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Setting up a national reference laboratory for Buruli ulcer: the case of Benin

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Summary

OBJECTIVE To report the experience of Benin, where Buruli ulcer (BU) is endemic, in the implementation of diagnostic laboratory services.

METHODS AND RESULTS There has been a gradual introduction of biologic diagnostic activities for BU comprising (1) training of a laboratory technician in a highly experienced reference laboratory; (2) acquiring indispensable laboratory start-up materials; (3) progressive development of diagnostic laboratory activities; (4) regular external quality assessment with an experienced reference laboratory and (5) decentralization of activities to various clinical diagnostic and treatment centres for BU in Benin.

CONCLUSION Setting up a reference laboratory for BU is a continuous process, which necessitates motivated personnel and the cooperation of an experienced external reference laboratory.

keywords Buruli ulcer, reference laboratory, Benin

Introduction

Buruli ulcer (BU) is the most frequent mycobacterial infection in humans after tuberculosis (TB) and leprosy. It exists in at least 30 countries, and the number of cases increases annually, especially in West Africa (Janssens *et al.* 2005). In Benin, cases are mostly residents of the central and southern regions, who make up more than 70% of the general population of Benin (Aguiar & Steunou 1997; Portaels *et al.* 2001; Debacker *et al.* 2004; Johnson *et al.* 2005). Due to the diversity of clinical forms and the numerous differential diagnoses of this disease, microbiological confirmation is sometimes necessary (Portaels *et al.* 2001). Culture of *Mycobacterium ulcerans*, the aetiologic agent of BU, also provides an indispensable basis for research, for example on antibiotic resistance of *M. ulcerans*. Antibiotic therapy is an alternative to or an adjunct treatment to surgery (Etuaful *et al.* 2005; van der Werf *et al.* 2005).

As with TB, the existence of an institution at a national level to ensure coordination of microbiological activities maintains the diagnostic quality of the laboratory (Rieder *et al.* 1998). We report the experience of Benin, a West African country endemic for BU, in the implementation of diagnostic laboratory services for BU.

Milestones in the implementation of laboratory activities

Created in 1997, the Programme National de Lutte contre l'Ulcère de Buruli (PNLUB, National Programme for the Fight against Buruli ulcer), is part of the Ministry of Public Health responsible for all BU activities in Benin. Since 1990, the number of BU cases has gradually increased to more than 1000 diagnosed in 2005. BU patients are exclusively found in the southern half of the country and managed in five centres for diagnosis and treatment of BU (CBTUB, Centre de Diagnostic et de Traitement de l'Ulcère de Buruli). The National BU Programme has recently adopted the WHO strategy of giving antibiotics to all cases and surgery when required, in all CDTUBs. Generally swab samples are taken from cases with 'open lesions' and tissue specimens from cases requiring surgical management.

The reference laboratory for mycobacteria (LRM, Laboratoire de Référence des Mycobactéries) is an institution of the National Programme for the fight against TB. It is situated in the economic capital of the country at approximately 150 km from the farthest CDTUB. Before establishing BU diagnostic activities, the LRM was solely a reference laboratory for TB. The initial priority of the new

section of the LRM laboratory was directed towards bacteriological diagnosis of BU.

To accomplish this aim, in 2002, a laboratory technician skilled in TB diagnosis was trained for 8 weeks at the Laboratory of Mycobacteriology at the Institute of Tropical Medicine in Antwerp (ITM) in diagnostic techniques for BU (direct smear examination, culture and molecular biology techniques such as PCR) (Portaels *et al.* 2001), as well as the theory of specimen collection. BU laboratory diagnosis was implemented at the LRM immediately after refresher training of this technician at ITM. Upon his return from Antwerp, he in turn trained the other six technicians.

Apart from large equipment in use for TB diagnostics, which could also be used for BU operations, new equipment (laminar flow incubator, refrigerated centrifuge), smaller devices, consumables and reagents were bought and installed with financial assistance from ITM and Accion Sanitaria y Desarrollo Social (ANESVAD). When BU operations at LRM began, staff comprised seven technicians and a medical microbiologist as chief of staff at LRM. By September 2007, this had increased to 11 technicians and two medical microbiologists working either on TB or on BU.

