



WORLD HEALTH ORGANIZATION  
ORGANISATION MONDIALE DE LA SANTE

WHO/CDS/CPE/GBUI/2002.6  
Distr.: General  
Original: English

***4<sup>TH</sup> WHO ADVISORY GROUP  
MEETING ON BURULI ULCER***

***REPORT***

***5–7 March 2001***

***WHO Headquarters, Geneva, Switzerland***

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**4<sup>TH</sup> WHO AD HOC ADVISORY GROUP MEETING ON BURULI ULCER**

*5–7 March, 2001 — WHO Headquarters, Geneva — Salle B*

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### 1. Research

A. The group reviewed the status of implementation of the 2000 meeting recommendations on research and noted the following. Refer to 2000 meeting report (WHO/CDS/CPE/GBUI/2000.2)

#### **Completed or in progress**

- Website
- Genome sequencing has started at the Pasteur Institute, Paris, France
- Reference culture at the Institute of Tropical Medicine, Antwerp, Belgium
- Tissue repository at the Armed Forces Institute of Pathology, Washington DC, USA
- Human response to infection
- Anthropologic studies

#### **Not yet done**

- Establishment of collaborating centres
- Artificial ecosystems (*different* centres)
- Gene expression library
- Development of fingerprinting systems
- Identification of new foci (laboratory diagnostics facilities)
- Behaviour modifications
- Serial BCG therapy/prophylaxis
- Historical collection

#### **Research priorities identified in 2001**

*Genomics* - emphasized the practical use of genomic information to solve problems with Buruli ulcer.

- Identification of *Mycobacterium ulcerans* specific antigens that may have diagnostic potential and/or may be useful to identify components of an effective immune response - identification of HLA recognition motifs.
- Reference point for evaluation of strain differences by limited comparative genomic
  - genomic plasticity (IS, pgrs, ppe)
  - re-occurrence versus recidivism
  - developments of spoligo typing
  - clinical correlates with genome variation. Are some isolated more virulent?
- Future use of DNA micro-assays to evaluate host response to *M. ulcerans*.

*Histopathology:*

- Better case definitions
- Use of newer immunohistochemical methods to evaluate immune response subsets of immune cells at different stages of the disease, whether re-occurrence is likely.
- Potential use of PGL-1 for better sensitivity for diagnosis.
- Mechanism for Japanese researchers interested in Buruli ulcer to get experience with histopathology in endemic countries.

### *Immune response*

- Elicit aid from WHO vaccine experts to review the potential role for multiple BCG vaccination to prevent Buruli ulcer
- Develop mycolactone-mutant as vaccine strain
- Use of newer immunochemical methods for the evaluation of immune response at different stages of the disease as well as identify subclinical infection.
- Evaluation of the role of co-infection with other tropical disease agents (malaria, schistosomes) on Buruli ulcer. Does co-infection with schistosomes shift TH1/TH2 response to *M. ulcerans* infection?
- Long-term follow-up of patients correlates of recurrence?
- Host immune factors (genetic)
- Establishment of serum bank with specimens from endemic areas. Uses include evaluation of potential antigens for diagnostic use, potential vaccine construction.

### *Environmental studies*

- Search for the reservoir is not over. Expand search, PCR "combing" and need to involve local ecologists.
- Evaluate the potential of culture methods from environmental microbiologists to culture *M. ulcerans*.
- Use of magnetic beads to increase sensitivity.
- Sample paired endemic/non-endemic regions (with similar ecology and cultural geography). Could we predict new areas?
- Evaluate what is known about epidemiology of *M. marinum* as a tool for identifying possible reservoirs.
- Develop model eco-systems to answer where *M. ulcerans* grows.

### *Miscellaneous*

- **Transmission:** Experimental work on transmission, i.e. can aerosol infect?
- **Treatment:** Is there a role for immune stimulation as a treatment adjunct? Evaluate use of lavamisol in animal model of infection.

## **2. Non-surgical treatment Group**

Based on the recommendations of the 2000 meeting, the trial of combination of streptomycin and rifampicin for early lesions will begin in Ghana by the end of April. Ethical approval from the Ministry of Health of Ghana has been given. Now awaiting ethical approval from WHO ethical committee.

Other approaches to treatment should be explored. However, such studies must meet rigorous scientific and ethical assessment before implementation. Protocols for treatment studies may be submitted to WHO and other Advisory Group members for review and comments.

### **3. Epidemiology and Control**

#### *Draft management manual*

The group reviewed the draft manual, revised the differential diagnosis, edited the surgical management, anaesthesia and nursing section. The group added a section on control and prevention in the introduction chapter.

#### *Epidemiology*

- World summary of incidence and prevalence for the past 5 years in tabular form
- Rough estimate generated with existing data
- Make available to countries the BU 01 and BU 02 forms as well as the Epi info software to assist in data collection and analysis.
- Mapping of affected communities based on country data
- Define missing countries
- Gaps can be filled with rapid assessment missions to missing countries.
- Reports should include numbers and incidence rates (compare to TB and leprosy). The risk group (denominators) must be defined.
- National minimum and maximum rates by province/region
- Economic information (cost data from many countries, direct and indirect costs - treatment, loss of income, long-term care, impact on domestic product), DALYs need to be defined.

#### *Training*

##### Initial training:

- In collaboration with the education sector, teaching of Buruli ulcer should be included in the curriculum of all levels of schooling (primary, secondary, medical and paramedical, public health, etc.)
- Internships for doctors, nurses and other paramedicals should include attachments to some of the referral treatment centres so that they can get first-hand experience with the disease.

##### Continuous education

- Options for rotation of health-care providers for a period of time in some of the referral centres to gain practical experience.
- For the already trained general surgeons/general practitioner practising in the endemic areas, include a theoretical and practical training with internships in the referral centres
- Promote the exchange of experiences between affected countries as well as other institutions and centres around the world.
- Diagnosis confirmation: Strengthen capacity of local laboratories to confirm the diagnosis of the disease depending on their levels.
- Promote collaboration and exchange of experiences among various research institutions and laboratories.

**Prevention:** Role of BCG needs serious consideration (see above).

**Needs assessments** should be conducted in affected countries and include: surgical and laboratory capacities, community support, personnel, materials, training needs, etc.

**Evaluation of surgery**: recurrence rates and extent of excision

**Incidence and prevalence in Africa**: AFRO report at next working group on

**Community-based educational materials** for disease control be developed. They should be standardized across countries, Ministries of Health to review and coordinate.

## **4. NGOs**

### **Public health approach**

Interventions for Buruli ulcer (BU) should consider a broader public health approach. For example, rather than dealing with BU, a broad public health approach should look at all the communicable disease issues at country/village level. This creates synergies for mobilizing resources and addressing the priorities as identified by 'target populations'.

### **Holistic approach**

Consider the holistic approach that deals with the person as a body, spirit and soul that is a system part of greater communities.

### **Strategies and economic consequences**

Consider the economic consequences of various strategies. For example, if considering a surgical intervention without social and community rehabilitation as a follow-up this can be more expensive and harmful.

### **Media**

Work with the media not as a one-off but rather in partnership so that appropriate messages are delivered to avoid stigmatization of the disease. Association/links with leprosy should not undermine intervention for either disease but rather should create synergies.

### **Co-ordination**

NGOs felt it was important to know who is working in which community. It was emphasized that NGOs need to know where each of them is working (in countries or within countries so that their efforts do not conflict and can work in harmony for the community)

Action: all national programmes, NGOs and research activities should send projects to WHO so that they are put on WHO Buruli ulcer website where everyone can access.

Co-ordination needed to be addressed among NGO through National Governments. Especially as it pertains to funding, training, and research.

An example of this was emphasized in a funding proposal request from Ghana, Côte d'Ivoire, and Benin to the European Union.

**Policy issues**

- WHO urged NGOs not to refer to Buruli ulcer as "leprosy of the 21<sup>st</sup> Century". While use of existing leprosy infrastructures (where possible) may be economical, it is unwise to use the educational approaches and references to leprosy for dealing with BU.
- Establish the burden of disease to build in sustainability. WHO perhaps needs to explore a plan that defines the targets through incidence and prevalence of BU.
- WHO role is to raise awareness at country level in countries where BU exists but where governments are not supporting.
- WHO proposes not to co-ordinate the NGOs but rather encourage them to co-ordinate themselves around the National Governments within which they are working. This is the proper and more direct context.

**Others**

- Treatment of patients with active disease and rehabilitation of people with deformities due to Buruli ulcer should go hand-in-hand. Rehabilitation should be physical, social and economic. If possible, rehabilitation should be done in the patient's familiar environment.
- Training of health personnel in the affected countries at local levels to promote early detection and, where possible, early treatment
- Active case-finding and treatment at local levels to reduce costs and disruption of families.
- Integration of specialized treatment centres into existing health centres to avoid stigmatization.
- Extension of IEC to non-endemic zones to enhance knowledge of BU and stimulate voluntary reporting.
- Suggestions were made to improve the comic on the Buruli ulcer.



## Update on Buruli ulcer control activities in Benin

*Dr Augustin Guédénon, National Buruli Ulcer Control Programme, Ministry of Health, Republic of Benin*

### I. The Republic of Benin

Geographical situation: Benin is on the Western Coast of Africa

Characteristics: The Republic of Benin consists of 12 *départements*, 77 communes, 517 areas, 3 400 villages and urban districts.

Population: 6 500 000

Surface area: 115 000 km<sup>2</sup>

### II. The Buruli ulcer situation in Benin

#### 2.1. Cases of Buruli ulcer (BU) reported in the year 2000

Centre	New cases	Recurrent cases	Total
CSNG*	280	20	300
DTC/BU, Lalo*	93	13	106
La Croix ZinviJ Hospital	43	6	49
Total	416	39	455

*Laboratory results for new cases at the CSNG*

	Number of cases sampled	Positive cases	%
ZN	132	85	64.4
Culture	72 / 60 pending	57	79.2
PCR	128 / 4 pending	102	79.7
Histopathology	27 / 105 pending	18	66.7

#### 2.2 Distribution of new cases by DTC/BU and by *Département*

	CSNG/Zou	DTC/BU Couffo	ZinviJ Hospital	Total
Atlant.	27	1	36	64
Collines	2	0	0	2
Couffo	8	69	0	77
Littoral	1	6	0	7
Mono	1	13	0	14
Ouémé	36	3	7	46
Plateau	14	0	0	14
Zou	185	1	0	186
Unspecified	6	0	0	6
Total	280	93	43	416

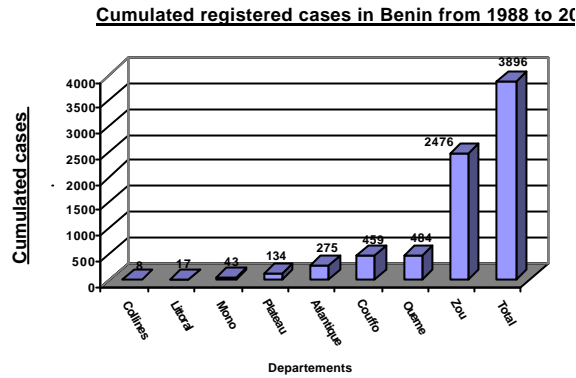
\* Gblmontin Health and Nutrition Centre, Zagnanado.

\* Diagnosis and Treatment Centre/Buruli ulcer, Lalo.

Cumulated total cases from 1988 to 1999	= 3 480
Total new cases in 2000	= 416
	-----
	3 896

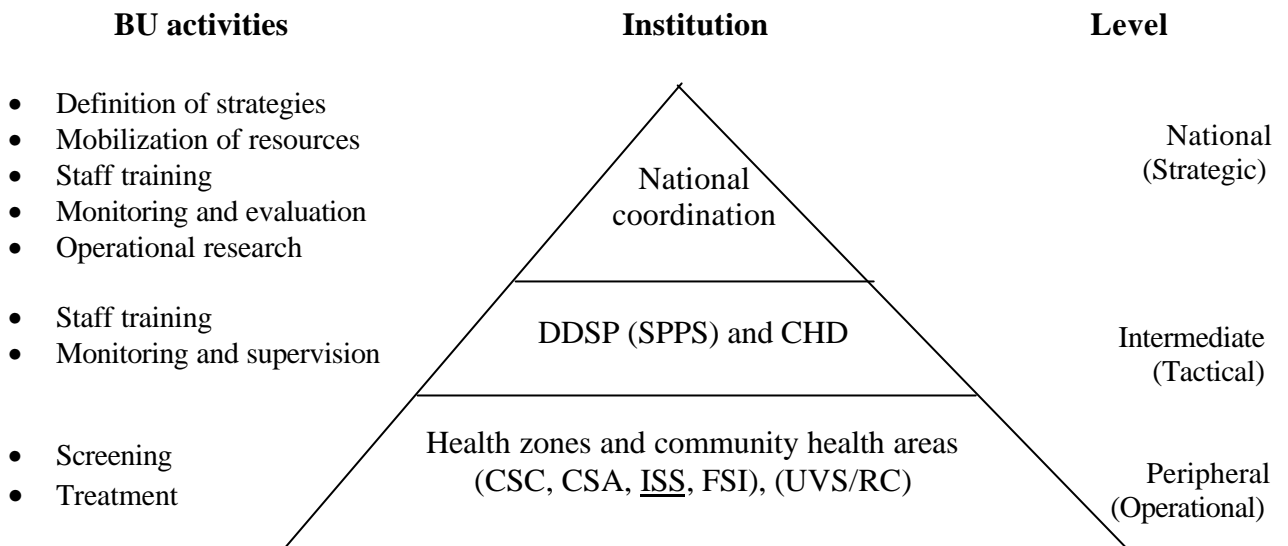
## 2.4 Distribution of cumulated cases by *dJ*partement

Cumulated cases reported in Benin from 1988 to 2000



## III. Key activities in the year 2000

### 3.1 Management of Buruli ulcer in the health pyramid



### **3.2 Strategic level activities**

#### *Definition of strategy:*

- Preparation of the policy document for Buruli ulcer management (April 2000)
- Preparation of the five-year plan (2001-2005) (in April 2000);

#### *Advocacy and resource mobilization*

- Draft agreement with MSF (prepared in 2000);
- Preparing draft protocol with FFL (preparation in 2000)

#### *Increased capacity at Lalo and National Buruli ulcer control programme coordination*

##### *Staff training:*

- Training of healers in Lalo
- Retraining of community contacts in Lalo
- Training of nurses in the health zones;

##### *Staff supervision:*

- Meeting to organize that activity
- Supervision conducted
- 2000 review meeting

##### *Monitoring and evaluation:*

- Monitoring activities in February 2000
- Evaluation of the 1st plan of action

##### *Coordination of operational research:*

- Joint supervision of 4 dissertations for doctorate in medicine

##### *Collaboration with WHO*

- Preparation of comics
- Participation in workshops

##### *Collaboration with IMT*

- Preparation of a guide for diagnosis of BU;
- Mapping of BU in Benin.

##### *Collaboration with other countries in the subregion*

- Study trip to Benin by colleagues from Guinea

### **3.3 Intermediate level activities**

One in 3 *dJpartements* has conducted this supervision

### **3.4 Operational level activities**

Passive screening of 416 new cases

Using the community contacts to inform the community, under the supervision of the teams from the DDC/BU

Treatment of 455 cases (new and recurrent)

Monitoring of cured patients

## **IV. Strong points and weak points**

### **Strong points**

- Clearly expressed political will and commitment
- Political stability
- Inclusion of the national Buruli ulcer control programme in the public investment project
- Support of many partners: WHO, MSF, IMT, FFL and AFRF
- Support expected from the EU

### **Weak points**

Low knowledge of the disease:

- Prevention?
- Drug treatment?

Low management

- Poor health services capacities
- Under recognition and under reporting of cases at the national level
- Weak or inadequate reporting of cases - lack of consistency throughout the centres
- Lack of tools for data recording

## **V. Prospects**

- Organization of a national prevalence survey
- Further establishment of treatment facilities
- Training and supervision of staff
- More advocacy among partners
- Participation in operational research

## **VI. Recommendations**

To WHO:

- Provide the treatment facilities with commutators
- Training field staff in the use of IMUB (?) software

To Partners:

- Continue to establish treatment facilities

To researchers

- Establish a drug treatment protocol

To endemic countries

- Translate political commitment into concrete action

## Update on Buruli ulcer control activities in Côte d'Ivoire

Professor Jean-Marie Kanga; Dr Djatch Edgard Kacou, National Mycobacterial Ulcer Programme (NMUP), Ministry of Public Health, Republic of Côte d'Ivoire  
Dr Koffi JJrJmie Yao, Zoukougbeu Health Center

### I. The epidemiological situation in 1999

5 endemic health districts, 1,351 cases reported

District	Number of cases
Bondoukou	94 (126*)
Bouaké	255 (1,211*)
Daloa	398 (1,544*)
Danané	172 (286*)
Yamoussoukro	432 (2,474*)

\* cumulated cases in 1997

### II. Activities conducted

2.1 Field visits to endemic zones including new zones in August and September 2000 (partner: WHO)

**Main result:** New zones visited

Sakassou (Bouaké) district and San Pedro (south-west Côte d'Ivoire)

2.2 Exploratory visit to the Sakassou (Bouaké region) with MAP International in early November 2000

**Principal result:** 2 levels of action proposed

- In the main town: upgrading of the general hospital
- In villages, training of nurses and community health workers, plus biped transport and treatment materials

2.3 Training workshop for community health assistants, teaching them how to inform the population and detect cases of Buruli ulcer in the zones of Zouan-Hounien (Danané) and Zoukougbeu (Daloa), end of November 2000 (partner: WHO)

**Principal result:** 30 community health workers and 2 local supervisors trained in each zone (a box of Buruli ulcer illustrations distributed to each *trainee*)

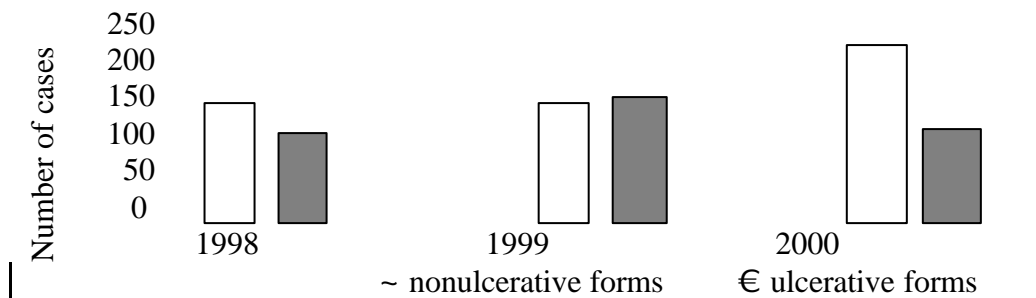
2.4 Buruli ulcer surveillance in Zoukougbeu (Daloa) using active and passive screening throughout 2000 (partner: WHO)

**Main result:** the impact of the screening campaign carried out in Zoukougbeu subprefecture in 1998 was evaluated

- fewer ulcerative cases detected
- more nonulcerative forms detected
- overall ratio of nonulcerative to ulcerative cases = 1.5 (446:308)
- the ratio for 2000 was 2.6 (197:76)

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\* cumulated cases end 1997



Comparative distribution of forms of Buruli ulcer reported in 1998, 1999 and 2000

### 2.5 Further clinical trial of multidrug therapy combining HBPM and antimycobacterials

**Main result:** 17 cases successfully treated after the first experimental case (article in press in the *Bull Soc Path Exo*)

#### Profiles of patients

- Case history prior to MDT

1 to 2 months: 3 cases  
 2 to 7 months: 3 cases  
 8 months to 5½ years: 11 cases

- Clinical aspects

No.	Nodule	Oedema	Ulceration	Multiple	Recurrence
1	-	+	+	-	-
2	-	-	+	-	-
3	-	+	+	-	-
4	-	+	+	-	+
5	+	-	+	+	+
6	-	+	+	+	+
7	-	+	+	+	-
8	-	+	+	+	+
9	-	+	+	+	+
10	-	-	+	+	+
11	-	+	+	-	+
12	-	+	+	+	+
13	+	-	+	+	+
14	-	+	+	+	-
15	-	+	+	+	-
16	-	+	+	+	+
17	-	+	+	-	-
G	2	13	17	11	10

- MDT used

ENOXA + RIF + KANA: 14 cases  
 ENOXA + RIF + AZI: 3 cases

- Treatment protocol

MDT alone: 6 cases  
 MDT + graft: 11cases

#### Results

- Rapid scarring in the 6 cases with MDT alone
 

On face:	2 cases
On arm:	2 cases
On leg:	1 case
On scrotum + abdomen:	1 case
Presence of oedema:	6 cases
Case of 7 months or more:	6 cases
- Persistence of nonulcerative nodules: 2 cases

### ***Photos***

*Cases of post-surgical recurrence treated with MTD alone*

*Cases treated with MDT and surgery*

*Case treated with MDT alone (x3)*

*Case with dissemination treated with MDT*

*Case treated with MDT and surgery*

## **III. Activities planned for 2001**

### **3.1 Trial of multidrug treatment on a large sample**

- Sample: 310 patients (expected efficacy: 80%  $\pm$  10%, " = 5%)
- Multicentre study
- No control group
- 5 treatment regimes:
  - ENOXA + RIF + ETH + CLARI
  - ENOXA + RIF + ETH + AZI
  - ENOXA + RIF + ETH + OFLO
  - ENOXA + RIF + CLARI + OFLO
  - ENOXA + RIF + AZI + OFLO

### 3.2 Further BU surveillance in Zoukougbeu

3.3 Extension of training of community health workers and local supervisors to other zones, plus distribution of BU picture boxes.

