essential breeding site of *M. ulcerans* and thus these areas should be opened and exposed to sunlight as well.

We are planning controlled in vivo studies in this regard and we hope that such adjunct therapy may allow a shortening of the cumbersome antibiotic therapy in use today.

***M. ulcerans* multi-strain genome sequencing project: progress and insights**

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The introduction of low cost whole genome sequencing technologies has opened the door to detailed genomic comparisons amongst members of the *Mycobacterium ulcerans* - *Mycobacterium marinum* complex. Using 454 pyrosequencing and Illumina short-read DNA sequencing technology we have established draft genome sequences for 14 members of the *M. ulcerans*-*M. marinum* complex, including isolates from human and animal sources from endemic areas throughout the world. Deep insights have been obtained for the first time into the genetic relatedness of *M. ulcerans* strains between - and most importantly - within different countries of Africa (Ghana and Benin). We have completed genome sequencing of 11 *M. ulcerans* strains from Africa (3 from Benin, 8 from Ghana) and six other *M. ulcerans* strains from different regions of the world using Illumina GAII DNA sequencing technology. This process has generated a very large DNA sequence database containing more than 15 billion nucleotides. Note that the *M. ulcerans* genome is approximately 6 million nucleotides in length. A specialized suite of software tools has been developed to analyze this large data set and we have now used comparative genomics to identify single nucleotide polymorphisms (SNPs) between the genomes of all these *M. ulcerans* strains. Importantly, this analysis has shown that we can distinguish strains at a very wide range of geographic (and temporal) scales, from global to the local village level. Every *M. ulcerans* strain thus far was distinguishable from every other by a unique profile of SNPs, even strains obtained from a very localized region of Ghana, less than 40 square kilometres in area could be identified as distinct. This finding represents a major breakthrough in aiding our understanding of *M. ulcerans* and Buruli ulcer as we can now follow the movement of *M. ulcerans* strains through human populations and through the wider environment.

Application of *M. ulcerans*-specific protein antigens for Buruli ulcer diagnosis and exposure assessment

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A rapid, serodiagnostic test would greatly assist clinicians confirm that a patient has Buruli ulcer, a chronic, necrotizing infection of subcutaneous tissue caused by *Mycobacterium ulcerans*. By comparative genomics we identified, then expressed and purified, 43 potential *M. ulcerans* specific antigens. Antibodies to these antigens in sera from *M. ulcerans* infected animals, and a limited Buruli ulcer patient/control serum bank were detected by Western Blot analysis. Specific antibody responses were found to five (MUP024, KRB, MUP045, ACP2, and MUL_2831) of the 43 proteins in these initial tests so they were then used to screen a larger collection of serum samples from 31 patients with confirmed Buruli ulcer and 55 healthy controls from the same endemic area. Equivalent proportions of patients (38.7 – 64.5%) and healthy controls (47.3 to 70.9%) had detectable serum IgG antibody responses to all five antigens, strongly suggesting a significant level of sub-clinical exposure to *M. ulcerans* amongst people living in Buruli ulcer affected areas. Although serodiagnostics may not differentiate patients from controls, they could be used as seroepidemiological tools to better understand the ecology of *M. ulcerans* and the frequency of subclinical exposure. Furthermore, the specificity of these *M. ulcerans* antigens could make them ideal targets in antigen capture tests for fine needle aspirate or biopsy specimens.

Mycolactone-dependent inhibition of adipokine production by primary human adipocytes.

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It has been postulated that mycolactone diffuses away from extracellular foci of *M. ulcerans* by passing through and destroying adipose tissue, contributing towards the observed pathology of Buruli ulcer. This seems an attractive hypothesis since mycolactone itself is very hydrophobic with lipid-like characteristics. However, only one study has examined its effects on isolated adipocytes (Dobos *et al*, Infect Immun 2001 69, p7184) utilising transformed SW872 liposarcoma cells. Here, mycolactone was shown to induce cell rounding, lifting and necrosis of the cells.

In recent years it has emerged that adipocytes have an important immune regulatory function and secrete proinflammatory adipokines in response to TNF and lipopolysaccharide (LPS). This raises the question of how *M. ulcerans* overcomes adipocyte-mediated immunity. To investigate this we expanded pre-adipocytes from surgical breast reconstruction tissue and then differentiated them into adipocytes *in vitro*. Cells were stimulated with LPS for 24hrs and MCP-1, IL-6 and IL-8 production was assessed. Mycolactone inhibited each in a dose-dependent manner and complete inhibition was seen at 10ng/ml. We previously showed that mycolactone inhibits the production of TNF, IL-6 and COX2 in primary human monocytes by specifically targeting the translation of these polypeptides (Simmonds *et al*, J Immunol 2009 182 p2194). Similarly, mycolactone did not inhibit steady state levels of IL-6 mRNA in adipocytes; supporting the notion that IL-6 production is inhibited by the same mechanism in both types of cell.

In terms of mycolactone's cytopathic activity towards primary adipocytes, we found that these cells tolerated exposure to mycolactone quite well (somewhat contrary to our expectations). At 24 and 48hrs there was no loss of cell viability and their morphology appeared normal. This prompted us to examine longer exposure times and, even after 5 days in the presence of 10ng/ml mycolactone, viability of the cells was still not affected. However, although the cells remained attached to the tissue culture surface, the cells had abnormal morphology and had lost many of their characteristic lipid droplets.

Our findings suggest that adipocyte immune function is effectively inhibited by mycolactone in Buruli ulcers, contributing to the overall immunosuppression observed. However, these non-dividing primary cells appear to be more resistant to mycolactone toxicity than transformed SW872 cells. Another difference between these cells is the presence of lipid droplets in the primary cells, however, these do not appear to prevent mycolactone entering the cytoplasm and interacting with its targets. We are currently quantifying the observed delipidation of adipocytes, since this could be an important pathological mechanism during ulcer progression.