After practical training at the ITM, the technician began direct smear examinations and culture of all patient samples sent to LRM from peripheral CDTUBs. Two tissue specimens per patient are sent in a transport medium from the largest treatment centre (Zagnanado). The other four centres submit only one specimen to LRM. No quality control system for swab specimens was in place during the study period. One of the two specimens from Zagnanado is sent to ITM, and both ITM and LRM directly examine and culture these samples for quality control (Portaels *et al.* 2001). For decontamination of specimens, both laboratories use the reverse Petroff method (decontamination by 1 N HCl neutralized by 1 N NaOH) and Löwenstein–Jensen medium (WHO 1999). Results are compared using the chi-square test.

The specimen transport medium used is a semi-solid Dubos medium with antibiotic supplement (PANTA, polymixin, amphotericin B, nalidixic acid, trimethoprim, azlocillin) (Portaels *et al.* 2001), which used to be prepared at ITM but is now made at LRM. This medium has the great advantage of not requiring any refrigeration, which offers some flexibility for the time between sample collection and analysis (Portaels *et al.* 2001). The medium is sent to the CDTUBs when requested. Every fortnight (not always adhered to) specimens are sent to LRM. From LRM, the specimens are sent (by express mail) to ITM as soon as possible. Specimens are kept at 4 °C until they are shipped.

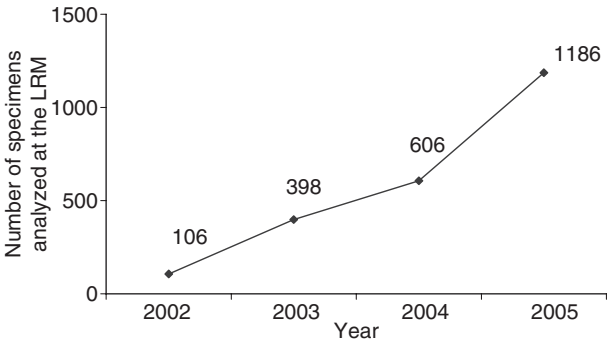

Figure 1 Evolution of the number of specimens analysed at the LRM.

After implementation of direct smear examination and culture for BU at LRM, PCR is to start to complete the series of diagnostic procedures for BU at LRM. During the preliminary phase (2002), laboratory investigations were only directed towards bacteriological diagnosis of BU, but 4 years on, operational research at LRM also aims to improve current diagnostic procedures.

Due to the rising number of BU cases in Benin, and concerns over accessibility of BU results, microscopy examination of slides was introduced at CDTUBs that have the technical capability. Their specimen collection procedures and transport conditions are being supervised, and their slides will be re-examined at LRM for quality control. Preparation of reagents remains centralized to LRM, as is the case for TB diagnostics.

Results

The number of samples received at LRM each year has been increasing steadily since 2002 (Figure 1), with a 10-fold increase to 1186 in 2005 (14 from Zinvie, 205 from Zagnanado, 682 from Pobe, 186 from Allada, 53 from Lalo and 46 from other regions to investigate the presence of BU in these regions).

Chi-square analysis revealed no significant difference between the results obtained by both laboratories in overall percentage of positive microscopy or culture (Table 1), although case-to-case comparison of test results differed importantly. In 2005, we also noted a significant difference in the levels of contamination (defined as the growth in the medium of bacteria other than mycobacteria), with a higher percentage of contamination at ITM ($P = 0.04$).