3.4 Biped transport for the community health workers and local supervisors who have undergone training.

## **IV. NGO activities**

### **4.1 Completion of the first ANESVAD project.**

- Rehabilitation of the dermatology operating theatre.
- Building of an operating theatre and annexes at Kongouanou.
- Strengthening of the Holy Family Centre at Yamousoukro

#### **4.2 Mission to identify new projects by ANESVAD.**

- Refurbishment of equipment at new treatment centres.
- Building of operating theatre and annexes at Kongouanou.

#### **4.3 Identification of projects by Map International (at the planning stage)**

### **V. Government contributions**

#### **5.1 Financial contribution**

- Initial allocation = 500 000 000 FCFA (714 286 US\$)
- Modified allocation = 375 000 000 FCFA (535 715 US\$)
- Amount paid out = 6 000 000 FCFA (8572 US\$), or 1.6%

#### **5.2 Technical contribution (under discussion)**

- Organization of a national treatment network
- Regulatory texts

### **VI. Problems and prospects**

#### **6.1 Main constraints:**

- Institutional and financial
- Political instability

#### **6.2 Prospects**

- Hopes: restoration of political and social stability
- Increasing interest from NGOs.

## **Buruli ulcer treatment activities at the Raoul Follereau Institute of Côte d'Ivoire**

*Professor Henri Assé, Raoul Follereau Institute, Adzopé, Côte d'Ivoire*

### **I. General description of the country**

Côte d'Ivoire is in the humid, subtropical region of West Africa. In 2000, it had a population of approximately 17 million, of whom 45% were under 15 years of age. The country has two very different types of vegetation: forest in the South and savannah in the North. It is also richly endowed with rivers, on which many dams have been built over the last 20 years, which is where the Buruli ulcer endemic zones have developed.

The health system in Côte d'Ivoire is a stepped pyramid with health centres at the base. In the endemic zones at present, religious health centres have specialized in treating patients. The most serious cases are referred, usually late, to the Raoul Follereau Institute of Côte d'Ivoire (the reference point for leprosy in the past, and now for Buruli ulcer).

### **II. Buruli ulcer in Côte d'Ivoire**

A census carried out in 1997 by the National Mycobacterial Buruli Ulcer Control Programme (NBUP) found 10,382 cumulated cases. The number of new cases each year is estimated at 2,000. The proportion of cases with sequelae is unknown. Observations suggest that, overall, the disease is on the increase once again and endemic zones are spreading. The same samples are processed separately in three laboratories, after which the researchers compare their results. Professor Carbonnelle regularly supervises our Laboratory in order to improve its level of performance.

The two centres have the following staff: 5 surgeons, 1 physician specialised in biology, 3 anaesthetists, 22 nurses, 5 laboratory technicians, 2 rehabilitation personnel

Because of its experience in treating the sequelae of leprosy, and the availability of staff trained in plastic surgery, the Raoul Follereau Institute in Côte d'Ivoire began to receive many patients presenting sequelae of Buruli ulcer in 1993. As of 1995, increasing numbers of progressive cases of Buruli ulcer arrived for treatment. Since then, every bed vacated by a leprosy patient is immediately filled by a Buruli ulcer patient. Between 1995 and 2000, we admitted 764 patients to the two centres.

Cases of Buruli ulcer at the Raoul Follereau Institute

<b>Year</b>	<b>Adzope centre</b>	<b>Manikro centre</b>	<b>Total</b>
<b>1995</b>	20	48	68
<b>1996</b>	20	105	125
<b>1997</b>	20	121	141
<b>1998</b>	31	110	141
<b>1999</b>	51	109	160
<b>2000</b>	61	68	129
<b>Total</b>	203	561	764

### **III. Presentation of the Raoul Follereau Institute, Côte d'Ivoire**

The Raoul Follereau Institute in Côte d'Ivoire has two centres:

- The Adzopé Centre, headquarters of the Raoul Follereau Institute;
- The Maniko Centre, an annex in the centre of the endemic zone.

The two centres have a combined capacity of 328 beds. Each has a laboratory equipped for biochemistry, haematology and microbiology. Each has two operating theatres, a rehabilitation unit with a workshop for orthotics and prosthetics, and a school for literacy training and remedial education for the children admitted to hospital.

The departments of the Raoul Follereau Institute include the research laboratory, which was entirely financed by the French Raoul Follereau Association in 1997, and the Microbiology Laboratory of the Follereau Institute, which is at the Adzopé Centre. It is equipped for carrying out all routine bacteriological tests, and in particular for studying the strains of *Mycobacterium ulcerans* in Côte d'Ivoire. It has an animal house with about 50 mice. It exists because the Raoul Follereau Institute in Côte d'Ivoire needed a Buruli ulcer research unit. It also provides diagnostic services for all the Institute's activities and for the hospitals of the region.

The laboratory's work on Buruli ulcer has been done in several stages:

- establishment of adequate culture medium for *Mycobacterium ulcerans*;
- identification of the strains of *Mycobacterium ulcerans* found in Côte d'Ivoire;
- research to produce effective multi-drug therapy against *Mycobacterium ulcerans*.

All of this was done in collaboration and in association with the laboratories of Professor Grosset in Paris and Professor Carbonnelle in Angers, thanks to financial assistance from the French Raoul Follereau Association.

### **IV. Buruli ulcer treatment at the Raoul Follereau Institute, Côte d'Ivoire**

This covers patients at all stages of the disease, but especially where there is ulceration and sequelae.

#### **1) active ulcers**

All such patients are admitted to hospital, where a laboratory test to determine clotting factors and serum proteins is performed along with the initial clinical examination. Swabs are systematically taken from the ulcer(s) for acid-fast bacilli.

Generally speaking, most patients admitted to the centre have a severe anaemia and hypoproteinaemia.

Immediate management consists of correcting these deficiencies by means of a calorie-reduced, protein-rich diet and, sometimes, blood transfusion.

This phase should be as short as possible in all patients since secondary infection or haemorrhage perpetuate anaemia. If there is no categorical contraindication to anaesthesia, excision is performed early. Ulcers are excised en bloc by cutting down to healthy tissue.

Repeat operations are necessary for ulcer excision when the patient is in poor condition and the ulcer very extensive. This is the case with most of our patients. It is an error in my opinion to attempt to excise very extensive ulcers in a single operation, and there has been disappointingly little discussion of post-operative sequelae in such cases.

There is no place for heroic surgery; it is more important to consider the patient's safety. Based on our experience 30 % of patients require more than one surgical procedure for ulcer excision.

There are several reasons for this figure:

- Very extensive ulcers
- Multiple ulcers
- Various anaesthetic risks
- Active infection at the margin of the initial excised area

After surgical excision, tulle gras dressings are applied in order to promote granulation. Dressings are changed every 2 to 3 days depending on the case. It is important, in order to prevent excessive retraction of the graft, to ensure that granulation is not too advanced before considering grafting.

The best way to cover the recipient site is with an expanded split skin graft. We use x 2 or x 4 precalibrated graft expanders which are easy to use and maintain. If conditions are ideal, graft healing is complete 10 days after grafting.

As with surgical excision, we are sometimes obliged to undertake grafting in several stages because of the patient's poor condition or the lack of a good donor site. Based on our experience, 20 % of patients require more than one grafting procedure.

### ***Wound healing***

The precarious financial situation in which our patients find themselves has a major effect on wound healing. Those admitted to our centres are financially destitute. This situation is a considerable burden on the care programme. Neither excision nor grafting can be done at a time that suits the patient. Funding from the Association Française Raoul Follereau has enabled us to treat 180 patients free of charge. Based on our experience, the average time for complete wound healing in these patients is 3.5 months; when the patient or family has to pay treatment costs the interval is 6 months.

A subsidy is therefore essential in order to reduce the morbidity and treatment costs associated with Buruli ulcer.

### **2) Sequelae of Buruli ulcer**

In addition to the active forms of the disease we treat all its functional and aesthetic sequelae. In 5 years, we have looked after 60 patients 80 % of whom presented functional sequelae involving contracture deformity and adherent scars.



## Buruli ulcer activities in Ghana in 2000

*Dr. George K. Amofah, Ministry of Health, Republic of Ghana*

### I. Epidemiological Situation

The first recorded case of Buruli ulcer in Ghana was in 1972 when one case from Amasaman, a village in Greater Accra region, was admitted to Korle Bu Teaching Hospital in Accra. In 1989, van der Werf published a series of 96 cases from an endemic focus in the Asante Akim North district of Ashanti region. A few unpublished cases had by then been picked up in the Amansie West district of the same region in 1988, which led to a district case search in 1991. It was, however, in 1993 that heightened media coverage of the disease in the district brought it to the attention of the ordinary man and the political authorities. The trend of reported new cases of the disease since 1993 is shown in Table 1 below.

Table 1: Number of reported new cases of Buruli lesions in Ghana, 1993-2000

Year	1993	1994	1995	1996	1997	1998	1999	2000
Total	280	241	233	214	120	230	1034	415

### II. Key activities

Implementation of Buruli ulcer control activities is decentralised at the district level. These activities include surveillance, IEC, dressing of ulcers, excision of preulcerative lesions, skin grafting of ulcers. Some special activities coordinated from the national level in 2000 include:

#### Review of case search

As at the beginning of last year, we had then completed a national case search and a very preliminary analysis had been made and presented at our meeting. Since then, much effort was put into making a thorough editing of the raw data and we have just finished with the final report which is being sent for publication in an international peer review journal. A total of 6,519 patients were identified (1999) with various forms of the disease and a national prevalence of active cases of 20.7 per 100,000 was registered. The results of the case search have enabled us to do the following:

- Develop a geographical mapping of the disease in Ghana using GIS
- Mobilisation of resources for Buruli ulcer control activities
- Allocation and targeting of resources to endemic districts
- Provision of technical support to endemic districts
- Shared data with international community

#### Presentation of Buruli ulcer to scientific communities

In order to stimulate more interest among the scientific community in Buruli Ulcer activities two major presentations were made first to the West African College of Surgeons who were meeting in Kumasi, and then to a group of scientists from the Noguchi Memorial Research Institute. The latter group was trying to find areas of possible research on Buruli Ulcer. This was followed by a research proposal development workshop that was supported by the WHO Global BU Initiative in Geneva.

### **Training of health staff from endemic districts**

Two health staff members from all the endemic districts were trained in the application and use of Phenytoin powder in the treatment of selected Buruli ulcers. They were also provided with quantities of Phenytoin powder provided by the Dreyfus Foundation in the USA.

### **Research collaboration**

A major research project was started during the year in partnership with CDC and Emory University, both of Atlanta, and WHO, Geneva. Our partners may give details of the project but essentially it involves undertaking a detailed case control multi-centre study in an attempt to find risk factors for the transmission of *Mycobacterium ulcerans* infection. Another component of the project involves attempting to find a diagnostic test that may be useful in field situations for persons infected with *M. ulcerans*.

### **Advisory committee meetings**

The National Advisory Committee met three times during the year to review progress of the implementation process of Buruli Ulcer control activities and to provide general strategic direction for the control and management of Buruli Ulcer in Ghana. The committee reviewed some of the research projects mentioned above and made technical inputs into their design and implementation. Members of the committee have also been involved in various advocacy activities in the country.

### **Field testing of WHO data collection tools**

For over a year now, we have been testing the data collection tools designed by the WHO Global BU Initiative in a number of districts. A presentation of the findings shall be made separately by Dr Kwame Asamoah.

### **Visitors**

Two major events took place during the year that provided a major boost to BU advocacy in Ghana. The first was a visit by Mr. Jack Dreyfus of Dreyfus Foundation USA and the second was a visit by Mr Yohei Sasakawa and a delegation from the Nippon Foundation, Tokyo, Japan. Both individuals met with the former President and Minister of Health as well as other senior officials of the MOH. The Dreyfus Foundation has since supported the application of Phenytoin powder in BU treatment after initial trials had shown that the drug speeds up the healing of certain Buruli ulcers. They are also supporting BU activities in two districts, Ga and Akuapim South, in Ghana. Support is mainly in the area of improving case management through the use of Phenytoin powder and IEC at the community level to the tune of about \$50,000 to be managed by the Health Foundation, Ghana.

## **III. Government support**

The year 2000 saw much effort being made to consolidate gains achieved in Buruli Ulcer control over the years. In line with the sectorwide policy of the MOH, a special effort was made to integrate Buruli Ulcer control activities with other disease control activities at the district level. To this end, planning and budgeting for BU activities were incorporated into the general district health planning and budgeting system called Medium Term Expenditure Framework.

The government has recently included mission hospitals in its budgetary allocation to enable them to provide services to the people in the communities. The central MOH periodically supports BU endemic districts with dressing materials. Recently, five regions benefited from such support.

#### **IV. NGO activities**

- HART, the US-based NGO that has been assisting Ghana with the surgical management of Buruli Ulcer cases, undertook another mission to an endemic district during the year. A team of plastic surgeons, anaesthetists and nurses performed a number of surgical operations on Buruli Ulcer patients at various stages of the disease including complex deformities. The NGO made a presentation of surgical equipment and dressing materials. HART is currently trying to establish a permanent presence in Accra, Ghana so that it can effectively coordinate its activities to support Buruli Ulcer control in Ghana.
- Dr Wayne Meyers visited Ghana in September 2000 on behalf of the American Leprosy Missions (ALM) to explore the possibility of the ALM supporting Buruli ulcer activities in Ghana. A follow- up visit is scheduled for April this year.
- AIFO supported BU activities in the Ga and Akuapim South districts in 1999 and the implementation of the activities spilled over into the year 2000.
- The Japanese Government is currently assisting with the construction of wards in the Amasaman Health Centre for the management of Buruli ulcer patients.
- Other NGO activities have been highlighted above.

#### **V. Constraints**

The key constraint is inadequate funding to meet the high cost of managing Buruli ulcer patients, particularly those admitted to hospitals. The incorporation of Buruli ulcer activities into the general health care planning and budgetary system is in an effort to ensure reasonable allocation of funds for BU activities at the district level. The MOH has also included mission hospitals in its allocation of funds for health activities.

The second main constraint is increasing basic health services at the community level, particularly in inaccessible areas. The ministry is currently implementing a CHIP initiative in 20 districts whereby community health nurses are placed in communities to take care of a cluster of nearby villages.

#### **VI. Planned activities for 2001**

1. Two studies are planned for this year. The first is the trial of antibiotics in the treatment of early lesions of Buruli ulcer. The Nippon Foundation, Tokyo, Japan; Association Française Raoul Follereau, Paris, France; the World Health Organization and the Ministry of Health, Ghana will support this study.
2. The second study will explore the use of cultured skin for grafting Buruli ulcers. This study will be conducted in May and June this year and will be supported by the Nippon Foundation and the Ministry of Health, Ghana under the coordination of WHO. Preliminary discussions with the Government of Ghana took place in September 2000 when a delegation from the Nippon Foundation, led by Mr Yohei Sasakawa, visited Ghana. The delegation visited a number of treatment centers, the Noguchi Memorial Institute for Medical Research, Accra and the National Plastic Surgery Unit, Ghana, Ministry of Health and WHO, Geneva.

3. Extension of theatre and other facilities at Amasaman and Akuapim South
4. Monitoring of the Phenytoin project in selected districts
5. Technical support visits to endemic districts
6. Advocacy activities with current government in BU control

## One-year field testing of Buruli ulcer data collection tools in Ghana

*Dr Kwame Asamoah, Ministry of Health, Republic of Ghana*

The programme has printed data collection tools as forms BU01 and BU02, which are somewhat similar to TB01 and TB07 of the TB programme. The BU01 collects detailed individual patient information and it is kept in the patient's file. The BU02 collates some patient information from BU01. There is also the community BU register used at the community level by the village health volunteers.

From the results of the national case search done in 1999, we identified four endemic districts in the country. These Buruli ulcer forms were introduced to these endemic districts. The reports are supposed to get to us at the central unit through the regional offices but unfortunately, for these pilot districts, most of the time we had to request 'silent' copies from the districts directly. This was because reporting from the regions was not only irregular but also no deadlines were set.

The CD2 reporting forms only collect the total number of cases and no other characteristics of the patients. During the year 2000 from this passive surveillance 7 regions (30 districts) reported cases totalling 415 to the National Surveillance Unit.

The pilot districts had their reports from BU01 and BU02 entered into the Buruli ulcer computer programme. GBU11 and our observations appear below:

**Easy installation:** It is a small programme that still leaves space when saved on a 1.44MB diskette. It is easily installed from a diskette and can use lower versions of Windows.

The programme is easy to learn and data entry is also easy. The BU01 has more data to enter than BU02 and therefore requires greater concentration when entering data.

**Reliable data:** Quite often some of our patients "shop around" for health services and by so doing, increase the total number of national reported cases. This programme can detect such cases in order to get a better picture of reported cases.

**Data analysis:** There is an inbuilt programme, which automatically does data analysis. The programme does the necessary analyses. It also provides room for one to do any other analyses required.

### I. Problems identified

- There is a need to have record of data entered. Some districts report no cases for a month or a given period and submit BU02 to that effect. There is no way to know through the programme whether that means nil returns or whether the district has not reported as yet.
- At the regional/national levels inbuilt analyses should be used to do the same analyses for the individual districts/regions.
- In the BU01 form, there is only one entry for source of water. A number of patients have reported more than one source of water for usage in their community. Also the 's' entry is not effective.

- It is important for the health centres and hospitals to fill in the BU01 form completely. This makes analysis complete and representative of the reported cases.

## **II. Operational problems**

- Through passive surveillance, 415 cases were reported while active surveillance in the 4 pilot districts recorded 496 cases. Our aim is to respect the chain of command and therefore get returns from the regions as we are presently doing for TB. This will help the regions to supervise the districts and make them accountable to the national level in terms of district performance.
- I hope that during this meeting we will look again at the data we want to collect and how easy it is to get them, e.g., data on confirmation of diagnosis. The present district hospital laboratories have manpower problems (quantity/quality). Personnel should be trained in ZN staining. Also, laboratories should be better equipped.
- There is a need for computers to expand this trial to the top ten Buruli ulcer endemic districts. We need to have a workshop with the regional public health authorities to improve laboratory confirmation and reporting. Laboratory confirmation of cases from Agroyesum in Japan revealed some cancer cases. The same has also been seen with our study with CDC/Emory University. We need to have a workshop with the regional public health authorities to improve this situation. In conclusion, this programme is very useful for surveillance and more training is needed to expand its usage. It will help in the future to do retrospective studies more easily.

## ***Mycobacterium ulcerans* disease in Papua New Guinea - Year 2000**

*Sister Joseph, Boram Hospital, Wewak, East Sepin Province, Papua New Guinea*

	<b>Average annual incidence of MBU in PNG</b>	<b>Incidence in 2000</b>
<b>Sepik river</b>	20	8
<b>Aitape</b>	3-4	1
<b>Vanimo</b>	8-10	3
<b>Kavieng</b>	1-2	0
<b>Port Moresby</b>	6	0
<b>Oro Province</b>	4	0

### **Sepik Cases**

- There has been a different case mix seen, though given the small numbers, it is not statistically significant.
- Usually the ratio incidence of the cases is children to adults 2.7 : 1.
- This year all the cases were children.
- The usual ratio is 1.6 boys to 1.1 girls.
- This year it is 3 girls to 1 boy.
- The commonest site as usual is the leg - six of the eight cases - the other two being the elbow and hand.

**MYCOBACTERIUM ULCERANS IN THE SEPIK.**

**SEASONAL INCIDENCE**

<b>Year</b>	<b>Jan</b>	<b>Feb</b>	<b>Mar</b>	<b>April</b>	<b>May</b>	<b>June</b>	<b>July</b>	<b>Aug</b>	<b>Sept</b>	<b>Oct</b>	<b>Nov</b>	<b>Dec</b>	<b>Total</b>
71							7	2	1	2	4	3	19
72	5	3	8	2	2	3	2	3	2	2	0	2	34
73	2	0	2	1	3	1	2	3	0	1	3	2	20
74	0	1	4	2	1	2	1	2	1	2	1	1	18
75	1	4	1										6
77				2	2	1	1	1	2	3	1	4	17
78	1	2	1	2	5	0	2	2	7	4	1	0	27
79	3	7	7	4	4								
87				3	5	2	1	1	1	0	0	0	13
88	0	0	2	2	1	0	0	2	2	3	8	5	25
89	3	2	2	4	2	2	1	0	0	1	2	1	20
90	0	1	1	0	0	2	2	4	1	3	1	2	17
91	2	0	0	0	0	4	1	2	0	1	1	1	12
92	1	0	2	0	0	1	1	0	1	0	0	2	8
93	5	4	1	4	0	0	1	3	1	1	3	1	24
94	2	6	3	2	1	1	3	0	3	1	3	1	26
95	1	2	2	1	3	6	0	1	0	0	0	0	16
96	0	1	0	3	0	0	2	1	1	3	0	4	15
97	3	5	4	3	4	0	2	3	2	4	0	0	30
98	1	1	0	0	0	0	0	0	0	0	0	2	4
99	0	0	1	1	2	0	0	0	0	0	0	1	5
00	0	0	1	1	1	2	1	0	1	0	0	1	8
<b>Total</b>	30	39	42	37	36	27	30	30	26	31	28	33	389

## ***Mycobacterium ulcerans* In Papua New Guinea - Year 2000**

The statistics were gathered from the usual areas, and this year with the appointment of a resident surgeon in Oro province it was possible to get the MBU statistics for the last ten years.

