BURULI ULCEr DISEASE IN CAMEROON REDISCOVERED

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Abstract. To assess the magnitude of the Buruli ulcer (BU) problem in Cameroon, we conducted a cross-sectional survey in the Nyong River basin and identified on clinical grounds a total of 436 cases of active or inactive BU (202 and 234, respectively). Swab specimens were taken from 162 active cases with ulcerative lesions and in 135 of these (83.3%) the clinical diagnosis was confirmed by the IS2404 polymerase chain reaction. Most lesions (93%) were located on the extremities, with lower limbs being twice as commonly involved as upper limbs. The age of patients with active BU ranged from 2 to 90 years with a median age of 14.5 years. Vaccination with bacilli Calmette-Guérin appeared to protect children against more severe forms of BU with multiple lesions. We conclude that in Cameroon BU is endemic, at least in the study area, and that a comprehensive control program for BU in Cameroon is urgently needed.

INTRODUCTION

*Mycobacterium ulcerans*, first isolated in 1948 by MacCollum and others,1 causes subcutaneous tissue necrosis giving rise to chronic, progressive ulcers. Even when these heal spontaneously, the damage to the tissues frequently results in severe permanent disability. Commonly, these ulcers are referred to as Buruli ulcers (BUs) because of the great number of cases found in a very limited area of Uganda called Buruli during the 1950s.2 Foci of BU have since been described in different regions of the world. A characteristic of these is that they are associated with slow flowing or stagnant water. Since 1980, this long-standing endemic disease seems to be strongly recrudescent, particularly in central and west Africa.3−7 Where it is highly prevalent, BU constitutes a serious burden on rural populations and on health system resources. Surgery is the recommended treatment with extensive excision of necrotic tissue, followed by skin grafting. Some antituberculous agents and several other antimicrobials are active against *M. ulcerans in vitro*, but are not consistently efficacious for treatment.8 Recognizing BU as an important emerging disease, in 1998 the World Health Organization (WHO) launched the Global Buruli Ulcer Initiative.

The name BU suggests that the disease appears only as ulcers of the skin; however, the clinical evolution of the disease is quite variable. Most authorities divide lesions of BU in the skin to three forms.9 The first is the non-ulcerative form, which appears as subcutaneous nodules, limited indurated plaques, or widespread edematous indurations. These lesions are usually not painful but may itch. Systemic inflammatory responses are minimal or absent. The second is the ulcerative form, (Figure 1), which is characterized by ulcers of widely varying sizes with undermined edges that tend to extend, but are well defined. Advanced or long-standing indurated plaques and edematous forms may ulcerate, but such ulcers are ragged and poorly defined. Secondary infections may provoke pain. The third is the end stage or healing forms. Healing may take many months and sometimes is punctuated by local recurrences of active disease. Healing usually begins in the nondependent area of the ulcer. Lesions tend to resolve spontaneously but the resulting scars often lead to disabling or disfiguring sequelae (Figure 1). Bone lesions also exist in BU. The damaged bone can be situated just under the lesion (contact bone lesion) or remote from the ulcer (metastatic bone lesion).

Although the modes of transmission of BU are incompletely understood, it has been reported that patients have had antecedent trauma at the site where the lesion later occurred.10 Traumas may be as slight as a hypodermic injection or as serious as a gunshot or a landmine wound. Circumstantial evidence suggests that trauma introduces the etiologic agent into the skin and the subcutaneous tissue from the contaminated surface of the skin.11 The skin surface may be contaminated with *M. ulcerans* by direct contact with water or other material (e.g., plants or mud) from swamps or ponds. Insects (e.g., waterbugs) are capable of contaminating human skin with *M. ulcerans* either by contact or by biting.12 Infection with *M. ulcerans* does not seem to be a risk factor for BU, but it is a risk factor for development of severe (disseminated osteomyelitis) forms of BU.

In Cameroon, BU was described for the first time in 1969 in 47 cases, all originating from a well-circumscribed area in the valley of the Nyong River in the Center Province between the villages of Ayos and Akonolinga.13−15 After its first description, this focus did not arouse any particular interest among either health authorities or public health professionals. Recently, however, District Leprosy Officers noted large, necrotizing chronic skin ulcers resistant to therapy in several children and adults in the same region. Enquiries among the local health personnel and the local population showed that they knew the clinical appearance and development of BU lesions very well. They were referred to in the local dialect as “Atom”. The great majority of patients with “Atom”, which was supposed to be caused by malediction, were under treatment by traditional healers. These findings suggested that BU, already described in the late 1960s in this area, had continued to exist or had reappeared. In this report, we present the results of a cross-sectional survey performed in August 2001 assessing the magnitude of the BU problem in the Nyong River basin in the Center Province of Cameroon.