Discussion

The role of the laboratory in the fight against BU is becoming more and more important, partly because of the

Table 1 Direct examination and culture: a comparison of results obtained at the LRM and the ITM on specimens received from Zagnanado CDTUB

	Direct examination			Culture					
	Percentage of positivity			Percentage of positivity			Percentage of contamination		
	LRM	ITM	*P*	LRM	ITM	*P*	LRM	ITM	*P*
2003 (n = 274)	58.8	58.8	1	37.4	33.3	0.32	17.2	14.7	0.41
2004 (n = 278)	42.1	35.9	0.14	30.0	23.1	0.07	22.7	22.4	0.92
2005* (n = 157)	42.0	35.7	0.25	15.3	19.1	0.37	8.3	15.9	0.04

*Part of the specimens of 2005.

growing number of cases detected each year since WHO recognized BU as an emerging health problem (WHO 2000). Although experienced health workers in endemic areas usually can make an accurate clinical diagnosis of BU, microbiological confirmation is essential to determine the precise prevalence and incidence of BU in a given area; to identify new foci, especially where health workers lack experience with BU; to help manage the disease by surgical and/or antimycobacterial treatment and to differentiate between relapse and reinfection after treatment.

Thus, setting up a reference laboratory to coordinate the microbiological activities for BU at a national level is crucial. In Benin, the existence of a TB reference laboratory and its choice for BU greatly facilitated the implementation, as the techniques employed in both diseases are similar. However, this choice must be backed up by material and human resources. Furthermore, when an existing laboratory diversifies, a robust organization must be put in place to maintain excellence for both old and new activities.

As some procedures required in BU diagnosis are not necessary for TB diagnosis, these must be taken into consideration when installing the new laboratory alongside the TB reference laboratory. Short-term training of a microbiologist or a technician is indispensable and must be followed by immediately putting into practice the new skills. A system for regular external quality assessment should be set up simultaneously (PHL *et al.* 2002) in collaboration with an external reference laboratory.

No significant differences between overall results from both laboratories were observed, but there were on a case-to-case basis. This could partly be explained by heterogeneous presence of mycobacteria in BU lesions (Portaels F & Meyers W, unpublished data). The discrepancy might be overcome if two portions of one homogenized tissue sample were analysed instead of two tissue samples. Even though there were fewer positive cultures in 2005 in both laboratories, the difference was not significant. These lower rates could be linked to the quality of specimens sent to the laboratories, or to the fact that some patients had been on antibiotic therapy before specimens were collected (Etuaful *et al.* 2005). Drug treatment requires different specimen collections – swab for 'open lesions' and fine needle aspiration for others.

In 2005, a higher percentage of sample contamination was registered at ITM (P = 0.04). This could be due to the difference in the delay of performing cultures between both laboratories. To date, there is no study on the expiry date of the transport medium used for BU specimens. However, it could affect the quality of the results (Eddyani M *et al.*, unpublished data), since contamination rates seem to be higher if specimens are kept too long in the transport medium, probably caused by a drop in antibiotic activity over time.

As shown in Figure 1, the workload of the LRM has increased 10-fold within 4 years. Adequate material and human resources must be available for all newly introduced activities to ensure the daily functioning of the reference laboratory. Because the sensitivity for direct examination and culture for BU is low compared to PCR, diagnosis by IS *2404* PCR will soon be implemented at LRM (Stienstra *et al.* 2003; Phillips *et al.* 2005). Moreover, in a reference laboratory for mycobacteria, research activities are necessary. Although LRM had initially concentrated on routine diagnostics, operational research is now undertaken. Limited finances and workforce would definitely hamper these activities. In Benin, the use of resources from the National Programme for the Fight against Buruli ulcer and from external partners allowed us to circumvent these financial difficulties.

In conclusion, the implementation of a reference laboratory for BU is a continuously evolving process, which necessitates motivated personnel and the assistance of an experienced reference laboratory to aid smooth and accurate running. The implementation of the reference laboratory for BU has been a success thanks to the existing experience in the laboratory on diagnosis of TB, and its excellent collaboration with the mycobacteria laboratory

at ITM. This cooperation is based not only on an agreement of equal partnership but also on reciprocal responsibility, it was built in the spirit of The New Partnership for Africa's Development, as stipulated in the Cotonou Declaration (Bouvier 2004; Portaels 2004).

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