Site	Males		Females		Total
	Adult	Child	Adult	Child	
<b>Knee</b>	1	3	1	1	6
<b>Leg</b>	1	5	2	2	10
<b>Foot</b>	0	0	1	2	3
<b>Shoulder</b>	1	0	0	2	3
<b>Back</b>	2	0	0	0	2
<b>Arm</b>	4	0	1	0	5
<b>Abdomen</b>	0	0	0	1	1
<b>Hip</b>	2	1	0	0	3
<b>Thigh</b>	2	3	0	0	5
<b>Total</b>	13	12	5	8	38

Three additional river trips were made to the Murik lakes, the Blackwater and Chambri Lakes. No new cases were seen. It seems as though all the cases eventually end up in Wewak General Hospital. It is also noticeable that most of the cases have been of the oedematous type, often with severe secondary infection, so that in several cases diagnosis was not immediately apparent.

During the year, six cases of Marjolin's ulcer - squamous cell carcinoma in an old MBU scar - have been operated on. They were all involving legs, and necessitated four amputations.

Further plans for this year include a trip up to the Hunstein ranges, which has been finalised for April and a presentation at the national medical symposium to provide update.



## Situation in Togo

*Dr Napo Tignokpa, National Buruli Ulcer Control Programme, Ministry of Health, Togo*

### General information on Togo

#### **I. Geography**

Togo is a West African country bordered by Burkina Faso to the North, Benin to the East, Ghana to the West and the Atlantic Ocean to the South.

#### **Population**

It has a population of 4,405,500, with a density of 72 inhabitants per km<sup>2</sup>, ranging from 27 per km<sup>2</sup> in the central region to 299 per km<sup>2</sup> in the coastal region. Demographic growth is 3.2%. Fifty percent of the total population is under 15 years of age, which makes it a young population.

#### **Administration**

Togo has five major administrative and economic regions, which run from North to South as follows:

- The savannah region
- Kara region
- Central region
- Plateaux region
- Maritime region.

Each region is divided into prefectures, of which there are 30, and 4 subprefectures. In terms of health, the country has six health regions, since the municipality of Lomé (the capital) became the sixth region in 1997, and it includes five health districts.

Birth rate in Togo: 45 per thousand

Gross mortality rate: 15 per thousand

Infant mortality rate: 20 per thousand

Life expectancy: 55 years at birth (54 for men and 56 for women)

#### **The economic situation**

Per capita income is low (US\$ 330 per capita per annum). Togo is classified among the least-developed countries (LDC).

#### **Education**

47.9% of adults are illiterate. 72% of children attend school, and there is great disparity between the sexes (more boys attend school than girls).

## **II. The health sector : brief description**

The health sector is a three-tiered pyramid:

- *The central level* consists of the office of the minister, five central directorates, 15 divisions and thirty-nine departments. The two university hospitals in Lomé constitute the *national level* of the referral system.
- *The intermediate or regional level* consists of five regional directorates and five regional hospitals, which are the second referral level. Lomé, the capital, is considered as the sixth region and is known as Lomé municipality.
- The peripheral or local level consists of 30 prefectural offices, 30 prefectural hospitals, 5 religious hospitals, a secondary hospital in the urban zone of Lomé, health and welfare centres, private clinics, private medical practices, dispensaries and health posts.

The prefectural hospitals, the religious hospitals and the secondary hospitals constitute the first referral level, but often are the first point of contact, as indeed are health facilities at the intermediate and central levels.

### **Buruli ulcer in Togo**

#### **I. The epidemiological situation of Buruli ulcer**

In Togo, cases of Buruli ulcer are reported in accordance with WHO criteria.

- 1995-1998: 33 cases were diagnosed and treated at the Saint Jean de Dieu Hospital in Afagnan and at the Tokoin University hospital in Lomé.
- In 1998: 3 cases were diagnosed, none with early lesions (nodules) and three with developed lesions
- In 1999: 48 cases were diagnosed, one with early lesions (nodules) and 47 with developed lesions (ulcers)
- In 2000: 18 cases were reported, 3 with early lesions and 15 with developed lesions.

The cumulated total is 102.

All those cases were reported in 12 of the 30 prefectures of Togo, namely:

- Tone in the savannah region
- Bassar and Kozah in Kara region
- Tchaoudjo, Sotouboua and Tchamba in the central region
- Moyen-Mono in the plateaux region
- Golfé, Avé, Lacs, Yoto and Vo in the maritime region.

#### **II. Main activities conducted in 2000**

##### **1. Organization of an international workshop on Buruli ulcer, from 14–17 August 2000, in Lomé.**

At the opening of that meeting, His Excellency the Minister of Health of Togo affirmed the desire of the Togolese Government to deal with Buruli ulcer. He said “I am asking you, the scientists, to come up with concrete proposals. Put forward effective programmes for the endemic regions. I can assure you that our States are completely committed to seeking and mobilizing resources to ensure that the treatment you recommend is made accessible to everyone free of charge. Ladies and gentlemen, honoured guests, it is on the basis of this strong political will and confident hope that I declare the Lomé workshop on Buruli ulcer open.”

## **2. Diagnosis of 14 cases of Buruli ulcer in the central region of the country.**

After that meeting got good media coverage, the entire population of Togo was made aware of the problem of Buruli ulcer. Sokodé regional hospital reported 14 cases. On the invitation of Dr Tignokpa, the DAHW representative visited those patients and provided 100 000 CFA francs to help pay for some prescriptions. The Minister of Health, the German Ambassador and the WHO Representative in Togo also travelled 340 km north of Lomé to Sokodé to see the cases of Buruli ulcer. The Minister gave patients money for food and the Ambassador asked the Togolese authorities to provide a status report on Buruli ulcer in the region, to enable him to request German funding. The request was submitted by the Minister. GTZ then agreed to take care of the Buruli ulcer patients in the central Region. The WHO Representative decided that a national survey would be beneficial. In February 2001, when the medical advisers from DAHW headquarters visited Togo, we managed to invite Prof. Portaels, from the Institute of Tropical Medicine in Antwerp, on 16–17 February, to help us persuade the headquarters of the German Association for Aid to Leprosy Patients to agree to fund the treatment of Buruli ulcer patients.

## **3. Diagnosis of 2 Buruli ulcer cases in Kara region.**

On 19 February, during the tour with officials from DAHW headquarters, we visited two new Buruli ulcer patients in Kara. We had believed that *Mycobacterium ulcerans* existed only in the maritime region of the country, but unfortunately four out of the five regions of the country are affected. No screening has yet been done on the fifth region.

**Government contribution:** Since treatment is essentially surgical, the Government provides beds in the surgery department of Tokoin university hospital.

## **III. Main problems**

1. We have not yet managed to conduct the epidemiological survey that was planned in the activities for the year 2000.
2. Since treatment of Buruli ulcer is very costly, we do not have the resources to treat the few patients we have.

## **Management of Buruli ulcer at Sokode University Hospital :** **The experience of Togo**

### **I. Cleaning of the wounds**

Surgery is used to clean the wound. Plaque, scabs and necrotic cells are cleansed under general anaesthetic. This is followed by daily dressing and antibiotic treatment.

### **II. Dressing of the wounds**

This entails several antiseptics:

- Dakin
- Oxygenated water and
- Honey or sugar.

Treatment continues until the wound is cleansed completely.

- Corticotulle or antibiotulle help with granulation of the wound.

### **III. Multidrug therapy**

This is a combination of three antibiotics. The course of begins on admission to hospital and continues until healing after the skin graft, though it is subject to change to take account of the wound and changes in antibiotics.

The antibiotics currently used at the SOKODE university hospital are:

No. 1

- |     |                |                        |                         |
|-----|----------------|------------------------|-------------------------|
| (a) | Pristinamycin: | e.g. Pyostacine 500 mg | 1 compress, twice daily |
| (b) | Ofloxacin:     | e.g. Oflocet 200 mg    | 1 compress twice daily  |
| (c) | Macrolid:      | e.g. Bactiflox 5000 mg | 1 compress twice daily  |

No. 2

Aminosides: e.g. Gentalline 80 mg, twice daily intramuscular  
e.g. Lincocine 500 mg, one capsule twice daily

No. 3

Chemical antibiotic: e.g. Flagyl Injection, 500 mg 1 phial 3 times daily

The course of multidrug treatment combines antibiotics 2 and 3 with one of the No. 1 antibiotics.

The antibiotics must be used in conjunction with pain killers and anti-inflammatories, the choice of which depends on the resources and general condition of the patient.

Antimalarials and nutrition supplements also are provided.

Once everything has been done to ensure complete granulation and cleanliness of the wound, skin grafting is performed.

#### **IV. Skin graft**

Skin grafting consists of covering the entire surface of the lesion with grafts separated by spaces that are covered by the expansion of those grafts.

Skin grafts are performed in the operating theatre under general anaesthetic.

Physiological solution is infiltrated subcutaneously under the surface to be removed (the outer side of either thigh, or hypocondrium); this makes for easy taking of grafts, which are kept in physiological solution to preserve their vitality. Once they have been removed, the surface to receive the graft is prepared by reopening the wound.

The grafts are always set across the entire surface of the wound. The grafted surface is covered with thick gauze compresses that foster the conservation, vitality and development of the grafts. The surface is then protected with sterile compresses and bandages. This dressing is kept for seven days at least. Dressings are changed until the wound heals (total coverage of the wound by the expanded grafts). This treatment protocol for Buruli Ulcer is expensive for patients. We shall thus estimate the approximate cost of treatment.

## Approximate cost of Buruli ulcer treatment at Sokode University Hospital

Treatment of Buruli ulcer lasts three months on average, depending on the state of the lesion and the general condition of the patient.

The patients admitted often require intensive care for the first five days. The approximate cost of Buruli ulcer treatment is therefore as follows:

### **I. Cost of the first five days (in CFA)**

Cost of the first five days:

- Pre-operation examination	=	12,000
- Dressings	=	20,000
- Surgical materials	=	20,000
- Antibiotics:		
* Pyostacine	=	15,575
* Gentalline 80 mg 10 vials	=	16,250
* Flagyl 500 mg 15 vials	=	39,375
- Analyic Temgesicq 1 box	=	5,045
- Olfenq anti-inflammatory	=	2,875
- Post-operation care		
- (cleansing of lesion)	=	8,000 on average
- Anti- malarials		
- (Quinimax 500 mg injection, 1 box)	=	4,600
- Nutritional supplements	=	5,000

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**Total cost** = **CFA150,000**

The cost of treatment depends on the development of the lesion.

### **II. The following days up to the skin graft**

Dressings and antibiotics are the essential elements, so patients spend an average of 8,000 francs per dressing for at least 30 days until the skin graft can be performed.

The CFA8,000 can purchase compresses, antiseptics, corticotulle, velpeau strips, and antibiotics. Dressings are changed every two days, which means that 15 dressings are applied every 30 days:  $CFA8,000 \times 15 = \underline{CFA120,000}$

### **III. Skin graft**

- Surgical materials	=	20,000
- Examination	=	5,000
- Surgery	=	10,000
- Antibiotics	=	10,000
- Pain killers	=	3,000
- Anti- inflammatories	=	4,000

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**Cost of graft** = **52,000**

#### **IV. From skin graft to healing**

The dressing is changed one week after the graft, and then every two days for at least 30 days, which means 15 dressings per month:  $CFA8,000 \times 15 = \underline{CFA120,000}$

#### **V. Cost of hospitalization**

Buruli ulcer requires individual hospitalization; the patient stays for at least three months in a room costing CFA1,500 per day:  $1,500 \text{ francs} \times 90 \text{ days} = \underline{CFA145\,000}$

#### **VI. Summary of the cost of treatment**

First 5 days	=	150,000
From intensive care to graft	=	125,000
Skin graft	=	52,000
Post-operation phase	=	120,000
Hospitalization costs	=	145,000
<b>TOTAL</b>	<b>=</b>	<b>587,000</b>

To this total of CFA587,000, which is only an approximation, must be added all the uncoded expenditure that the patient incurs to have the disease cured.

Since this figure of 587,000 francs is far beyond the means of the patients, the management of Sokode University Hospital has to contribute, by covering costs such as hospitalization and drugs.

## **Preliminary evaluation of the Buruli ulcer situation in the prefectures of N'zerekore, Lola and Yomou in Guinée forestière**

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### **I. Introduction**

Buruli ulcer is a skin infection that constitutes a public health problem because treatment is very expensive and the sequelae are often disabling. It is found in certain areas near rivers and lakes and on wetlands.

Thanks to collaboration with Professor Françoise Portaels, head of department at the mycobacteria laboratory at the Antwerp Institute of Tropical Medicine, the regional hospital of N'Zérékoré diagnosed and confirmed the first indigenous case of Buruli ulcer in 1995.

Four other cases were reported to the morbid anatomy department of Donka University Hospital between 1993 and 1997.

The Republic of Côte d'Ivoire, which borders Guinée Forestière to the west, is a highly endemic zone (10,382 cumulated cases between 1980 and 1997). Liberia and Sierra Leone to the south are also endemic countries. The endemic zones in these three countries are all close to Guinée Forestière. The location of the forest region means that it is potentially exposed to Buruli ulcer because of ecological similarities, similar occupations of the population, their migration and the massive influx of refugees from Liberia and Sierra Leone.

The declaration of the Yamoussoukoro Conference in Côte d'Ivoire recommended epidemiological surveillance and control of the disease to all the endemic countries. These activities cannot be initiated without prior evaluation.

This is the rationale for the preliminary evaluation of Buruli ulcer in Guinée Forestière. It is a preliminary prevalence survey across the national territory, and an essential element from which positive results are needed for preparation of the national programme.

### **II. Objective**

**1. General objective:** To assess the extent of Buruli ulcer in Guinée Forestière.

#### **2. Specific objectives**

- To determine the prevalence of Buruli ulcer in the survey zones;
- To determine awareness of the disease in the community;
- To provide treatment for the patients identified.

### **III Description of the study area**

#### **1. Guinea**

The Republic of Guinea is on the coast of West Africa between 7° and 13° North latitude and between 8° and 15° West longitude.

To the north are Senegal and Mali, Liberia and Sierra Leone are to the South, Côte d'Ivoire to the east and Guinea-Bissau and the Atlantic Ocean to the west.

It covers an area of 245,857 km<sup>2</sup> and has a population of 7,000,000, with a population density of 28 per km<sup>2</sup>. The growth rate is 2.7%.

Guinea is entirely in the tropical zone, with two seasons: a rainy season and a dry season. The heavy rains frequently cause flooding and create large bodies of water.

#### **2. Guinée Forestière**

##### **Geography and environmental change**

South-east Guinea is called Guinée Forestière, though it is densely forested only in relatively small areas. The forest, which was once very extensive, has receded drastically because of forest clearing, slash and burn agriculture and frequent forest fires. The major ecological changes in Guinée Forestière must be borne in mind.

- 1968 to 1984: The active phase of the revolutionary period, especially of the socialist cultural revolution, when student towns were built, and there was intensive agriculture in the plain and in the marshlands. Many reservoirs also were built.

80% of the population of Guinée Forestière worked on the land and paid taxes in kind. Most opted for rice growing, living in wet-lands, where there was no mountain forest and no cultural facilities.

- 1984 to present: The end of the first president's rule led to the abolition of payment of taxation in kind and the closure of agronomy faculties. This ought to have been of benefit to the environment, but unfortunately two other changes worsened the ecological situation.

The opening of the country to the outside world resulted in cash crops such as coffee, cocoa, palm oil and rubber. This destroyed reserves in the plains and disrupted the environment. Rice growing, which calls for the damming up of large amounts of water, further upset the ecological balance.

The influx of refugees from Liberia and Sierra Leone has been another important factor in environmental change in Guinée Forestière.

##### **Administration and demography**

Guinée Forestière consists of seven prefectures, six of which are in the administrative region of N'Zérékoré.

The survey was conducted in three subprefectures (Palé, Guéassou and Bowé) in three different prefectures: N'Zérékoré, Lola and Yomou. The population of the survey zones in 1999 is estimated at 42,458, and that of the three prefectures (each the equivalent of a health district) is 1,524,676.

## IV. Material and methods

### 1. Sampling, duration and period

The survey site was chosen on the basis of the first case discovered in Guinée Forestière. All the inhabitants of these study locations were included in the study. The survey was conducted in December 1999 and January 2000 in N'Zérékoré and Yomou, and in Lola in March and April 2000.

### 2. Survey staff

The survey staff consisted of three subgroups: a mobile team with a principal researcher going out on the field, a local group consisting of hospital staff in the prefecture and staff of the health centre where the survey was done, and an external consultant to evaluate the study.

### 3. Methodology

The study consisted of a retrospective survey using the databases of the health centres in the study zones, and a prospective survey consisting of a systematic examination of the population studied.

Community awareness of the disease was assessed by interviewing 10 people in the area who had a thorough knowledge of the place.

All those activities were reinforced by programmes broadcast by N'Zérékoré rural radio.

Data were gathered on forms modelled after WHO forms, which were tested prior to the study (the survey sheet, the questionnaire on awareness of the disease, the form on observation of nodules, the form on observation of ulcers and the form on management of drugs and dressings).

## V. Results

Table 1: Progression of cases of Buruli ulcer in Guinée Forestière

Year	Female	Male	Total	%
1995	1	1	2	0.9
1996	1	0	1	0.45
1997	1	1	2	0.9
1998	7	10	17	7.69
1999	62	61	123	55.66
2000	45	31	76	34.39
Total	117	104	221	100

Table 2: Development of Buruli ulcer cases in Guinée Forestière by place of origin of patients

Place of origin	1995	1996	1997	1998	1999	2000	Total	%
Lola	1	0	0	0	12	41	54	24.43
N'Zérékoré	1	1	1	17	73	28	121	54.75
Yomou	0	0	0	0	36	1	37	16.74
Beyla	0	0	1	0	2	5	8	3.63
Macenta	0	0	0	0	0	1	1	0.45
<b>Total</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>17</b>	<b>123</b>	<b>76</b>	<b>221</b>	<b>100</b>

Table 3: Distribution of cases by sex and age

Age	Female	Male	Total	%
0-24 years	45	47	95	42.98
25-54 years	49	41	90	40.73
55 years and over	20	16	36	16.29
<b>Total</b>	<b>117</b>	<b>104</b>	<b>221</b>	<b>100</b>

Table 4: Distribution of Buruli ulcer cases by clinical form and sex

Form	Female	Male	Total	%
Nodule	8	7	15	6.79
Oedema	2	0	2	0.9
Ulcer	89	90	179	80.99
Scarring	18	7	25	11.32
<b>Total</b>	<b>117</b>	<b>104</b>	<b>221</b>	<b>100</b>

Table 5: Physical condition of patients during the survey

Physical condition	Female	Male	Total	%
Amputation	2	1	3	1.36
Disability without amputation	8	9	17	7.69
Patients without disability	107	94	201	90.95
<b>Total</b>	<b>117</b>	<b>104</b>	<b>221</b>	<b>100</b>

## **VI. Community awareness of the disease**

### **Definition of the disease**

Interviews in the three zones (Palé, Guéassou and Bowé) came up with the same definition: kolo in Palé and Bowé, and klo in Guéassou, meaning a chronic wound that cannot be cured by the usual forms of treatment. The wound always reappears after three to five months.

### **Transmission**

Concepts vary from place to place. By and large, the interviewees believed that the disease was caused by a spell, usually a sacred object put in the path of the victim.

In Guéassou, the interviewees held that the victim was not necessarily the intended target, but simply an innocent person who happened to take the path before the intended victim. In Palé and Bowé it was believed that the evil spell was so specific that no other person could contract the disease by mistake. In all three zones, it was believed that in special cases the spell could affect the victim from a distance.

### **Contagion**

In all three zones, interviews showed that there was no person-to-person contagion. However, in two villages, the interviewees mentioned two family precedents, which they called contamination, in that some people became ill after others had been ill for a long time.

### **Treatment**

There were various opinions. In Palé, it was felt that the disease could be treated at the clinic as long as the evil spell was neutralized first with traditional medicine. In Guéassou, however, only traditional medicine was recognized as a treatment. In Bowé, opinions were divided.

### **People at risk**

The interviewees believed that everyone was at risk of an evil spell, irrespective of sex, age or race.

### **Evaluation of the survey by WHO external consultant**

The objectives of the evaluation were:

1. Evaluation of the results of the survey through a field trip and interviews with the political, administrative and health authorities;
2. Discussion of the draft plan of action in terms of results with the national focal point.

The results of the evaluation were:

1. Clinical confirmation of the patients visited;
2. Determination of the seriousness of Buruli ulcer in the zone surveyed: active screening showed between 4.9 and 77.5 cases per population of 100,000;
3. Draft plan of action with priorities;
4. The urgent need to provide treatment for the patients with ulcers (179 cases found during the course of the study).

## **VII. Constraints**

The absence of a national Buruli ulcer control programme during the study caused a number of difficulties, namely:

- Lack of a national control policy;
- Lack of logistic support;
- Poor awareness of the disease among medical staff;
- Late detection of patients;
- The absence of treatment facilities;
- Organizational problems.

## **VIII. Assets**

Although few, there were some assets, such as:

- Motivated staff;
- Considerable technical support from WHO, the Institute of Tropical Medicine in Antwerp, Belgium, and the Armed Forces Pathology Institute in Washington, United States;
- Collaboration with endemic countries in West Africa (Benin, Togo, Ghana and Côte d'Ivoire).

## **IX. Projects**

The study showed the following prospects for action:

- Treatment of the 179 patients with ulcers;
- Establishment of a national Buruli ulcer control programme;
- Development of a national control policy;
- Development of a five-year plan (2002–2006);
- Establishment of treatment facilities;
- Study trips to enhance skills;
- Continuing technical and financial support from WHO.

## **X. Conclusion**

The results of the study show that Buruli ulcer is a public health problem in Guinée Forestière and could be one for the rest of the country too. The rate of active screening is very high, with a resurgence of the disease. 42.98% of cases are children, and the disease seems to be present in every prefecture of Guinée Forestière. The distribution of clinical forms shows a probable relation between the disease and environmental change. The very motivated field team and N'Zérékoré rural radio are the two factors contributing to the success of the study. It was possible to identify patients in other locations thanks to the information and education programmes broadcast by N'Zérékoré rural radio.

## Infection of animals with *Mycobacterium ulcerans*

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The experimental infection of animals with *M. ulcerans* has a long history. Animals were successfully inoculated with the mycobacteria taken directly from lesions before it was cultivated on artificial media and its cultural characteristics determined. MacCallum and his co-workers in the paper that first described *M. ulcerans*, reported that the organism was initially cultivated in mice and that experimental lesions could be produced by intraperitoneal and hind-limb inoculation.[1] Following this historic paper, Meleney and Johnson in 1950 were successful in cultivating the mycobacterium from the leg of a six year-old boy who had been with his missionary parents in what was then the Belgian Congo.[2] However injections of tissue digest into the peritoneum and groin of guinea pigs did not result in any lesions.

Clancey, in Uganda, inoculated mice with suspensions of tissue and cultures both intraperitoneally and via the foot-pad. Male white rats were also inoculated through the intraperitoneal, intratesticular and foot-pad routes.[3] Male rabbits were similarly inoculated, also by intravenous and intramuscular routes, and male guinea pigs through the intraperitoneal, intramuscular, intratesticular and foot-pad routes. Infection from tissue inoculum occurred in the foot-pads of rats and mice; intraperitoneal and scrotal infection occurred after intraperitoneal injection of a culture suspension and intratesticular, scrotal and hind-limb infection occurred after the intratesticular inoculation of rats with a culture suspension. No lesions were produced by any method in rabbits or guinea pigs.

A leading article in the Medical Journal of Australia in 1975 mentions that numerous animals including rodents, cows, possums, chick embryos and amphibia have been infected experimentally, but no natural animal reservoir has been detected to date.[4] Bolliger, Forbes and Kirkland, in Sydney, have, however, successfully inoculated *M. ulcerans* into possums (*Trichosurus vulpecula*) and have found that uninoculated possums in the same room but in separate cages also developed infection. [5] Marcus, Stottmeier and Morrow successfully inoculated anole lizards (*Anolis carolinensis*) with *M. ulcerans* by the subcutaneous route, producing both necrotizing and granulomatous lesions. [6]

The first spontaneous *M. ulcerans* infection of free-living animals other than humans was reported by Mitchell, Jerrett and Slee. They described ulcers in seven koalas (*Phascolarctos cinereus*) living on Raymond Island in the Gippsland lakes, near Bairnsdale in south-eastern Australia. [7]

Between 1993 and 1995, a large human outbreak of *M. ulcerans* infection in residents of East Cowes, Phillip Island (near Melbourne, Australia) occurred. [8] Epidemiologic and laboratory investigations suggested that the source of this outbreak was a golf-course irrigation system and nearby swamp. [9,10] It is presumed that human exposure followed direct or indirect exposure to aerosols arising from water contaminated with *M. ulcerans*. There is a large koala sanctuary within 5 km of the outbreak region, but no koalas with *M. ulcerans* infection were reported from Phillip Island during this period. Isolated human cases continued to occur in East Cowes until October 1998.

In July 1996 an ailing brushtail possum (*Trichosurus vulpecula*) was captured in East Cowes. The animal was put down for humanitarian reasons. A presumptive diagnosis of *M. ulcerans* infection was made because of the presence of skin ulcers. Histology suggested *M. ulcerans*

infection and numerous acid fast bacilli were seen. No fresh material was available for culture, but multiple formalinised samples were subjected to IS 2404 PCR (specific for *M. ulcerans*).[11] All tested negative, suggesting an alternative mycobacterial infection. Therefore, although brushtail possums (*Trichosurus vulpecula*) are experimentally susceptible, no proven natural *M. ulcerans* infection in the species have yet been documented.

In January 1998, an adult ringtail possum (*Pseudocheirus peregrinus*) from East Cowes was found to have lesions consistent with *M. ulcerans* infection but no material from this animal was subjected to PCR or culture to confirm the diagnosis. In May 1998, another ringtail possum from East Cowes was found with ulcers on its nose and hind feet. This time, IS 2404 PCR confirmed that the infection was *M. ulcerans*. Two more ringtail possums with IS 2404 PCR-confirmed *M. ulcerans* infection were identified in East Cowes in January 2000. No new infections have occurred in humans in East Cowes since 1998, and the human epidemic in this region peaked in 1994. Recent environmental testing suggests that the originally implicated water sources are no longer contaminated [9]. Taken together, these data imply that the incubation period for *M. ulcerans* infection appears to be longer in ringtail possums than it is in humans. An alternative hypothesis is that ringtail possums have been recently exposed to a new, unidentified source of *M. ulcerans* in East Cowes that is not yet causing human infections.

In November 1997, an alpaca from an alpaca farm near Lakes Entrance in East Gippsland, (near Bairnsdale, south eastern Australia) was noted to have a large ulcer on one leg. An alpaca is a South American hoofed mammal related to camels. These animals are not native to Australia and have recently been imported for their high quality wool. Mycobacteria were seen on a swab and *M. ulcerans* was confirmed by culture and IS2404 PCR. No other natural infections in alpacas or other ruminants have been reported in Australia to our knowledge.

Infection of these mammals is most likely incidental and the animals have no role as direct vectors of the disease, although it is possible that they may serve to disseminate the organism. The recent identification of *M. ulcerans* in aquatic insects and vertebrates may be of epidemiological significance and confirmation and correlation of results is an urgent requirement.

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## Treatment of Buruli ulcer with hyperbaric oxygenation

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### I. Buruli ulcer

Buruli ulcer (BU) caused by *M. ulcerans* is a skin disease that takes various forms. Approximately 40% of cases are ulcerative, the other 60% being nonulcerative. A study conducted in Benin has shown that up to 20% of patients today may develop serious forms with bone involvement (1,2). Treatment is mainly surgical and consists of excision of the necrotic tissue, followed in most cases by autografts. Bone involvement is particularly difficult to treat, and here again it is essentially surgical, with curettage of the affected bones. Non-surgical forms of treatment have been considered.

Treatment with antimycobacterials has generally been disappointing, except for certain early forms (3). A recent study in mice, however, has produced encouraging results from rifampicin or rifabutin used in association with amikacin (4). Heat treatment has also been tried, and is effective in some cases (5).

Given the gravity of certain forms and the difficulty of treating them, even with surgery, other alternatives have been sought, including hyperbaric oxygenation (HBO) (6,7).

### II. Hyperbaric oxygenation

HBO is known to be effective against certain bacterial diseases (8) including mycobacterial diseases (9, 10, 11).

Two studies of the effect of HBO on the multiplication of *M. ulcerans* in mice after experimental infection have been published (6, 7). One of them (6) showed that mice experimentally infected with *M. ulcerans* responded to HBO treatment. However, the authors stressed that HBO does not cure the disease but rather delays the onset of symptoms. The second publication (6) (?) shows that HBO treatment can be effective against experimental infection of mice with *M. ulcerans*, on condition that it is used combined with other forms of treatment such as rifampicin and heat. In both publications, the authors explain the effect of HBO in terms of stimulation of the immune response through high O<sub>2</sub> concentration, which seems to enhance cell-mediated immunity or stimulate the granulation of tissue. The efficacy of HBO might also be explained by the sensitivity of *M. ulcerans* to high concentrations of O<sub>2</sub>. We have shown that *M. ulcerans* multiplies better *in vitro* when exposed to atmospheres containing between 2 and 5% O<sub>2</sub> (12).

### **III. HBO treatment of BU patients**

To our knowledge, only one case of BU to date has been treated with HBO (13).

A four year-old Angolan child arrived in Germany with a disseminated form of BU, skin ulcers on the left knee and right hip, and bone involvement on the proximal and distal metaphyses of the left and right tibia, and the bones in the joint of the right foot. The child was treated with surgery and antimycobacterial multidrug therapy.

Three months later there was a relapse, and the patient was treated with HBO (27 sessions over 33 days).

After excision of the ulcers and grafting, there was very rapid granulation of the tissues using HBO, and all the lesions were healed three weeks later. A new swelling appeared on the left hand and was treated surgically and with antimycobacterial multidrug therapy. HBO was continued, and once again rapid granulation was observed. All the lesions had closed six weeks after the start of HBO treatment combined with surgery and antimycobacterial multidrug therapy. A new swelling appeared on the left knee without skin involvement. X-ray showed osteomyelitis of the distal metaphysis of the left femur. A bone marrow biopsy was taken, and BU was confirmed by PCR.

A whole body X-ray was then done, which showed osteomyelitis of the diaphysis and metaphysis of the left humerus, with no involvement of the soft tissues.

The conclusion is that this first case of BU treated with HBO showed that HBO led to rapid granulation of tissue, but never stopped the progress or dissemination of the disease. It was a difficult case with disseminated BU and multiple bone involvement. It may be that HBO would have a much more effective therapeutic outcome on less serious forms.

### **IV. Hyperbaric oxygenation and possible action by the Rotary Club for BU treatment**

The efficacy of HBO in treating BU patients has not yet been demonstrated. The case we have just presented was a complex one, in which HBO did not prevent dissemination of the disease with bone involvement. The first thing to do would be to demonstrate the efficacy of HBO as an adjuvant treatment for BU. This should be done with patients presenting different stages of BU.

In the first instance, it might be more practical in scientific terms and more affordable to demonstrate the effectiveness of HBO on a small number of patients treated in Italy.

If HBO proved effective, it might then be used in a well-equipped centre in an endemic zone.

These proposals should be discussed in greater detail at a subsequent meeting, and treatment protocols should be developed. The choice of patients to be treated in Italy also should be discussed.

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## ***M. ulcerans* infection in humans and animals in Australia, March 2000 – March 2001**

*P.D.R. Johnson, F. Oppedisano, M Smith, JA Hayman, Sheena Broughton et al.*

Most incident cases of *M. ulcerans* infection in Australia are now diagnosed by PCR. This service is performed by the Microbiological Research Unit at the Royal Children's Hospital in Melbourne. However, our data-collection is not complete, and we may miss some cases. Whenever possible, we confirm our PCR diagnoses by culture (see abstract on PCR). To our knowledge, only Queensland and Victoria have active endemic foci of *M. ulcerans* transmission at present.

### **I. Report from Victoria and Queensland**

#### **Victoria**

Humans: 5 new cases (1 Phillip Island, 2 Frankston-Langwarrin, 1 Port-Arlington -St. Leonards, 1 other).

Possoms: 3 new cases, all from East Cowes, Phillip Island.

Trends in Victoria: Confirmation of new focus on western side of Port Phillip Bay.

Emergence of disease in possums at Phillip Island; re-emergence of cases in humans at Frankston-Langwarrin.

#### **Queensland**

Humans: 11 new cases (Mossman region 8, Townsville 2, other 1).

Trends in Queensland: Emergence of new focus in Townsville; continued activity in Mossman region.

### ***In vitro* susceptibility of *Mycobacterium ulcerans* to Azithromycin\***

*P.D.R. Johnson, F. Oppedisano, I. Bastian et al.*

Buruli ulcer (*Mycobacterium ulcerans* infection) is an emerging infectious disease. In West Africa, tens of thousands of cases have occurred since the 1980s. The epidemic commonly affects children and is not attributable to the recent increase in HIV infection. Surgical excision is the mainstay of therapy but there is increasing interest in the use of adjuvant drug therapy, either to limit the extent of resection or to prevent recurrence. Azithromycin is an effective anti-mycobacterial drug which achieves high tissue levels and has proven efficacy against MAC. Azithromycin also has the advantages of daily or weekly dosing, and a relative lack of drug interactions. However, little is known about the activity of azithromycin against *M. ulcerans*. We conducted preliminary experiments to establish the optimal inoculum size and the optimal growth-medium pH for assessment of susceptibility to azithromycin using the radiometric BACTEC 460 system. We studied one recently obtained Victorian and one African clinical isolate of *M. ulcerans*. Experiments were conducted with 3 inocula (100 µl of a McFarland 1.0 suspension diluted 1:1, 1:5 or 1:10) at pH 6.8 and 7.4. The 1:5 dilution appeared to be most effective in separating susceptibility from resistance. Alkaline pH did not appear to alter *in vitro* drug efficacy. In duplicate experiments, growth curves over 8 weeks established that both isolates were sensitive in the 1-2 µg/ml range. Tissue levels of azithromycin are likely to exceed these concentrations in humans. We are now proceeding to test 20 additional clinical isolates obtained from Africa, Queensland and Victoria. Further studies will be required to establish the clinical efficacy of azithromycin in treating Buruli ulcer, most probably in combination with drugs such as rifampicin and ethambutol. We thank Pfizer for their financial support.

## **PCR compared with ZN smear and culture for the diagnosis of *M. ulcerans* infection**

*P.D.R. Johnson, F. Oppedisano, M Smith, JA Hayman, R Robins-Browne et al.*

Culture confirmation of *M. ulcerans* infection traditionally takes 8-12 weeks. A diagnostic PCR based on the *M. ulcerans*-specific insertion sequence IS2404 has been provided by the Microbiological Research Unit at RCH since 1995. We now report our clinical experience with PCR compared with ZN-smear and culture for the diagnosis of *M. ulcerans* infection. Between September 1995 and 1999 we received specimens from 119 individuals; multiple specimens were received from 13 of these. Six specimens were paraffin tissue blocks, 63 were dry swabs, 6 were duplicate samples of fresh tissue and dry swabs, and 42 were fresh tissue specimens. The mean age of patients was 42 years. Eighty-eight individuals lived in Victoria, 34 in Queensland, 1 in the Northern Territory and one in West Africa. The subsequent results are based on 79 of these individuals on whom both ZN smear and mycobacterial culture data are available. Of the 79 individuals, 30 tested positive by PCR and 49 negative. Twenty-six of the 30 patients with positive PCR results also had positive ZN smears, but 4 were smear negative. Of the 49 individuals with negative results by PCR, 6 were ZN smear positive. Of these, only 1 was culture-positive for *M. ulcerans*. PCR therefore had a sensitivity of 98.7% compared with culture, and in the other culture-confirmed cases, was slightly more sensitive than ZN smear. There were 6 patients who had a positive PCR but negative cultures. Histological specimens from these patients were reported as typical of *M. ulcerans* infection and they were all treated surgically. All of the 24 remaining patients with positive PCRs had their diagnosis confirmed by culture. IS2404 PCR appears to be generally more sensitive than both ZN and culture, although 1 culture-diagnosis was initially missed by PCR. Specificity in this study appeared to reach 100%.

## Toxin, vaccination and sequencing

*Dr Pam Small, University of Tennessee, Knoxville, TN, USA*

1. Isolation and characterization of mycolactone from a wide range of MU isolates from different geographical areas
2. Second vaccination trial using an avirulent ML- MU
3. Construction of a BAC library of *M. ulcerans* DNA, finger-printing of these clones, obtaining sequence from both ends of 1152 clones, and BLAST comparison of these with MTB genomes. Assembly of the physical map is in progress.

1) In collaboration with Professor Françoise Portaels and Armand Mve-Obiang we have investigated the toxin producing capabilities of 27 *M. ulcerans* strains. Of these strains, 18 were from Benin, 5 were from Australia, 2 from Mexico and 1 from China and Japan each. Toxin was isolated from organic extracts of sterile culture filtrate (SF) and bacterial cell mass for all of these strains and analyzed by mass spectroscopy for the presence of mycolactone or mycolactone-related molecules. These molecules were further assayed on L929 fibroblasts for cytopathicity and in guinea pigs for typical *M. ulcerans* pathology. These studies show that all isolates except for 1 Australian strain produced mycolactone or a mycolactone-related molecule.

Mass spectroscopy analysis showed that all African isolates assayed contained a major mycolactone (mycolactone A/B) peak at 765 (743+Na species). Four of the 5 Australian isolates produced a ML-related molecule designated mycolactone C with a mass of 749 as a major peak with a minor peak at 765.

The remaining Australian isolate was ML- and was negative both in cell culture and guinea pig studies for cytopathicity. The Japanese and Chinese isolates produced a major mycolactone D at with a mass of 781 but a 765 peak was also present. Neither of the two Mexican strains produced any detectable mycolactone, but mycolactone-related molecules were present. However, in one of the Mexican strains toxin production decreased through time suggesting genetic instability in these genes. All strains tested with the exception of 1 Australian strain were cytotoxic; presence of mycolactone A/B correlated with greatest potency. *In vivo* virulence studies are in progress. Preliminary evidence from these suggests that the Australian, Mexican and Asian strains are less virulent than the African ones.

**In summary**, All MU except for one isolate produced mycolactone A/B (743) or a mycolactone-related molecule. All African strains produced mycolactone A/B as a major lipid species. However, there appears to be variability in the potency of toxin species produced by MU in different geographic areas.

2) In a second vaccination study, 20 guinea pigs were vaccinated with an avirulent ML-isolate 1615A in the inguinal region and challenged with the parental virulent strain using intradermal infection. None of the 5 vaccinated guinea pigs developed lesions as opposed to the nonvaccinated animals although there was transient erythema observed at the challenge site. These guinea pigs were followed-up for 6 months and cultured at 2,4,8 and 24 weeks. No MU were recovered from vaccinated pigs after 8 weeks.

**In summary**, this second study confirms work shown last year suggesting that vaccination with a ML- mutant confers protective immunity to subsequent challenge by MU in a guinea pig model of infection. We are in the process of trying to produce a strain with a defined lesion in mycolactone genes.

We had two problems in this initial stage of the work. First, the plugs we initially made either did not have an adequate number of cells or we did not get adequate lysis because the yield of DNA was too low. This problem was solved using a protocol from Stewart Cole and a higher concentration of bacteria. Secondly, we were unable to get clones as large as we had wanted. Because of this, assembling the physical map has been slower than expected. This information has been provided to Tim Stinear to facilitate the MU Genome project.

We have so far obtained 1,962 DNA sequences from the ends of 1152 BAC clones. Using DNA/Protein comparisons, we have assigned these sequences to particular categories such as “energy production and conversion”, “amino acid metabolism”, etc.

We have also compared these sequences with sequences in the MTB database. With a few exceptions, all of our 1,962 sequences show significant homology to genes in MTB though the extent of homology varies.

In most cases, however, the sequence homology is over 70%. One notable exception is in the polyketide genes where the homology between MU and MTB sequence is between 30 and 50%. This is not surprising because these are very mutable genes.

Among the MU genes we so far identified, it is interesting to note that MU has several genes present in MTB that are putative virulence determinants in MTB. These include isocitrate lyase and the *mce-1*, *mce-2* and a putative phospholipase.

It is not surprising that the MU genome is very similar to that of TB in the large amount of DNA encoding genes involved in lipid metabolism. A list of genes/ category is included in Table 1.

Table 1. Comprehensive Mycobacterial Comparisons\*

<b>Functional category</b>	<b>#putative sequences</b>
Energy production and conversion	84
Amino acid transport and metabolism	64
Coenzyme metabolism	30
Lipid metabolism	130
Translation, ribosomal structure and biogenesis	46
Transcription	19
DNA replication, recombination and repair	24
Cell envelope biogenesis, outer membrane	16
Signal transduction mechanisms	12
Cell motility and secretion	5
Post-translational modification, protein turnover	33
Chaperones	
Inorganic ion transport and metabolism	37
General function only	78
Function unknown	46
Currently unclassified	394**

\*\* These include a *plc* gene, many PPE and PE-PGRS family proteins, several MmpL family membrane proteins

\*\*Please keep in mind that this is only the assignment of the sequence. This is less than 1/4 of the genome. A much more complete picture will emerge as the genome sequencing project moves forward.

++ This information has been provided to Dr Tim Stinear as a contribution to the genome sequencing project in Paris.



*Dr Harold King, Emory University, School of Medicine, Atlanta GA, USA*

## **I. Seroprevalence of *Mycobacterium ulcerans* antibody in Persons with Healed Buruli Ulcer Disease and their Household Contacts in a Highly Endemic Area of Ghana.**

Stacey L. Kihlstrom<sup>1</sup>, Karen M. Dobos<sup>1</sup>, Mark R.W. Evans<sup>2</sup>, Harry Thangaraj<sup>2</sup>, Richard Phillips<sup>3</sup>, Ohene Adjei<sup>4</sup>, Mark H. Wansbrough-Jones<sup>2</sup> and C. Harold King<sup>1</sup>, <sup>1</sup>Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA; <sup>2</sup>Division of Infectious Diseases, St. George's Hospital Medical School, London, United Kingdom; <sup>3</sup>Department of Medicine, Komfo-Anokye Teaching Hospital, Kumasi, Ghana; <sup>4</sup>Department of Medical Microbiology, School of Medical Sciences, U.S.T., Kumasi, Ghana.

Buruli Ulcer disease (BU), caused by *Mycobacterium ulcerans*, is rapidly becoming an important public health concern in Africa. Endemic areas are primarily in tropical climates of Africa with a rising incidence of disease specifically in the West African countries of Ghana, Togo, Benin, and Cote d'Ivoire. BU was first recognized in the Ashanti region of Ghana in 1980. In 1993, 24% of the Ghanaian villages in the Amansie West District of the Ashanti Region were reported to have BU. The two most endemic villages, found in the southern half of the district along the Offin river, included Tontokrom (22% prevalence) and Bonsasso (7.8% prevalence). Current surveillance of BU relies mainly on clinical presentation and is almost certainly an underestimation of the true prevalence of disease due to lack of sensitive diagnostic tools to detect *M. ulcerans* infection early. The purpose of this study was to detect the antibody response of persons in a highly endemic region to the *Mycobacterium ulcerans* culture filtrate proteins (MUCF) as a measure of seroprevalence of BU. Either sera or plasma samples were obtained from individuals with healed BU (cases) and their household contacts with no evidence of BU (controls) between 1998-1999. Sera from West African TB patients were also obtained from the WHO TB serum bank. Specimens were tested by ELISA and Western blot containing the total MUCF. A mean BU index (sample OD/cut-off OD) was used to distinguish between patients who tested positive (mean BU index >1) or negative for the disease. Overall seroprevalence for BU by the ELISA was 27% (19 of 70) and 36% (25 of 69) of the total sample population (cases and controls together) in 1998 and 1999, respectively. Of the 20 West African TB controls analyzed, 7 (35%) were considered seropositive for BU by ELISA. Sero-reactivity of each individual's sera/plasma towards two MUCF proteins previously identified as diagnostic candidates (38 and 70 kDa) were then recorded. Overall seroprevalence of *M. ulcerans* antibody to the 38 and/or 70 kDa in the sample population was 63% (44 of 70) in 1998 and 36.2% (25 of 69) in 1999. Thirty-five percent (7 of 20) of the West African TB control cases elicited a response to either the 38 and/or 70 kDa MUCF protein as well. In 1998, 80.9% (34 of 42) of cases were sero-positive to 38 and/or 70 kDa ( $\chi^2 p = 0.0109$  for case-control comparison; OR = 1.6). Similarly, 50% (20 of 40) of the cases in 1999 elicited an antibody response to the 38 and/or 70 kDa ( $\chi^2 p = 0.0052$  for case-control comparison; OR = 3.3). The mean BU index was significantly greater (1998 Student's T  $p = 0.0146$ ; 1999 Student's T  $p = 0.0065$ ) in BU patients with an antibody response to either the 38 and/or 70 kDa serodiagnostic proteins. No such correlation was seen when sera from West African TB patients were tested, (Student's T  $p = 0.193$ ). This study suggests that seroprevalence using the 38 and 70 kDa serodiagnostic candidate antigens may enhance clinical surveillance of BU in endemic regions of Ghana.

## **II. Surveillance, Serodiagnosis, and Identification of Modifiable Risk Factors and Host Factors for Buruli Ulcer Disease Caused by *Mycobacterium ulcerans* in Ghana.**

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We initiated active surveillance for Buruli Ulcer disease (BU) during the fall of 2000 in three highly endemic districts in The Republic of Ghana (Amansie West, Upper Denkyira, and Asante Akim North). The active surveillance strategy for BU was modeled after that currently used by the Guinea Worm Eradication Program (GWEP) to monitor incident dracunculiasis infections. Cases of BU identified through active surveillance were enrolled in a case-control study to identify modifiable risk factors for infection so that prevention strategies could be developed. After case enrollment and hypothesis generation, environmental samples (vegetation, soil, water, and potential insect vectors) were collected in a systematic random manner from case and non-case peridomestic areas for subsequent analysis for the presence of *M. ulcerans* and identification of potential environmental reservoirs that may correlate to risk factors of BU in Ghana. As part of the case-control study, serum samples were obtained from consenting subjects for the development of an ELISA-based diagnostic test for *M. ulcerans* infection. Whole blood and urine specimens were also collected to identify host factors and study other infectious diseases as risk factors in developing BU. All consenting participants in the case-control study also received burulin and tuberculin skin tests to measure delayed type hypersensitivity to these antigens, and were also interviewed regarding potential environmental risk factors for BU. Wound swabs and elliptical or punch biopsies were obtained from persons with nodules and active ulcers for case confirmation by AFB staining, histopathology, culture and PCR. To evaluate possible cross-reactivity of the *M. ulcerans* serologic test with *Mycobacterium tuberculosis* infection, serum specimens were also obtained from persons with pulmonary tuberculosis (TB) and household controls living in Amansie West, Upper Denkyira, and Asante Akim North districts. TB patients and healthy persons living in West Mamprusi, a district with few reported cases of BU will be collected in March of 2001 for comparison. Preliminary analysis of the humoral immune response of cases and controls to the *M. ulcerans* culture filtrate antigens (used to define a reference bank of confirmed BU cases) will be presented and laboratory confirmation of disease protocols will be discussed.

## **III. Definition of *Mycobacterium ulcerans* Culture Filtrate Proteins**

Karen. M. Dobos, Stacey. L. Kihlstrom and C. Harold. King, <sup>1</sup>*Division of Infectious Diseases, Department of Medicine, Emory University School of Medicine, Atlanta, GA, USA*

We have shown that persons with Buruli Ulcer Disease (BU) produce a specific antibody response to the proteins secreted in the culture filtrate of *Mycobacterium ulcerans* (MUCF) strain S-WT. Because these proteins may therefore be important diagnostic and vaccine candidates, a complete analysis of the protein composition of this fraction, including a comparison with diverse strains, is needed. Using two-dimensional polyacrylamide gel electrophoresis, detailed maps of the culture filtrate proteins of three distinct *M. ulcerans* strains: S-WT, S-Y, and 94-816, were generated. The number of secreted proteins differed between the three strains; strain S-WT exhibited over 150 protein spots, and strains S-Y and

94-816 demonstrated less than 100 protein spots each. The coupling of this electrophoretic technique with Western blot analysis allowed the identification and mapping of 15 proteins from strain S-WT recognized by either human BU patient sera or rabbit immune polyclonal antisera. However, fewer than 6 of the 15 identified proteins were identified from strains S-Y and 94-816 by this method, indicating that there may be strain variation in either the expression or immunologic reactivity of the secreted proteins of *M. ulcerans*. Further molecular characterization of abundant proteins within the MUCF of strain S-WT was achieved by N-terminal amino acid sequencing by Edman degradation. One of these proteins, with a molecular mass of 38-kDa, corresponded to a putative diagnostic antigen for BU, and contained at its N-terminus a proline rich sequence that frequently precedes mycobacterial glycosylation sites. This protein has been purified from the MUCF and is currently being structurally characterized and analyzed for its serodiagnostic potential. In addition, a 28-kDa protein with a sequence identical to MPT 51, a 31-kDa protein with an amino acid sequence identical to that of antigen 85A and 85B of *M. tuberculosis*, and a 24 kDa protein corresponding to super oxide dismutase, were identified. N-terminal amino acid sequence analysis of the remaining 15 proteins and mapping of these proteins to strains of *M. ulcerans* from various geographic regions are currently underway. These detailed proteomic maps of *M. ulcerans* will be useful in the identification and analysis of serodiagnostic and vaccine candidates for this emerging mycobacterial disease.

#### **4. *Mycobacterium ulcerans* cytotoxicity in an adipose cell model.**

Karen M. Dobos<sup>1\*</sup>, Pamela L. Small<sup>2</sup>, Manon Deslauriers<sup>3</sup>, Fredrick D. Quinn<sup>3</sup> and C. Harold King<sup>1</sup> <sup>1</sup>Department of Medicine, Emory University School of Medicine, Emory University, Atlanta, GA 30303, <sup>2</sup>Department of Microbiology, University of Tennessee, Knoxville, TN 37996 and <sup>3</sup>Division of AIDS, STD, and TB Laboratory Research, NCID, CDC, Atlanta, GA 30333, USA

*M. ulcerans* is the etiologic agent of Buruli ulcer, a disease characterized by adipose tissue destruction and ulcer formation. We developed a cellular model of toxicity using a human adipose cell line (SW872) to examine *M. ulcerans* infection and the entire repertoire of *M. ulcerans* culture filtrate products (MUCF) from 3 strains of *M. ulcerans*. Although infected SW872 cells did not harbor intracellular bacteria as demonstrated by transmission electron microscopy, characteristic necrotic and apoptotic phenotypes were observed. Similar results were seen when the MUCF was tested in this cell line. Quantitation of necrosis by lactate dehydrogenase release and apoptosis by the presence of sequestered cellular DNA-histone complexes in SW872 cell monolayers treated with the MUCF demonstrated that the ratio of necrosis and apoptosis was strain dependent. The necrotic phenotype was explained by the presence of mycolactone in the MUCF. However, apoptosis was only partially reflective of this mycolactone content. Furthermore, apoptosis of SW872 cells was significantly reduced when the MUCF was proteinase K and heat-treated, suggesting the action of proteinaceous products. Our data confirm the presence of additional cytotoxic factors in *M. ulcerans* potentially involved in the pathogenesis of Buruli ulcer disease.

**Research conducted at Emory University was funded in part by the Department of Health and Human Services, Public Health Service, CDC cooperative agreement #U50/CCU416560 and the Emory Medical Care Foundation.**



## Cytokine responses to stimulation with *Mycobacterium ulcerans* antigens using an ex vivo whole blood assay.

Dr Mark Wansbrough-Jones, St George's Hospital Medical School, London, England

We have attempted to clarify elements of the cell mediated immune response to *M. ulcerans* in humans using a whole blood antigen stimulation assay. In patients with tuberculosis, whole blood samples incubated *in vitro* with PPD produce gamma interferon which can be measured by ELISA assay. Such gamma interferon production correlates to some extent with results of tuberculin skin tests. We have used a similar *in vitro* assay to compare the response in blood from Ghanaian patients with *M. ulcerans* disease to *M. ulcerans* sonicate, *M. tuberculosis* antigen, PPD and *M. avium* antigen. As previously reported, in the first set of 37 patients with active or healed lesions, 9 (24%) responded to *M. ulcerans* antigen in terms of gamma interferon production. In the next study, *M. ulcerans* organisms from which soluble antigen was prepared were grown in protein-free medium before sonication like the burulin used in skin tests and cytokine release by blood from patients with healed ulcers was compared with that from household controls. Only 5 of the 52 patients showed significant gamma interferon production. The same 5 patients' blood samples responded to *M. tuberculosis* antigen but gamma interferon production was less. No significant responses to burulin were found among 24 household controls exposed to the same environment as patients. These results strongly suggest that a small proportion of patients with healed *M. ulcerans* disease have mounted a specific TH1 type immune response to *M. ulcerans*. IL-4 concentration was measured in all the same supernates and none was detected.

We were not able to correlate these findings on cytokine release with histology of lesions from patients but it is known that granulomatous inflammation appears late so it is possible that development of an effective immune response is delayed by cytokines released on first contact with *M. ulcerans*. For example, IL-10 is known to have inhibitory properties on the inflammatory response under some conditions. Therefore, we went on to measure IL-10 in the plasma from whole blood stimulated by burulin. The results indicated high levels of IL-10 (978 $\pm$ 76pg/ml; mean  $\pm$  SE) release in samples from 46 subjects with healed *M. ulcerans* disease and samples from 32 patients with active disease had raised but significantly lower levels (696 $\pm$ 98pg/ml) ( $p$ <0.05). Furthermore, samples from both groups had significantly higher levels of IL-10 than those observed in household controls (391 $\pm$ 63pg/ml).

Although *in vitro* stimulated IL-10 levels in household contacts did not differ significantly from those in normal school children from a non-endemic area, there was greater variation among the household contacts suggesting some degree of exposure to *M. ulcerans* antigens. Since IL-10 is an anti-inflammatory cytokine known to suppress production of pro-inflammatory cytokines including gamma interferon, this finding could explain why the immune response may be delayed after natural exposure to *M. ulcerans*. Further studies are needed to establish the specificity of this response and to identify antigens responsible for stimulating IL-10 release since this may influence the choice of vaccine antigens.

The work was funded by the Wellcome Trust.

# BCG vaccination in the control of Buruli ulcer

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## I. Introduction

Control of Buruli ulcer (BU), like most public health problems, involves multiple, often interrelated, socioeconomic, environmental and biomedical issues. There is every reason to believe that improvements in socioeconomic and environmental factors would control BU; however, salutary changes in the ecosystems of the major endemic foci, in the short-term, are improbable. This makes biomedical approaches the only practical recourse. Surveillance and treatment will not reduce the incidence of BU because the disease is seldom contagious, leaving immunoprophylaxis as the only current rational strategy for control of the disease.

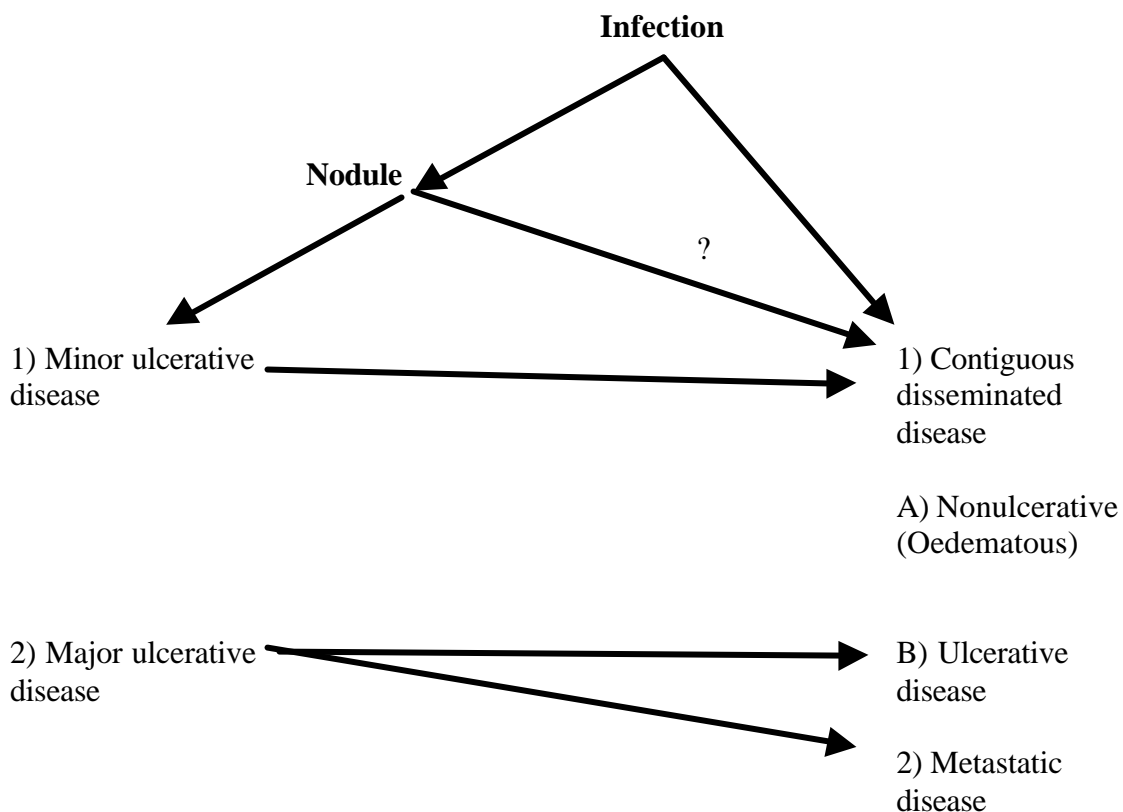
Recognizing that the history of vaccination against mycobacterial infections, which dates back to Robert Koch, is filled with failures, disappointments and controversies, the task of developing immunoprophylaxis methods for BU will be formidable, and the outcome uncertain. Nevertheless, the subject is worth serious deliberation.

## II. Background

The clinical and pathologic features of BU range from minor self-healing ulcers to widely disseminated, disabling, even life-threatening disease, with host response factors seeming to play an important role in determining the form of disease each patient develops.

The following outline of the spectrum of active BU may provide a basis for working out strategies for immunization intervention:

A Simplified Schema of the Natural History of Buruli Ulcer Disease



- 
- Minor ulcerative:** small, limited, with early self-healing.  
**Major ulcerative:** small to large, widely undermined, well demarcated, with late healing or possible dissemination.  
**Contiguous disseminated:** small or large edematous plaque that may ulcerate late.  
**Metastatic:** spread from initial lesion to distant site, usually in skin and/or bone.

Histopathologic correlations of lesions and clinical forms include:

- Minor ulcerative:** Acid-fast bacilli (AFB) limited to central necrotic slough, with scarring of surrounding dermis.  
**Major ulcerative:** AFB in necrotic base and adjacent areas, with undermining.  
**Contiguous disseminated:** widespread contiguous coagulation necrosis, with AFB in panniculus and fascia.  
**Metastatic:** typical changes of BU at distant skin sites, or *Mycobacterium ulcerans*-specific osteomyelitis.  
**Healing:** early organization - development of infiltrations of loosely arranged lymphocytes, epithelioid cells and giant cells.  
AFB scarce or absent  
late: development of well organized delayed-type hypersensitivity (tuberculoid) type of granuloma, followed by scarring.

### III. Rationale for immunization strategies

#### Humoral immunity

Following introduction of *M. ulcerans* into the skin or, more likely, subcutaneous tissue, there is a variable latent period during which the organism proliferates and elaborates a small amount of a diffusible toxin that destroys cells, especially adipocytes. Speculatively, this necrosis provides nutrients and creates local hypoxia, a milieu that favors more rapid growth of the etiologic agent. As this nidus of infection develops, the locus enlarges and becomes increasingly isolated from homeostatic and defense mechanisms such as contact with immunocompetent cells.

At this stage, pre-existent humoral antibody to virulence factor(s) such as *M. ulcerans* toxin should abort the infection in the preclinical or early stage; however, convalescent serum does not seem to inhibit toxin action *in vitro*. There is an urgent need for research on the development of immunogens that stimulate active humoral immunity to *M. ulcerans* or its virulence factors. Hyperimmune serum could be a useful adjunct in treatment of advanced life-threatening disseminated disease.

### **Cell-mediated immunity (CMI)**

After the initial stage, necrosis of tissue advances for variable periods and during this stage the burulin skin test is usually nonreactive, suggesting that specific CMI is deficient. Eventually as bacillary growth subsides, healing begins and the burulin test usually becomes positive, at least in the localized forms of the disease. This suggests that antigen components, perhaps from the bacterial cell wall, are detected by the host immune system and a Th-1 or delayed-type hypersensitivity response begins to destroy *M. ulcerans* bacilli, and promoting healing. Although there is evidence of immunologic events developing during infection with *M. ulcerans*, the potential immunogenic activity of the organism has received little attention.

## **IV. BCG Vaccination**

### **Efficacy in Buruli ulcer**

Reports on well documented prospective studies in Uganda indicate that a single BCG vaccination may protect against *M. ulcerans* disease at a rate of up to 75%. These studies revealed that protection began to wane after 6 months but lasted in some measure up to a year. Furthermore, in those vaccinated patients who developed BU, onset was delayed. Other observers note that lesions in BCG-vaccinated individuals appear to be more limited and to heal earlier than in those who were not vaccinated.

While there are probably several variables that determine susceptibility to BU in vaccinated individuals, level of exposure to *M. ulcerans* may be important. For example, in experimental studies in mice, BCG conferred immunity to low dose but not to large dose challenges with *M. ulcerans*.

## **V. Vaccination policies**

Worldwide, a total of 138 countries vaccinate with BCG at birth. This includes 46 countries in Africa. All the African countries vaccinate only at birth, and in 1995 vaccine coverage in infants was approximately 65%. Booster vaccination with BCG is policy in 40 countries, mostly in Europe, but none in Africa. WHO does not encourage booster vaccination at this time. The basis for this position may be because there has been inadequate evaluation of booster vaccination, and the potentially dangerous side effects in AIDS patients (e.g. BCG-osis).

### **Experience with Booster BCG for Tuberculosis and Leprosy**

BCG vaccination at birth or within the first two months of age protects against childhood tuberculosis (especially disseminated and meningitic forms). Efficacy for pulmonary tuberculosis in adults ranges from 0% to 80%. Protection rates with booster BCG for tuberculosis are controversial.

In leprosy, single BCG vaccination protection rates range from 20% to 80%. The protective effect of single and booster BCG vaccination has been evaluated in one extensive study (involving 121,020 individuals) in Malawi over a 5 to 9 year follow-up for both leprosy and tuberculosis. While a single BCG vaccination gave 50% protection, a second BCG vaccination gave a further protection of about 50% for leprosy, but not for tuberculosis. All age groups were included in this study, but protection appeared greatest in those vaccinated before 15 years of age.

## **VI. Conclusions**

1. The histopathology and immunology of Buruli ulcer support the concept that the host response determines, to a major degree, the natural history of each *M. ulcerans* infection.
2. Prior infection or a single BCG vaccination appears to decrease risk of infection by *M. ulcerans*.
3. Based on a projection of studies on leprosy, BCG vaccination with a booster vaccination may represent the best readily available and currently acceptable strategy for controlling Buruli ulcer.

## **VII. Suggestions for recommendations to the GBUI**

1. Assign priority for further consideration of the role of BCG vaccination in the control of Buruli ulcer disease.
2. Conduct an analysis of records in highly endemic areas to determine possible influences of BCG vaccination on Buruli ulcer disease.
3. If deemed advisable, select sites for a pilot trial of the effect of BCG vaccination, including booster interventions, on Buruli ulcer disease. (West African countries would seem to be the most suitable.)
4. Establish guidelines for BCG vaccination trials.

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## **I. Case-Control Study to Identify Modifiable Risk Factors for Buruli Ulcer Disease, Ghana, 2000**

*Pratima L. Raghunathan, Ellen A. Whitney, Jeannette Guarner, Thomas H. Taylor Jr., J. Anderson Comer, Jordan W. Tappero, David A. Ashford, Sam Bugri, George Amofah,, Kwame Asamoah, David Ofori-Adjei, Daniel Boakye, Anthony AblordeyY. Stienstra, W. van der Graaf, T. van der Werf, Groningen University Hospital, Groningen, K. Dobos, S. L. Kihlstrom, C. King.*

Buruli ulcer disease (BUD), a cutaneous infection caused by *Mycobacterium ulcerans*, is a major contributor to long-term disability in rural West Africa. BUD prevalence has been reported to range from 4 to 24% in endemic villages. Treatment is primarily surgical, and lengthy hospitalization imposes serious financial burdens on local healthcare systems. Prevention strategies cannot be developed, because the source and mode of transmission remain unknown, and risk factors for the disease are not well-characterized. We conducted a case-control study to identify modifiable risk factors for Buruli ulcer disease in Ashanti Akim North, Amansie West, and Upper Denkyira Districts of Ghana. To identify case-patients, we enhanced hospital- and community-based surveillance for BUD from September - November, 2000. Probable cases were diagnosed clinically according to the WHO criteria for past or present Buruli ulcer disease. We administered standardized questionnaires, obtained blood and urine specimens, and performed skin biopsies on suspected Buruli ulcer patients. Cases are being confirmed by positive *M. ulcerans* culture; characteristic histopathology; presence of acid-fast bacilli; or detection of *M. ulcerans* DNA by polymerase-chain reaction. We enrolled 159 probable case-patients, 150 age- and community-matched controls, and 155 family controls. From preliminary analysis of 159 probable cases, BUD patients were predominantly under 15 years (71%) with equal representation of the sexes. The vast majority of patients were from farming families (79%), had primary school or no education (73%), and lived in houses constructed from mud (73%). Lesions appeared primarily on the extremities (53% leg, 35% arm), but males were significantly more likely than females to have lesions on the trunk ( $p < 0.02$ ). Most (70%) case-patients had active ulcers, although nearly one-fourth (23%) had preulcerative lesions only. Case confirmation and analyses for BUD risk factors are in progress.

## **II. Histopathologic Analysis of Buruli Ulcer Disease**

*Jeannette Guarner, Sherif Zaki, Jordan W. Tappero, David A. Ashford, Sam Bugri, George Amofah, Kwame Asamoah*

Histopathology is one of four methods being used to confirm suspected cases of Buruli ulcer in the case-control study investigation in Ghana. Thus far, histopathologic study has been performed on 100 specimens from probable BUD case-patients. Acid-fast bacilli (AFB) have been found in 49 (49%) of the specimens. Of these 49 cases, 12 (24%) presented clinically as nodules, 4 (8%) had edema or plaques, 26 (53%) had ulcers, and 7 (14%) had a combination of nodules and ulcers. The amount and type of inflammation varied considerably between the cases; however, nodule specimens tended to have less inflammation and more bacilli, compared to ulcers. The bacilli were usually found in the necrotic subcutaneous material; rarely, they were found in the keratin or in the neutrophilic exudate of the ulcer. In the

confirmed Buruli ulcer cases, two inflammatory reactions were observed. A combination of neutrophils and mononuclear cells tended to accompany the necrosis of dermal collagen and subcutaneous adipose tissues, while a reactive mononuclear cell infiltrate was usually present in the superficial dermis. Granulomatous inflammation was found in these cases but it was usually not the main inflammatory reaction. The clinical diagnosis of Buruli ulcer could not be confirmed in 51 cases (51%), as acid-fast bacilli were not identified (14 nodules, 1 plaque, 32 ulcers and 4 nodules and ulcers). In at least 4 nodule specimens other diagnoses have been confirmed: 2 filariasis, 1 keratin cyst and 1 hemangioma. One case with a nodule had abundant granulomatous inflammation and fungal elements in the giant cells. Two cases with ulcers had squamous cell carcinoma. In these three cases, acid-fast bacilli were not identified; however, Buruli ulcer cannot be completely ruled out, since other diagnostic test results are pending. Histopathologic results will be compared with *M. ulcerans* culture, PCR, and AFB to determine sensitivity, specificity, and positive predictive value of the four diagnostic tests in this study population.

### **III. Burden of Buruli Ulcer Disease in Upper Denkyira District, Ghana, 1994-2000**

*Pratima L. Raghunathan, Ellen A. Whitney, Jordan W. Tappero, David A. Ashford Sam Bugri, George Amofah, Kwame Asamoah, Erasmus Klutse Y. Stienstra, W. van der Graaf, T. van der Werf, K. Dobos, S. L. Kihlstrom, C. King*

Buruli ulcer disease (BUD) is a rapidly emerging mycobacterial infection in West Africa. In Ghana, passive BUD surveillance has been conducted since 1994, but true incidence is unknown. To assess the burden of BUD in an endemic district of Ghana, we reviewed passive BUD surveillance data, and from September through November 2000, we enhanced hospital- and community-based active surveillance for BUD in Ghana's Upper Denkyira District (population 107,642). We defined a probable case of BUD as a characteristic active ulcer or preulcerative condition (nodule, plaque, or edema). Passive surveillance in Upper Denkyira District detected 501 probable BUD cases from 1994 to 1999 (annual median=85); no seasonal trends were detected. For comparison, 585 tuberculosis cases (annual median=70) and 13 leprosy cases (annual median=1) were reported concurrently. Using active surveillance, we identified 71 probable BUD cases. In contrast, a median of 18 BUD cases was reported during the same period over the 6 previous years in the passive surveillance system. From the active surveillance data, we estimate a crude annual BUD incidence rate of 28 cases per 10,000 population in this district. Active surveillance detected 3.6-fold greater probable BUD cases than the passive system, suggesting that Buruli ulcer may be underreported by over 70% in this location. The annual burden of BUD rivaled or exceeded that of tuberculosis and leprosy in this district; therefore, BUD control strategies are urgently needed.

## Genome Sequence Analysis of *Mycobacterium ulcerans*

Timothy Stinear, Roland Brosch, Karin Eiglmeier, Thierry Garnier and Stewart T. Cole, Pasteur Institute, Paris, France

Genomics, the systematic analysis of the complete genetic make-up of an organism, is making a major impact on biology and medicine in general, and has revolutionised mycobacteriological research in particular. Complete genome sequences have been determined for *Mycobacterium tuberculosis* and *Mycobacterium leprae* and the information gained from the analysis of the corresponding gene and protein sequences has provided fresh insight into the biochemistry, physiology and genetics of these important pathogens. The availability of this knowledge has opened up a host of new avenues for research in chemotherapy by enabling better use of existing antibiotics and inhibitors, facilitating the discovery of novel targets, and allowing new drugs to be developed along rational lines. The fields of immunodiagnosics and vaccine development are also benefiting from the complete definition of the antigenic repertoire of these mycobacteria, and a series of novel vaccine candidates derived from the genomic information is now being tested in animal models.

The time has come to apply the genomic approach to *M. ulcerans* as this will elucidate the epidemiology, biology and pathogenesis of this important emerging pathogen, and enable new prophylactic and therapeutic approaches to be conceived.

Within the framework of the Génopole programme at the Institut Pasteur, we will sequence the 4.4 Mb genome of an epidemic strain of *M. ulcerans*. About 40,000 reads from a whole genome shotgun library will be obtained using PE3700 automated sequencers then assembled into contigs using the programs Phrap and GAP4. Gap closure will then be undertaken using primer walking, and the resultant contiguous sequence subjected to bioinformatic analysis and exhaustive comparisons with the genome sequences of other mycobacteria.

The expected deliverables from this project are:

- Definition of complete sets of genes, enzymes, proteins and antigens of *M. ulcerans*
- Identification of species-specific functions that could serve as novel drug targets
- Identification of species-specific proteins for use as immunodiagnostic reagents
- Leads for developing therapy for Buruli ulcer
- Tools for detecting infection
- Improved molecular epidemiology
- Insight into the process by which an environmental bacterium becomes a pathogen
- Definition of the route to obligate pathogenesis in mycobacteria



## **The potential role of hyperbaric oxygen in the treatment of Buruli ulcer**

*Dr Franco Poggio, Rotary International, Milan, Italy*

The pathogenic agent *Mycobacterium ulcerans*, which causes Buruli ulcer, is a microaerophile that develops slowly, in an environment with less than the usual amount of oxygen. Animal experiments have shown that morbidity and mortality are reduced when hyperbaric oxygen therapy is associated with the usual forms of treatment.

It would therefore be worth testing this kind of treatment on humans.

For many years, it has been known that hyperbaric oxygen therapy for human subjects nourishes the tissues around lesions, prevents oedema, stimulates the granulation of tissue on surfaces requiring skin graft, and fosters free grafts.

The Milan Aquileia Rotary Club has offered to provide, for 2000-2001, a sealed chamber for hyperbaric oxygen therapy, for this type of experiment.

This should be done in an African hospital where there is a medical and surgical team specialized in treating Buruli ulcer, possessing a well-equipped laboratory for analysis, and if possible, a telemedicine link.

If appropriate, the hospital could bring together patients selected in accordance with medical and scientific criteria, and coming from other, less well-equipped medical facilities.

This solution would be an alternative to admission to Italian and other European facilities.

After a period of treatment under the supervision of physicians specialized in hyperbaric oxygen therapy (preferably Rotary volunteers), who would undertake to pass their knowledge on to their African colleagues, it would be possible to assess the usefulness of hyperbaric oxygen therapy in association with surgery, antibiotic treatment and routine care.

Once a team of experts in the different areas connected with the disease had been formed, the sealed chamber for hyperbaric oxygen therapy could (since this is a mobile unit) be taken to other hospitals (e.g. in Côte d'Ivoire) for a further period of treatment.



## Phenolic Glycolipid-I(PGL-I) in tissue of *Mycobacterium ulcerans* infected patients

Mikihisa Yajima, Milanga Mwanatambwe, Samuel Etuaful, Yukiko Fukunishi, Kenji Hibiya  
Keiji Suzuki, Nobutaka Yamada and Goro Asano

It has become obvious that one of the principal goals of Buruli ulcer research should be the development of tests permitting early detection of both individuals at risk and those already affected by early forms of the disease. Such tests, if available, will lead to timely treatment of the disease and hence, adequate prevention of its disabling consequences. Envelopes of mycobacteria are rich in carbohydrate-based antigens. Among these, phenolic glycolipids(PGLs) have given rise to remarkable interest as possible tools for early serodiagnosis of mycobacteriosis(1). Of particular interest is the phenolic glycolipid-1(PGL-1) of *Mycobacterium leprae*. The terminal sugar of its unique trisaccharide, is believed to make the molecule specific for *Mycobacterium leprae*(2). Historically, Hunter and Brennan(3) were the first to observe the occurrence of the molecule in an abundant quantity in tissue surrounding foci of infection by *Mycobacterium leprae*. From these observations, possible implications for immunogenicity and pathogenicity of the molecules were made. Its role in the pathogenesis of leprosy is still the subject of controversy. Being capable to scavenge oxygen radicals, the molecule has been suggested to help the leprosy bacilli avoid the action of lysosomal enzymes in macrophages(4). Along with a complex glycoprotein of *M. Leprae*, PGL-1 was also suggested to have lymphocyte suppression effect *in vitro*(5). In the field of serodiagnosis of leprosy, the molecule has been widely investigated and has gained worldwide acceptance(6). It is established that the antigen elicits early and abundant production of IgM antibodies(7). Simplified serological tests have been elaborated and are available for mass screening(8) and field works on leprosy. The molecule has also been used in the sero-surveillance of leprosy patients under MDT(9). Given that mycobacterial cell wall molecules are always capable of carrying out myriad functions, we sought to investigate the possible presence of PGL-1 in tissue of patients clinically diagnosed with Buruli ulcer.

### I. Materials and Methods

Skin flaps were surgically obtained from 30 patients clinically diagnosed with Buruli ulcer at the St. Martin's Catholic Hospital, Agroyesum, Ghana where Buruli ulcer is endemic. Formalin-fixed and paraffin-embedded tissues were routinely processed for H&E and AFB stains(Fite-Faraco and Harada). The tissue was further processed for immunostaining according to a protocol used at the Pathology laboratory of the National Leprosarium Tama in Tokyo(10). PGL-1 was kindly provided by the National Institute for Research on leprosy of Japan and was prepared according to a protocol already described(11). The antibody bridging technique were performed by the ABC methods.

## II. Results

Clinical form	Patients	Histopathology	
		Acid fast bacilli Stain(+)	PGL-1(+) by immunostain
Plaques	3	1	1
Nodules	10	5	5
Ulcerated nodules	1	0	0
Deep ulcer bed	7	4	4
Healing ulcer	9	2	2

**Acid fast bacilli stains** : Fite-Faraco & Harada stains

**PGL-1**:Phenolyc lycolipid

## III. Considerations

The results above suggest that PGL-1 may not be specific to one member of the *Mycobacterium* genus and that *Mycobacterium ulcerans* will probably express the antigen.

Any possible role Phenolyc glycolipids may play in the pathogenesis of Buruli ulcer should be further investigated.

If found in sera of Buruli ulcer patients, we may suggest its use for purpose similar to those applied for leprosy, such as early serodiagnosis, probably in association with histopathology, particularly since commercialized simple kits of the antigen are already available.

## Beliefs and attitudes towards Buruli ulcer in Ghana

*Ymkje Stienstra*

This study focuses on three aspects of beliefs and attitudes:

- 1) Perceived cause of the disease
- 2) Help-seeking behaviour and views on treatment
- 3) Stigma

### **I. Methods**

The study was done at three locations: Agogo, Agroyesum and Dunkwa. Based on a case-control study in Ghana, our impression is that Buruli Ulcer is less endemic in Agogo than in Agroyesum and Dunkwa.

Thirty-three Buruli Ulcer patients admitted in the hospitals were included and also for each Buruli patient one age- and sex-matched control patient, admitted for diseases other than Buruli Ulcer. The interviews were developed with an English framework. Interpreters asked the questions in Twi. The interviews lasted about one hour and were semi-structured. In the beginning more open questions were asked, followed by more specific questions. Also a quantitative study was done on stigma. In this quantitative study an individual stigma score was made by scoring answers to a set of questions. This is adapted from a study on stigma of onchocercal skin disease in Africa, including Ghana. Internal validation of items was done with Cronbach's alpha reliability analysis.

### **II. Results**

#### **Perceived causes of the disease:**

Of the 66 respondents 39 mentioned bad personal hygiene as one cause of developing Buruli Ulcer. Environment was often mentioned, including walking in swampy areas and insects., Fifteen respondents mentioned close contact with a patient as a cause, witchcraft was mentioned by 39 respondents and a curse was also mentioned often. I would like to illustrate the way of thinking about witchcraft with the following two citations of patients in this study. "Witches are known for spreading mysterious diseases in Ghanaian society, like tuberculosis, Buruli and leprosy." "People in my village think I'm bewitched if I'm admitted for a longer period. Witches usually cause diseases that are difficult to cure."

#### **Help-seeking behaviour and views on treatment:**

Financial difficulties were often mentioned by respondents, especially the high indirect costs, which prevent them from coming to hospital earlier. Next Buruli patients reported a fear of the effects of treatment. Some patients mentioned a fear of amputation. Sometimes family members even advised patients not to go to the hospital, because of the possibility of amputation. Fear of surgery and the duration of treatment was another fear leading to patients' delay. Other reasons for patients' delay is reluctance to seek treatment outside the own community and the social stigma of the disease.

## **Stigma**

In the quantitative part of the study a set of questions was asked to Buruli patients and control patients. Some of them will be shown during the presentation. With this quantitative data we concluded that control subjects had higher stigma scores than study subjects. The stigma scores in Agogo were higher than in Agroyesum and Dunkwa. The quantitative data can be supported by the qualitative data.

## **III. Conclusions**

- Witchcraft and curses play an important role in the way of thinking in communities.
- Lack of knowledge about the treatment is a cause of patients' delay.
- In the higher endemic areas the stigma scores are lower. Education might have a role to play in lowering stigma scores.

## Manual on the management of Buruli ulcer

*John A. Buntine, Cornell's Specialists' Centre, Victoria, Australia*

It may be a surprise that I have become editor of the surgical manual. This is because English is my first language, I have had some relevant experience and Kingsley could not take on the role, being a WHO employee. Nevertheless, Kingsley has continued to be most active with respect to the manual.

Editing the manual has been far from easy but I have enjoyed the task. My contribution to the content has been small. I have tried to put together the work of others in a reasonably consistent style. An attempt has also been made to reduce the manual to a useful size.

Difficulties peculiar to the subject arise from a lack of fundamental knowledge of the disease and from the need to include information consistent with the needs of both village communities and major hospitals. I know of no other disease about which transmission is so poorly understood. It is extraordinary that the infection does not spread directly from one person to another. Also, the clinical role of antibiotics, which are effective *in vitro* is still debated. This is perhaps fortunate because in some areas where the disease is prevalent the possibly most active antibiotics cannot be afforded. It is virtually impossible to apply the principles of evidence-based medicine to *M. ulcerans* infection. Perhaps an effective and inexpensive means of immunisation will be found eventually?

Study of *M. ulcerans* infection rekindles the romance of medicine of the past, that of finding the source of the causative organisms, of working out how the disease is caught and of searching for effective treatments. Presently, we understand a lot about *M. ulcerans* as an organism but little about its transmission and about the effective use of antibiotics.

Surely, treatment by radical excision is but a passing phase. Surely, antibiotic therapy or some form of immunisation will eventually become the major modality of treatment.

A number of conditions, such as keloid, are presently treated by intralesional injections. If *M. ulcerans* bacilli are protected from antibiotics because they grow in dead tissue (mainly subcutaneous tissues), would injection of a relatively cheap and non-toxic antibiotic, such as streptomycin, directly into the dead tissue be an alternative to radical excision? As we have dealt with the minor epidemic near Melbourne by the application of public health measures, I currently do not have an opportunity to study intralesional antibiotic injection and, as this is no more than an idea, nothing has been said about it in the manual. I should also mention that the public health measures which were effective at Phillip Island could not be applied elsewhere except, possibly, in an occasional situation.

I am unsure how far we have progressed with the illustrations and photographs. Fortunately, one of my sons can arrange to transmit high-definition colour photographs by e-mail.

I am waiting with more than a little trepidation for your responses to the draft manual as it presently stands. I am wondering how many feel that I have missed or misrepresented the essentials of what they said or wrote.

Finally, I would like to suggest that a small pocket version of the manual be written in basic English and French, mainly for use by health workers other than doctors.

Please let me know your views.



## ANESVAD

*Mr Andrés Ginés and Mrs Verónica Malda, ANESVAD, Bilbao, Spain*

The ANESVAD Foundation is a nongovernmental and non-profit organization, which falls under the umbrella of the National Coordinator of NGOs for Development, which carries out health and social projects in least-developed countries.

This Foundation - one of the oldest NGOs in Spain - was founded in Bilbao in 1968, and its initial aim consisted of providing healthcare and social assistance to poor and deprived patients and their families. In 1970, it was established as a national association and subsequently, it was declared as a "public utility entity" and was awarded the Cross of the Civil Order of Charity. In 1992, when ANESVAD had already been working for 25 years in the field of development, it became a foundation. It was classified by a ministerial order as "a private charity institution to provide assistance", and falls under the purview of the Works and Social Affairs Ministry.

ANESVAD is financed by payments and donations made by over 135,000 partners and collaborators, from all over Spain. Companies and councils also provide us with some of their profits, and we count on the support of the Spanish Agency for International Cooperation. Thanks to their generosity, ANESVAD can execute its main health and social work objectives in those areas and undeveloped villages in order to achieve total social integration and self-sufficiency in most deprived countries. This work is carried out in close collaboration with Jesuit missionaries and other holy orders, with secular organizations and NGOs from the countries we are working in. Through the applications for assistance that we receive, we can identify the needs they have and help them.

ANESVAD began its international work following the footsteps of Fr. Javier Olazabal, S.J., a Jesuit from Bilbao, who went voluntarily to practice his apostolate to the Culion leprosy-island (The Philippines), considered then as one of the biggest leper colonies in the world, with a population of over 14,000 villagers, lepers, their families, health workers and missionaries, who were obliged to live in very poor conditions. They did not have supplies of drinking water, electricity or medicines; child mortality rate reached 80%, etc. Thanks to the help that ANESVAD provided them since 1971, carrying out health and development programs in Culion, two new treatments to cure leprosy were developed, multidrug therapy (MDT) and ELISA, and many health, social, educational, socio-economic rehabilitation and community development programs were also carried out. Nowadays, Culion has been integrated in the Philippine archipelago. The fear of leprosy has disappeared thanks to the effective medical treatments and reintegration of the patient in his/her community, his/her ability to obtain a dignified job and be integrated in a community life. As this development has been a success and has gotten international recognition, this experience in combating leprosy was extended to other places where this disease afflicted people, such as the leper colonies in south-east Asia and Central America. ANESVAD has recently taken up a new challenge in Ivory Coast (Africa): the battle against BURULI which affects mainly children (70% of the people affected by this disease) and leads to severe lesions and physical and psychological problems.

ANESVAD has helped 28 Asian, Latin American and African countries. Some of the Asian countries assisted by ANESVAD are: The Philippines, Vietnam, India, China, Cambodia,

Taiwan, Thailand and Burma. This is therefore one of the NGOs with the greatest outreach in south-east Asia. Apart from this important work carried out in Asia, ANESVAD began assisting Latin America: Argentina, Ecuador, Bolivia, Peru, Colombia, Nicaragua, Honduras, Mexico, the Dominican Republic, Brazil, Venezuela and El Salvador. In Africa, ANESVAD assists people from Burundi, Democratic Republic of Congo, Ghana, Cameroon, Madagascar, Ivory Coast and Liberia.

ANESVAD develops a wide scope of projects. These projects are related to sanitary action (construction and maintenance of medical dispensaries and hospitals; treatments to eradicate leprosy; continuous supplies of medicines; etc.), social projects (assistance and social integration to victims of wars; assistance to refugees and immigrants; construction of centers where training programs to establish a right set of values for their life are conducted; construction of water wells, dwellings, infrastructures and occupational workshops; social integration programs for single mothers and abandoned women; construction of orphanages and special assistance programs for orphans; etc.) and educational projects (training programs; construction of schools; educational programs; construction of special education schools; scholarships for the youth, etc.)

Another important task is sending medicines that the Health Department of ANESVAD offers through CEMED (Medicines Center for the Least-Developed Countries), in Villagarcía de Campos (Valladolid). In this center, medicines donated by laboratories and pharmacies are collected, to be distributed in the countries that need them urgently.

On the other hand, the Educational Department for Development sensitizes the Spanish population about the problems that the undeveloped countries have to face, through videos, magazines, brochures and mass media, giving full details about the situation of poverty of the inhabitants, and their desire to lead a better life.

At ANESVAD, we commit ourselves to continue working in accordance with our mission. Experience has shown us that thanks to the efforts made by all the members of ANESVAD, there have been positive and lasting changes, where we have offered our help and our services to carry out development programs.

## **The Buruli ulcer Project in Ghana: Support from the Italian Association Amici di Raoul Follereau (AIFO) in 2000**

*Dr George Abram and Dr Enzo Zecchini, AIFO, Bologna, Italy*

In keeping with the renewed agreement with the Government of Ghana, AIFO has decided to help with the care of patients affected by Buruli Ulcer, concentrating their intervention in selected districts of Ghana. The Ga District was the first choice, as it is the most needy.

The project is located in a rural area not far from the outskirts of Accra, the capital city of Ghana, at Amasaman, the administrative centre of Ga District. The Ga District was created quite recently and therefore lacks many essential commodities, such as buildings for the administration and health centres. The rural area, besides the lack of roads, electricity and pipe-borne water, is suffering a constant degradation due to uncontrolled excavation of sand: greenery is removed, sand is carried away, and all that is left is just barren soil which turns into marshes during the rainy season and slowly becomes a swamp.

### **I. Problems**

Nowadays, notwithstanding the efforts of the government and due to the passive attitude of the population, both patients and their families are very reluctant to refer their cases to the health authorities. Some of the reasons for this noncompliant behaviour and neglect may be rooted in the belief that the disease is linked to a supernatural cause, which is a reminder of the former attitude of the population towards leprosy. Therefore, people have been referring to shrines and shamans rather than doctors and hospitals. The experience that official medicine could not do much to heal the wounds was another cause of distrust and it deepened superstitious beliefs. Another drawback is the fact that nodules and ulcers are usually painless, so the patient is not urged to seek relief, and not from a doctor in any case.

### **II. Possible Solution**

The ulcer, as said before, can be prevented by early surgery. But it may also be successfully treated with the standard procedures, which require daily care: cleansing, disinfection, trimming, dressing and skin grafting. The ulcer slowly shrinks and heals, leaving a scar more or less visible depending on the type of treatment and whether intervention is early or late. Recently, some drugs have been experimented with encouraging results. We are preparing the printing of a manual, in which some of the successes of these trials will be outlined.

### **III. The project**

We have been discussing with the Ministry of Health in general and with Dr George Amofah in particular the possibility of creating a model project, or prototype, that may be then applied to other districts in different ways according to their needs and the gravity of the situation. In many districts of Ghana, where the health system is working and there are hospitals, it is possible to intervene both at the onset of the disease and during infection. Unfortunately, in the Ga District there is only one health centre, which lacks an operating theatre and wards where Buruli patients can be admitted after the intervention to remove the nodule or surgery to clear the ulcer, or to avoid further damage caused by the disease.

At present, those who have the necessary financial means are sent to the University Hospital in Accra, but very few can afford it due to the high costs involved: travel expenses, assistance and meals. The family is responsible for meals, medical expenses, etc. The project, therefore, consisted initially of building a small ward and an operating theatre. Construction of the hospital is now completed and plans for the other aspects of the project are being studied and prepared.

The plan is to have an integrated programme for the overall running of the project. It spans from health education activities to training of rural health personnel, to household management of ulcers and improvement of living conditions. Two four-wheel drive pick-ups have been purchased for household treatment and the health education campaign. Many refresher courses have been started, others will be carried out during the current year and the manual will soon become a reality.

## American Leprosy Missions (ALM)

*Dr Paul Saunderson, ALM, Greenville, USA*

### I. Leprosy

ALM has been involved in funding leprosy work for almost a century, with an interest in both the **public health aspects** of the disease and the **care and rehabilitation** of those affected by it. We are gratified by the recent rapid decline in the prevalence of leprosy, realizing, however, that this is no time for complacency: ALM is committed to ongoing efforts to free the world of leprosy, so that our major expenditure will continue to be on leprosy control, prevention of disability, rehabilitation and research, for the foreseeable future.

### II. Buruli ulcer

ALM's Board has agreed to expenditure in the field of Buruli Ulcer for four reasons:

- 1) The needs in leprosy may decrease over the next decade, freeing funds for other purposes.
- 2) Economic growth (especially in the US) may allow significant growth in ALM's program budget, with the possibility of taking on new projects.
- 3) Buruli Ulcer is biologically related to leprosy, but perhaps even more significantly, from ALM's point of view, it causes very similar social problems, so that our experience in this area may be of relevance.
- 4) Dr Wayne Meyers is a consultant to the Board of ALM and therefore his interest, experience and expertise in the field of Buruli Ulcer make it a natural development for us.

We are at an exploratory stage. We have contacts in Ghana and Ivory Coast and expect to fund some projects in 2001. I will visit both countries in April with Dr Wayne Meyers. Support will increase as funds become available and as we find appropriate project partners with whom to work.

The following issues are currently important to us as we select project partners, but we are flexible and willing to learn from the experience of others:

- 1) Co-ordination with other donors, including WHO and ILEP partners, to avoid duplication and promote service provision as widely as possible in endemic areas.
- 2) The logical places to begin support seem to be hospitals or health centers that can provide diagnostic services and surgical treatment. Once those services are available in an area, other work, such as public education, early diagnosis in the community, rehabilitation and research can be developed and supported.
- 3) Typically, our primary partners are Christian NGO's (for example, mission or church hospitals) through whom we can assist other projects, including government health services, as necessary. Specifically, in Ghana we have links with Presbyterian hospitals, including Agogo Hospital and with the Teaching Hospital in Kumasi. In Ivory Coast our links at present are with MAP International as local co-ordinator, but we are still searching for the most appropriate treatment centers to work with.



## The French Raoul Follereau Association (AFRF)

*Pierre Olphe Galliard, AFRF, Paris, France*

The French Raoul Follereau Association (AFRF) is specialized in leprosy control. It is therefore a partner of several West African governments in implementation of national leprosy control plans. It also pays close attention to changes in the health status of the populations concerned. In 1995, it was alerted several times to the grave condition of certain patients in Côte d'Ivoire and Benin, resulting from a disease which, in those days, was little known: Buruli ulcer.

Given the scale of the epidemic, and once its medical committee had given a favourable opinion, AFRF decided to tackle that disease. The first step was more or less automatic, in that leprosy and Buruli ulcer patients are treated at the same facilities.

As of 1995, AFRF affirmed its desire to help public and private health facilities in Benin and Côte d'Ivoire to comfort the victims of Buruli ulcer as it was already doing for leprosy patients. In parallel, a working session was organized at AFRF headquarters that brought together several specialists interested in the subject; these included Professor Grosset, Doctors Scherpbier and Asiedu of WHO and Professor Kanga, responsible for Buruli ulcer control in Côte d'Ivoire. Two conclusions arose from the meeting, namely:

The disease was spreading at a tremendous speed in both Benin and Côte d'Ivoire, Medicine is ill-equipped to fight the disease; the ecology, epidemiology and transmission of *Mycobacterium ulcerans* are obscure. Given the lack of medical treatment, a cure could be effected only by surgery.

The AFRF has therefore shown ever-increasing determination to tackle Buruli ulcer, just as it tackles leprosy.

From 1995–1996 onward, support from AFRF has been in three main areas:

1. support to facilities treating patients;
2. support for research programmes;
3. support for the spread of knowledge and resource mobilization, launched by the government and WHO.

### **I. Support for facilities that treat patients**

The long experience of AFRF in leprosy control has brought it into close contact with private and public facilities specialized in treatment of leprosy patients. That is how the centres of Zagnanado and Davougou in Benin and the Raoul Follereau Institute of Adzope/Manikro in Côte d'Ivoire, both in highly endemic zones, have come to admit increasing numbers of Buruli ulcer patients: 205 underwent surgery at Zagnanado in 1995, 231 in 1996 and 300 in 2000. The AFRF provides 75,000 Euros a year to purchase the drugs and expendibles needed for those operations, which are now performed under the supervision of a professor of surgery who, with our support, provides the centre with his technical assistance.

At the Raoul Follereau Institute in Adzope/Manikro in Côte d'Ivoire, 171 patients were treated between July 1999 and June 2000. In two years, over 160,000 Euros were provided by AFRF for drugs, dressings and essential supplies.

Similarly, medical and surgical missions are organized in the two facilities, under the guidance of Professor Christophe Oberlin, a surgeon and member of the AFRF Medical Committee.

In Benin, at the request of the government, our commitment is now taking on a new dimension with the establishment at Pobè of a centre specialized in the treatment of Buruli ulcer. It is the extension of a leprosy treatment centre, with an operating theatre and extended wards. The investment of more than 150,000 Euros will be financed entirely by AFRF, along with the operating costs, which include the salary of the physician responsible for managing the new facility. Epidemiological surveys conducted in 1998 and 1999 thanks to AFRF funding, managed by Dr Augustin Guédénon, showed a high prevalence of Buruli ulcer in that region of Oueme, in the east of Benin. For various reasons, the patients found it difficult to get to other centres in the country. With the Pobè centre, they will therefore have surgical rather than medical treatment in the vicinity.

## **II. Support for research programmes**

In 1995, a research programme was set up that involved the Raoul Follereau Institute of Adzope, the bacteriology laboratory of the Medical Faculty of Pitié-Salpêtrière (Professors Jacques Grosset et Baohong Ji) and the bacteriology laboratory of the Medical Faculty of Angers (Professor Bernard Carbonelle). For this purpose, a laboratory was specially equipped and initiated at the Raoul Follereau Institute of Adzope, under the supervision of Dr Henri Kouakou. The laboratory also has an animal house, where *Mycobacterium ulcerans* can be cultured in the paws of mice.

The laboratory, which was opened in the second half of 1996, thanks to an investment of 120,000 Euros, is now running smoothly. Professor Carbonelle visits regularly and there are interesting scientific exchanges. The main objective of the laboratory is to help evaluate antibiotics that could serve as a basis for medical treatment of Buruli ulcer. AFRF contributes to the running of the laboratory, especially by supplying antibiotics and other products needed to further research.

On that basis, a pilot clinical trial of antibiotic treatment of patients has been underway in the Raoul Follereau Institute since the end of 2000, as was already mentioned elsewhere. This activity will be extended to Ghana, through collaboration with WHO and the Ghanaian officials responsible for Buruli ulcer control. Our contribution is of 27,500 Euros.

In order to improve knowledge about the ecology of *M. ulcerans*, research for this bacterium in the environment has been started with a budget of 65,000 Euros.

Finally, AFRF has contributed 52,000 Euros to sequencing of the genome of *M. ulcerans*, a project directed by Professor Stewart Cole of the Institute Pasteur in Paris, with the assistance of Dr Tim Stinear. This is in addition to the work underway at Pitié Salpêtrière on *Mycobacterium leprae* and *Mycobacterium ulcerans*, for a provisional budget of over 160,000 Euros.

### **III. Support for the dissemination of knowledge and mobilization activities, by governments and WHO**

The commitment of AFRF has brought it into dialogue, and even into partnership, with WHO, in Buruli ulcer control activities. AFRF has always responded to the requests that have been made, for example, during the organization at Yamoussoukro in July 1998 of an international conference that brought together the three heads of State and 150 delegates from the scientific world.

In addition to this very high-level event, AFRF has helped finance WHO initiatives for endemic countries, especially the French version of a reference work on the subject, using four-colour process printing, for almost 17,000 Euros. Contribution to the French translation of a guide for physicians and nurses is being considered at present.

Similarly, members of the Medical Commission concerned with *M. ulcerans* maintain close links with WHO officials and African practitioners and research workers.

Through its presence on the field and its close relationships with health workers, AFRF was alerted as early as 1994-1995 to the gravity of the Buruli ulcer epidemic in Benin and Côte d'Ivoire. It has responded with the resources available, and it has strove to make its donors aware of the extreme suffering this disease causes. AFRF has been providing approximately 300,000 Euros every year for nearly five years, to those who suffer from this disease, and to those who are working to eradicate it. By so doing, it is faithful to its vocation to combat "leprosy and all related diseases".



## **Aide aux Lépreux Emmaüs – Switzerland (ALES)**

*Patricia Beauverd, René Staeheli, Dr Richard Hehl, ALES, Bern, Switzerland*

As an NGO seeking to control leprosy and tuberculosis, Aide aux Lépreux Emmaüs – Suisse (Aide for leprosy patients, Emmaüs – Switzerland) (ALES) has naturally taken an interest in Buruli ulcer since it belongs to the same category of diseases as leprosy and TB.

It was in 1999 that ALES really became aware of this disease, through the work done by its partners in the International Federation of Anti-Leprosy Associations (ILEP), who were trying to deal with the disease in several African countries. As ILEP coordinator for Cameroon and the Central African Republic, ALES has therefore gathered information on the field in order to see if it should become involved.

A strategy, however, can be formulated only through a serious study or epidemiological survey. For the moment, the rest is only speculation. If it was found that Buruli ulcer really was a public health problem in Cameroon and the Central African Republic, the facilities for dealing with leprosy could be appropriate, given the similarity of treatment.

A thesis on Buruli ulcer by medical students in Cameroon a few years earlier, had described Cameroon as an endemic country. However, a superficial survey conducted in the Centre, South, West and Coastal provinces in July 1999, showed that the disease is little known or not known at all by public health staff or leprosy specialists. On the other hand, the symptoms of the disease seem to be recognized, but diagnosed as a “tropical sloughing ulcer”. Some regions seem to have favourable conditions for the disease, suggesting that in some cases what had been diagnosed as tropical sloughing ulcer might actually be Buruli ulcer.

In the Central African Republic the disease is hardly known, and no data are available. At present, the extent of the problem cannot be assessed. A survey conducted at the beginning of this year provided the following information: it is quite possible that patients with Buruli ulcer have consulted, but usually at the nodule stage, when ulceration is limited. In such cases, the ulcer has often been mistaken for a sloughing ulcer, especially when located on the extremities.

With its similarity to leprosy and tuberculosis, Buruli ulcer can to a large extent be treated at facilities for leprosy treatment. For this reason, ALES took the initiative of starting preventive work in Cameroon by distributing leaflets to the medical staff of public health services, and by providing information on the disease throughout the year 2000, during tours of inspection for leprosy conducted by the physician responsible for our Regional Office for Africa, in Yaoundé. The opening of an ALES office in the Central African Republic this year means that the same work that was done in Cameroon in 2000 can now be done .

If a serious survey were to show that Buruli ulcer was a public health problem, then the following steps would have to be taken in both countries:

1. Drawing the attention of the political and administrative authorities, health workers at all levels, training institutes (universities, nursing schools, institutions, nongovernmental organizations, agencies and associations, the Pasteur Institute and laboratories) to the danger of this disease and the need to prevent it (early diagnosis, medical treatment and education in order to minimize its progression and complications).
2. Linking with the leprosy and tuberculosis programmes, so as to include a Buruli ulcer control programme in collaboration with the ministries of health.
3. Ensuring training and supervision of staff, with a referral system for centres that can provide surgical treatment.
4. Providing information and awareness training materials.
5. Providing surgical treatment.
6. Working together with dermatology and orthopaedic surgery services.

## **The Buruli ulcer control activities of the Luxembourg Raoul Follereau Foundation in Benin**

*Professor Henri-Valère T. Kiniffo and Mr Robert Kohll, FFL, Luxembourg*

In 1998 the Raoul Follereau Foundation of Luxembourg (FFL), through the Raoul Follereau Association of Benin (ARFB), conducted a feasibility study (a prevalence study) to help with Buruli ulcer control in Benin. That study concentrated on a few towns in the north of the Atlantique département, one of the four endemic départements in Benin.

The results of that study were presented by Professor Henri-Valère T. Kiniffo, president of the ARFB, at the Yamoussoukro conference in July 1998.

In 1999, cooperation agreements were signed by FFL and the Government of Benin (Ministry of Public Health), thus opening the way to close collaboration. After a number of preliminary studies, the FFL decided to build a Buruli ulcer diagnosis and treatment centre from scratch, in Allada, 50 kilometres from Cotonou, on the grounds of the subprefectural health centre. That choice of site was guided by the following factors:

- the Plan of Action of the Benin Health Ministry;
- the presence of qualified medical staff;
- the proximity of major endemic foci;
- the accessibility of the site, and
- the hierarchical level of the subprefectural health centre in the health system.

The facilities to be established, and financed entirely by FFL, are as follows:

- a surgery unit;
- a technical unit;
- a hospital unit;
- an administrative unit;
- annexes.

The plans for the centre have been approved and authorized by the health ministry, in conformity with prevailing rules. The building contract went to three local companies, which started work in February 2001 and should finish construction in the following seven or eight months. Admission of the first patient is scheduled for November 2001.

The surgery unit consists of two operating theatres and a room for minor surgery, a laboratory and a room for anaesthesia and intensive care. The hospital unit has 30 beds.

In addition to building the facilities, FFL has undertaken to provide equipment and necessary medical supplies, transport (an ambulance and a liaison vehicle) and a supply of drugs. As soon as the building work is done, the FFL will donate them to the State of Benin.

For its part, the Ministry of Public Health has undertaken to build latrines and an incinerator, to provide the 30 hospital beds, to appoint and maintain the medical, paramedical and ancillary staff and to pay the running costs. The Health Ministry will maintain the centre as a medical unit once Buruli ulcer is eliminated.

In addition to the Buruli ulcer diagnosis and treatment centre at Allada, the Luxembourg Raoul Follereau Foundation in 1999 provided support to the Gbemonten Nutritional Centre (in Zou département) by providing equipment and drugs for Buruli ulcer control. In 2001, medical supplies and drugs were provided for the Camilliens hospital in Zinvié (Atlantique département) and these are now being supplied.

In conclusion, the Buruli ulcer control activities of FFL have been conducted in line with the Plan of Action drawn up by the Ministry of Public Health of Benin.

Through the ARFB, its representative, FFL, attends meetings of the steering committee that was set up by the National Buruli Ulcer Control Programme.

The Allada centre will be recognized as the national reference centre for Buruli ulcer.

## Presentation summary from Humanitarian Aid Relief Team (HART)

*Ms Alicia Jackson and Dr Kimball Crofts, HART, Provo, Utah, USA*

The 4<sup>th</sup> Annual Ad Hoc Meeting on the Global Buruli ulcer Initiative convened on March 5-7, 2001 at the World Health Organization Headquarters in Geneva, Switzerland. Alicia Jackson and Kimball M. Crofts, MD presented a report to the convened body on the organization of HART, its activities in international health care delivery and its surgical treatment of BUD.

HART (Humanitarian Aid Relief Team) was founded in 1992 when a small group of students at Brigham Young University in Utah congregated to discuss how they could make an impact on the international health care system. Initially, the focus was on the delivery of health care goods to Russia. From 1993 to 1995 hundreds of thousands of dollars of medical supplies were delivered to Russia. No medical services were undertaken at that time.

In 1995, a connection was established with a certain individual in Ghana who directed HART's efforts away from Russia to the surgical treatment and management of a relatively new disease process caused by the *Mycobacterium ulcerans* organism known as Buruli ulcer. Additionally, HART began to address concomitantly the medical needs the local traditional birth attendants. Not long after the first project in 1995 in the Ashanti Region, the TBA program was dropped in favor of the more demanding and pressing Buruli ulcer disease.

Our mission is to study and address international health care delivery issues, collaborate with local medical personnel, educate and train, deliver medical supplies and develop long term local self-sustainability. The Ghana Project was focused on direct surgical relief, medical supplies, an outreach education program and training and collection of research data. With that in mind we proceeded to set up our first mission in Agroyesum, Ghana. From 1995-1997 two surgical missions were undertaken. A total of 116 cases were addressed surgically. A dermatome and mesher were procured for the hospital in Agroyesum. They are now self-sufficient with a self-sustaining program for treating BUD.

Our next project was centered in the Central region. From 1997-1999 four missions were executed. A total of 305 cases were surgically managed. It should be noted that when we made an effort to familiarise ourselves with the local conditions prior to the arrival of the medical team, we learned that they had very little and that they were antiquated, but had excellent help. This information helped us to address their needs better.

In setting up of the surgical mission, we brought with us loads of donated medico-surgical products that were subsequently set up for use in the OR and Pre and Post-operative areas. Once the OR and facility were ready to go we made our way to the Buruli Ward or Isolation Ward to evaluate and triage the many patients in waiting. Over 250 patients pleaded for care.... We got to ~80 of them in 7 days of surgery – good but not adequate to address the problem. From this we determined that we were spending about \$2,600 per patient – very costly indeed. Hence the need for education and self-sustainability.

We then took a good, hard look at our organization and decided that we had a few problems that needed to be addressed: our surgery missions resulted in mostly debulking, we were temporarily overwhelming their system, our triage was inadequate, follow-up poor, data collection lacking and exchange of treatment ideas wanting.

Serious reflection and introspection led to a new approach, which I will attempt to clearly evince: First, our new focus is: To improve total healthcare delivery through the understanding and development of existing healthcare modalities.

Our next measure was to establish a Ghanaian NGO and Board of Directors in Accra, Ghana to work closely with our American Board of Directors. This would give us better local control of activities. We also developed the idea of an Area Development Team to help prepare a facility for surgery and medical treatment of BUD.

Our entire team concept: Surgical Treatment Team

## An Annual Report on Volunteer Actions for Buruli ulcer Children

*Yuki Shimomura & Tetsuya Fujikura, Kobe International University, Japan*

Ever since Kobe International University (KIU) launched its volunteer project to save children from Buruli ulcer in 1999, our activities have gained considerable recognition in the Kobe region. This report covers our advocacy activities from April 2000 to March 2001.

### I. Photo Exhibitions and Charity Drive

Photo exhibitions have served two purposes. One is to let the public know about the disease and the sufferings that it brings to the people of the endemic regions, and another is to enhance our charity drive. Project SCOBU and KIU Student Volunteers are jointly organized to sponsor five separate exhibitions in various areas in the Kobe region. We run an exhibition usually for two days consecutively at every place:

- at Campus Square (12–13 October 1999 and 14–15 October 2000)
- at Patio, Suma-ku Kobe (19–20 October 1999)
- at the Center Court, Seishin-chuo station (12–13 October 2000)
- at Kobe Sangyo Shinko Center (20 October 2000)
- at Tarumi Municipal Health Office (21–22 December 2000)
- at Kobe Culture Hall ( 7 February 2001)

Those photo exhibitions have proven to be effective for our advocacy activities, but they are not without limitations. While exhibiting photos is one of the surest ways to approach the people of the region, they remain “regional.” To solve the problem, we needed to go public through the existing networks of other organizations. Christian churches and YMCA are such examples.

### II. Christmas Charity Drive

As a Christian school, Kobe International University has a chapel on its campus and provides daily services to the students and staff. The chapel has sponsored a Christmas Charity Drive for BU children for two consecutive years from 1999 and collected considerable donations not just from the church, but also from other Christian organizations:

- at St. Michael’s Chapel on Kobe International University campus.
- at St. Luke Church, Saitama

Furthermore, Christian News Services in Japan kindly agreed to run articles for our cause. The first article on BU appeared in the Anglican Monthly News on 25 October 1999, immediately followed by the second article in the Christian Chronicle, a bi-weekly newspaper. Both news agencies did the same in 2000 to support our advocacy activities.

YMCA Nishinomiya invited Project SCOBU to speak on BU on 16 February 2001. The members of the steering committee had shown a great interest in our activities and kindly provided other possible options to them. The Project will also give a talk at the Kobe Rotary Club on 13 March 2001 on BU. We sincerely hope that this will widen the scope of our activities in the future.

### **III. Media and Advocacy Activities**

The media has played an important role in our activities. Various articles on the first International Symposium '99 appeared in three major newspapers. The news made a regional NHK news program in that year. In the year 2000, we witnessed a new phase of development. The student volunteer mission to Ghana on which I will report later appeared on national network news in December, while Osaka Central Cable Network approached the Project and volunteered to put the suffering of BU children on air in the Osaka region. Through the powerful tool of the media, I am confident that our advocacy activities are slowly but surely winning recognition.

#### **What did we do with the donations?**

##### *(1) Côte d'Ivoire*

A shelter at St. Michel Catholic Hospital, Zoukougbe in Côte d'Ivoire was completed in September 2000 with the donations from the people of Kobe and KIU Project SCOBU.

##### *(2) Portable medical kits and washing machines*

We were able to provide six portable medical kits to two hospitals in Ghana that would enable the medical staff of the outreach clinic to carry them easily and give first aid to patients on the spot. Our understanding suggests that the system of an outreach clinic will continue to play a major role in the early detection of the disease. Washing machines are also important to sustain the sanitary condition of the hospitals in the region. These were all possible with the kind donations not just from the people of Kobe but also from anonymous donors. Another outcome of this was an international student volunteer mission to Ghana.

#### **Ghana Mission (September, 2000)**

Mr. Tomomi Seto, an undergraduate of KIU, along with Mr. Kazuyuki Fukunishi, a graduate of KIU and currently a Ph.d. student at the University of Wales at Aberystwyth, visited Ghana as a KIU International Volunteer Mission. The university has been sending students to various regions of Asia under its international service learning programs. Ghana Mission 2000 marked the very first time that the university had officially sent its students to the African continent. The aim of their mission was to deliver our goodwill in the form of portable medical kits and washing machines and to conduct fieldwork to gather our own information on BU children.

#### **International Symposium 2000 (20 October 2000)**

The biggest and foremost event for our advocacy efforts was KIU international symposium 2000. As was done in 1999, four distinguished guest speakers were invited. The key-note speaker for the symposium was Dr. Wayne M. Meyers (Chief, US Armed Forces Institute of Pathology) who provided a pathological insight on the disease to the 300-strong audience, while Dr. Asiedu Kingsley (WHO Coordinator, Buruli ulcer Initiative) gave a brief report on WHO's activities on BU. A shocking report came from Dr. Ernestina Mensah-Quainoo (District Director of Health Services, Ministry of Health, Ghana) whose experience in her district clinic made a deep impact on the audience. Mr. Koji Kawai (Director, JICA Hyogo International Center) provided a broader vision on what we can do to help people in need. Of course, we could not have had such a success symposium without the coordination of Dr. Goro Asano (President, Nippon Medical School) and Dr. Yukiko Fukunishi (Director-General, National Sanatorium Matsuoka Hoyoen). They have been the guiding lights for our activities. Dr. Milanga Mwanatanbwe of the Nippon Medical School has been serving the Project as a liaison to African embassies in Japan since 1999.

The success of International Symposium 2000 is also due to the patrons of the event. Five African embassies in Japan gracefully expressed their support for our activities. In addition to embassies of Burkina Faso, Republic of Ghana, Democratic Republic of Congo, those of the Republic of Côte d'Ivoire and the Republic of Madagascar came forward to support our actions in Japan. Furthermore, four ambassadors attended the symposium.

Strongest support in Japan came from the Provincial Government of Hyogo and the City of Kobe that are still struggling with the aftermath of the immense earthquake. From the media, we recognize Nippon Hoso Kyokai (NHK), Kobe Office, the Mainichi Newspapers Co., Osaka Headquarter, The Asahi Newspapers Co., Osaka Headquarter, The Kobe Newspaper Co. Headquarter, The Yomiuri Newspapers Co. Osaka Headquarter, and Sun Television. We are all grateful for their kind and sincere support.

#### *New Developments*

In addition, there were new developments in the Project's activities. A subject on ODA and International Cooperation with Practicum in volunteer actions was introduced to the KIU academic curriculum. Students were given a series of lectures on ODA and International Cooperation. They were also required to participate in volunteer activities outside class.

The most unique development was our sponsoring of a charity music concert for BU children. On 7 February 2001, Trio Primabella (violin: Masko Kida, Cello: Jiro Tanaka, and piano: Tomoe Kuroki) and 14 year-old violinist Aya Shimozu played magnificent music for the cause of saving children from BU at the Kobe Culture Hall. The concert drew audiences totalling over 680 people and Trio Primabella held the audience in a trance.

#### *T-shirts for Assisting Advocacy Activities in Endemic Regions*

Before leaving Japan for this meeting, we sent 200 T-shirts to WHO of which 100 were written "Seek Treatment Early" in English and the other 100 in French. By wearing T-shirts, we are hoping that medical staff and others may promote awareness of the existence of the disease among people of endemic regions. We recognize that 200 T-shirts may not be enough, but we believe that this is one way of showing our appreciation to WHO's continuing fight against Buruli ulcer. As for the T-shirts, I trust there will be more to come in near future.



*Dr Edouard Yao, MAP International, West Africa, Abidjan, Côte d'Ivoire*

### **I. The origins of MAP International**

MAP International (Medical Assistance Programme) is a religious nongovernmental organization that was founded in the United States of America in 1954, within the Christian medical society. In 1965 it left the CMS and became a separate nongovernmental organization.

### **II. Mission**

The mission of MAP International is to provide for the integral health of people living in poor communities in the world, without discrimination of race, ethnicity, sex or religion. Comprehensive or total health is defined by MAP International as the ability of individuals, families and communities to work together to provide a sustainable basis for their physical, social, psychological, economic, environmental and spiritual well-being.

### **III. Areas of intervention of MAP International**

1. Disease prevention and eradication.
2. Promotion of community health development.
3. Provision of essential drugs and teaching people to use drugs rationally.

### **IV. Main strategy**

Partnership with all those working in health and related sectors, at all levels, using a holistic approach.

Offices throughout the world:

- Latin America: Quito, Ecuador
- Eastern and Southern Africa: Nairobi, Kenya
- West and Central Africa: Abidjan, Côte d'Ivoire
- North America: Brunswick, Georgia.

Programmes in West and Central Africa

- Eradication of guinea worm
- HIV/AIDS;
- Community health (training, promotion, operational research)
- Comprehensive health
- Essential drugs and education in their use.

## **V. Concern for endemic Buruli ulcer**

The urgency of the situation

The desire of the national programme to extend its intervention

The potential contribution

- strategic position
- 45 years experience in:
  - essential drugs and biomedical equipment
  - comprehensive health
  - community approach

## **VI. Conclusion**

MAP was glad to be invited to this consultation, which allows it to learn more about the disease and control, and about the areas in which its expertise could be of use. The modest contribution we can make is at the disposal of WHO and all partners.

## Sasakawa Memorial Health Foundation

*Prof. Kenzo Kiikuni & Mrs Kay Yamaguchi, Sasakawa Memorial Health Foundation, Tokyo, Japan*

Dear Colleagues, Ladies and Gentlemen:

As you already know, The Nippon Foundation responded positively to the call by WHO (the then DG, Dr. Hiroshi Nakajima) to launch a global initiative to combat a newly spreading Buruli Ulcer in 1998. Within a few months this developed into the establishment of the Global Buruli Ulcer Initiative which is now in its fourth year. As I reported in this meeting last year, Sasakawa Memorial Health Foundation is playing the part of coordinating the collaboration between The Nippon Foundation and WHO following the example of the leprosy elimination programme.

The Nippon Foundation's initial interest in Buruli ulcer was sparked from personal observations by its chairperson Ms. Ayako Sono, as she traveled in some countries of Western Africa in the mid 1990s. She saw many young people suffer from devastating skin ulcers that often cause a lifelong damage and asked if there was any cure. Just as the concern for the elimination of leprosy grew out of the personal experience of the founder of the Foundation, Mr. Ryoichi Sasakawa, personal commitments can sometimes become a driving force for actions. Unfortunately, a definite cure for Buruli ulcer has not yet been identified, but awareness among the endemic countries as well as in international health and medical community has increased greatly and the world is better informed about the disease. The members here today and the WHO Buruli ulcer team can be proud of this result.

The major role of The Nippon Foundation in this initiative is to create a platform for various partners, including political and public health leaders of countries, international agencies and international and national NGOs to effectively plan and develop collaboration. The Nippon Foundation's contribution should be a catalyst to enable exchange and joint action to produce speedy results to benefit the affected population.

Scientific breakthroughs, improved field treatment and intensified public awareness about the disease are the major challenges that we face today. Patients of Buruli ulcer are often found among the vulnerable population of countries. For them, access to health and other services is often very limited. Buruli ulcer is known to attack children who are a particularly vulnerable section of population. The Nippon Foundation's interests lie in responding to the basic health needs of people. Therefore, The Nippon Foundation will continue funding the WHO Global Buruli Ulcer Initiative. The amount they have provided amounts to 1.1 million US dollars over four years, including 2001.

In addition to offering a platform or being a catalyst for effective interventions, The Nippon Foundation is exploring possible intervention in the surgical process with the application of cultured bio-skin. You will recall that I briefly mentioned this interest at the meeting last year. Since then we have continued our discussion with the medical team of the National Nagoya University Medical School of Japan. Some of you will recall that Professor M. Ueda's presentation to the surgical treatment subcommittee of BU, June 2000. At the June meeting a question was directed at Prof. Ueda by some of the participants. They said "the technique is attractive but when will it reach the countries burdened with Buruli ulcer?" We took this seriously. After the June meeting, Prof. Ueda briefly visited Ghana and saw some patients. Later, in September last year, Dr. Hata, who is with us today, and his colleague from the

plastic surgery department of Nagoya University Medical School, visited Amasaman hospital and Agroyesum hospital, in Ghana, to familiarize themselves with Buruli ulcer and its patients as well as to meet the surgeons in the Korle-Bu teaching hospital of the University of Ghana. A WHO-commissioned specialist is finalizing the protocol. The Nagoya University team, WHO and the Ministry of Health of Ghana are making every effort to fully satisfy the ethical standards that are required of this project. The Nippon Foundation and the Nagoya University team are aware that this is not a solution to Buruli ulcer but it is hoped that if it is proved that this can be applied to Buruli ulcer, it will, at least, bring about improvements in surgical treatment, which will lead to a better quality of life for patients. Applying cultured allogenic skin is an intermediate process. Whether the project will reach the ultimate stage of autologous bio-skin is not clear at the moment. The technology already exists and there are abundant examples of successful results in Japan and elsewhere. If this brings better results for Buruli ulcer patients, there should be a way. Every precaution will be taken ethically and medically.

Today at this meeting, I am quite impressed to see so many partners, new and old, joining in the Buruli ulcer initiative. I congratulate WHO and its Buruli ulcer team for successfully intimating the prospective partners. The ball is in the court of the partners. Let us respond to the call of the field, whether by case finding, treatment, public awareness, health education, training, drug trial or surgical intervention. Let us send the ball back with positive actions before we meet next in March 2002.

Thank you.

## Management of Buruli ulcer (BU) in precarious area

*Drs Vincent Stoffel et Benoît Barthelmé, Projet Humanitaire Afrique Nord Sud (PHANS), France*

The Projet Humanitaire Afrique Nord Sud (PHANS), a French NGO, has intervened in the rural district of Bonou, Department of Oueme, Benin, since 1998

Rural district Bonou: 32 000 inhabitants  
Increase in population: 3% per year  
Under 15 years: 50%  
One doctor, five health centres  
No water conveying, no electric current

### I. BU: a great public health problem

BU is a disease caused by *mycobacterium ulcerans*, an acid-fast bacillus related to those of tuberculosis and leprosy. It usually begins by nonulcerative skin lesions (nodule, plaque or oedema) which, if left untreated, lead to massive skin ulcerations  
Non superinfected BU is a painless disease.

#### Photos

*Nonulcerative: oedema*

*Nonulcerative: plaque*

*Ulcerative: the previous plaque, seven days later*

*Ulcerative: a typical lesion with, by the destruction of the fat panniculus, undetermined edges*

*Development: scars without sequelae, retractile scars, deformities, amputation of limbs or loss of organs (eye, genitalia, breast)*

### II. Burden of BU

- In the Bonou district (Benin), the number of cases exceeds that of tuberculosis and leprosy
- 70% of the BU affect the children under the age of 15 years
- The economical burden is important

#### Photos, illustrations

*Economical burden of BU: the vicious circle*

*The Atchonsa's health centre in 1998*

*The new Atchonsa's health centre since 1999 (no pipe-borne, no electric current)*

*The health note-book (memory of consulting people, tool of communication between health personnel)*

Without laboratory, history and physical examination are often sufficient, in endemic area, to make a reasonably accurate diagnosis

All the techniques have to be adapted to the local situation: it is the guarantee of their lasting success

2.1 Water is filtered through ceramic cartridge and treated with Ag ions. Sterilization of the instruments in a self-cooker on an oil-stove with a quality check by strip

2.2 Burning of medical wastes to master the dirty circuit

2.3 Techniques of anaesthetic :

- Local or regional with lidocaine
- General with ketamine in :
  - Intra-venous
  - Intra-muscular
  - Intra-rectal in children

2.4 Technique of general anaesthetic with ketamine in intra-rectal in children

### **III. Surgery is often the treatment of choice in BU**

Incision of an abscess (superinfected BU)

Excision of a non superinfected lesion with primary closure (rarely)

Excision of a non superinfected lesion with skin graft at the first go or in a second time (often)

Referral for amputation (unfortunately)

#### Photos

*Incision of an abscess (superinfected BU)*

*Excision of a non superinfected lesion*

*Skin graft in a second time (often)*

*Referral for amputation (unfortunately)*

3.1 The perioperative care is as important as the technical act:

- Antitetanic vaccination
- Chloroquine
- Comorbidity treatment
- Wound dressings ...

#### Photos

*The perioperative care is as important as the technical act*

*Results after skin graft (one year later)*

3.2 The curative secondary prevention is insufficient without :

- Inhabitants information
- Early detection of BU (targetting of pupils)
- Tertiary prevention in order to decrease the prevalence of disadvantage
- Health personnel training « in loco »

Don't ever forget that, in Africa, the disease is two-dimensional through:

- its meaning explained by the traditional therapist
- its cause explained by the doctor

Photo – Don't ever forget that, in Africa, the disease is two-dimensional

Ahmadou kourouma wrote in « Allah n'est pas obligé »

Finally, the BU management in precarious area needs:

- The respect of the cultural difference
- The use of adapted techniques
- The detection of nonulcerative BU (i.e.c.+++)
- The training of the health personnel
- The assessment of the actions
- A partnership north-south



## **Support for the National Buruli ulcer control programme in Benin, Lalo, Lalo Prefecture, Mono Department**

*Médecins Sans Frontières, Luxembourg section*

In July 1998, MSF Luxembourg signed a three-year cooperation agreement with the Government of Benin, to help with Buruli ulcer control in the country and improve treatment of patients while contributing to awareness training and research.

On 14 December 1998, the Buruli ulcer diagnosis and treatment centre at Lalo in Mono département was opened, and the national Buruli ulcer control programme for Benin was launched.

The MSF Luxembourg project in Benin has three main areas, which are its principle objectives:

### **Treatment**

The Lalo diagnosis and treatment centre needs better reception and accommodation facilities in order to improve diagnosis and early treatment of patients suffering from the disease.

### **Prevention**

MSF Luxembourg is concerned with both health education for families affected and with awareness training for the population and health workers. The painless progression of this disease in its early stages poses problems for awareness training and early consultation. It has been shown that early diagnosis can considerably reduce the period of hospitalization.

### **Research**

The purpose is two-fold:

to help with research on the best way of organizing services for patients and treatment in particular, to participate in research to determine the vector of the disease and the best form of treatment.

The specific results achieved by MSF Luxembourg in three years of field work are as follows:

- **Treatment**

It should be noted that so far not enough is known about the disease, and that to date, surgery has been the only really effective way of stopping the progress of Buruli ulcer in patients. Surgery, of course, means difficult access to care due to cost, lack of medical staff and limited capacity to admit patients for the long periods needed.

In recent years, MSF Luxembourg has diagnosed 264 new cases and treated almost 400. In 2000 alone, 382 operations were conducted, 35% of them skin grafts, 35% excisions, and the others consisting of cleansing, curettage of the bone and mobilization under general anaesthetic.

- **Preventive and promotional activities:**

*Initial and in-service training:*

The MSF Luxembourg team has trained 12 physicians, 12 nurses, six health workers and 36 community workers, in addition to teachers and traditional practitioners, while giving further training to community workers and members of the Lalo Buruli ulcer team.

*Information, education and communication:*

An information, education and awareness training campaign for the population was carried out by health workers, community workers, teachers and rural radio.

- **Research:**

Samples were regularly sent to the Institute of Tropical Medicine in Antwerp for diagnostic confirmation; 81.8% of the results were positive.

A sociological survey of knowledge, attitudes and practices (KAP) was conducted, which confirmed that the population of Lalo was well aware of the disease and that traditions influenced the population's perception of Buruli ulcer.

### **Future activities**

- a BU prevalence survey in Mono;
- a survey on disseminated forms of the disease and forms with bone involvement,
- survey on BU treatment with traditional medicine;
- survey of the socio-economic impact of the disease.

The work done so far indicates that efforts should be concentrated on the following areas:

- Consolidating gains, which means keeping up the current impetus of activities, developing new research tools, further investigating the unanswered scientific questions and investing in staff training for the project;
- Developing operational research in the interests of early treatment of cases and quality comprehensive care;
- Better standardization of treatment protocols, management tools and follow-up for patients;
- Studying how to find external subsidies for treatment of patients., In most cases, neither patients nor the local health system can cover treatment costs.

In conclusion, this structural support project, in all its aspects, is a major challenge for MSF and for science, and it gives us the opportunity to make a valuable contribution to improving knowledge of the disease in the interest of better treatment for patients. As is the case with all tasks of this magnitude, it calls for belief, commitment, resources and synergies.

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