MATERIALS AND METHODS

Delimitation of the survey zone. Following the notification of suspect BU cases in Ayos and Akonolinga, a pilot study was done in these two places. Clinical history and clinical examination of the notified cases suggested BU lesions. After laboratory confirmation of some of these clinically diagnosed
cases, a cross-sectional survey was initiated. For this survey an area stretching along the Nyong River and some of its main tributaries, approximately 100 km long and 10–30 km wide (Figure 2) with a population of approximately 98,500, was initially identified as the endemic region. The area has a population density of less than 50 people per km² and many villages have fewer than 2,000 residents. The impoverished population of the area lives mainly by subsistence agriculture and informal commerce.

During a preparatory six-week period, assisted by the District Leprosy Officers, we asked health personnel and the local residents in all settlements and villages in the presumptive affected area systematically about known suspected cases of BU (Atom). Villages and settlements along the Nyong River and its tributaries served as starting points for the investigation. Subsequently, more remote villages along the different roads and tracks leading away from the river were visited until in at least two successively visited villages no suspect cases were reported.

**Case definition.** For the purpose of the survey, cases were defined clinically as active BU and inactive BU. Active BU was defined as 1) a nodule or plaque or edema consistent with BU, 2) chronic ulcers with subcutaneous tissue necrosis, undermined edges and peripheral induration, which were painless unless secondarily infected, with no constitutional symptoms, except when osteomyelitis was present, or 3) healing ulcers with a history and healing aspect consistent with BU. Inactive (healed) BU were defined as scar tissue on a former lesion deeply sunk into surrounding healthy skin (stellate aspect), with or without complications (e.g., contractures and/or ankylosis) leading to invalidity. A new case was defined as a patient with no previous history of or treatment of BU, and a recurrent case was defined as a patient presenting within one year from the end of the last treatment with a new lesion at the same or a different site.

**Data collection.** During a two-week survey, all settlements and villages identified in the first enquiry as having possible cases of BU were visited. Records were made of each patient after informed consent was given on a pre-established data form adapted from the Buruli ulcer form recommended by WHO. The study was reviewed and approved by the Ministry of Health in Cameroon. Information was collected on identity, residence, presence of other cases in the family or among people who had lived or were living in the same household, duration of lesions, and previous treatment. Possible risk factors and protective factors, such as distance of residence from the Nyong River, sources of water, and vaccination with bacilli Calmette-Guérin (BCG) were also recorded.

The patients were thoroughly examined and the lesions were recorded according to the data form. Lesions were defined as active or inactive, and the location of the lesion was marked on a drawing of a human body. The largest diameter of ulcerative lesions was measured. The presence of complications was also reported. A photograph of the affected part of the body was taken with the patient’s permission.

**Sample collection.** Swab specimens were taken from all cases with active BU excluding pre-ulcerative lesions and ulcers in an advanced stage of healing. Samples were taken with a cotton swab from under the undermined edges of the ulcers or areas of drainage. Smears were sent twice a week to the Centre Pasteur du Cameroun for Ziehl-Neelsen staining and microscopic examination to identify acid-fast bacilli. Samples
for polymerase chain reaction (PCR) detection of *M. ulcerans* DNA were stored in a cool box and maintained at 4°C until they were sent to the Institute of Tropical Medicine in Antwerp and/or to the Swiss Tropical Institute in Basel.

**IS2404 PCR.** DNA was released from the dry swabs essentially as described previously using heat (15 minutes at 95°C) and alkaline lysis (0.2% sodium dodecyl sulfate, 0.05 M NaOH). Additional proteinase K digestion and vortexing with glass beads was found to improve the release of DNA. After extraction and precipitation of total DNA, IS2404 PCR amplification was done for 35 cycles essentially as described previously using HotStart DNA Polymerase (Qiagen, Basel, Switzerland). Inhibition of the PCR was overcome in a number of cases by repeating the PCR using a 1:10 dilution of the template solution. The three-room PCR principal was applied and negative controls were included to avoid or detect potential cross-contaminations. Inhibition of the PCR was overcome in a number of cases by repeating the PCR using a 1:10 dilution of the template solution. The size of the amplification products was verified further by Southern blotting using an IS2404 probe.

**Statistical analysis.** Data were collected and analyzed using the Epi-Info software program (version 6.04fr, Centers for Disease Control and Prevention, Atlanta, GA). The chi-square test was used to elicit associations between dichotomous variables. Continuous variables were analyzed using the Student’s *t*-test or analysis of variance as appropriate. Two-tailed tests were used, with *P* < 0.05 considered significant.

**RESULTS**

In response to recent reports of District Leprosy Officers on patients with large, necrotizing chronic skin ulcers in the area of Cameroon where BU had been described in 1969, a pilot study was conducted. In this study, our clinical examination identified 20 patients with lesions highly suggestive of BU. Seven of 10 analyzed swabs from the undermined edges of the lesions proved to be positive for the presence of *M. ulcerans* DNA in the diagnostic IS2404 PCR assay, and Ziehl-
Neelsen staining confirmed the presence of acid-fast bacilli in four of them. All patients were living in villages along the Nyong River in the health districts of Ayos and Akonolinga.

A subsequent cross-sectional survey performed in August 2001 identified on clinical grounds 436 BU cases in 83 different villages and settlements. Of these 436 cases 202 (46%) presented with active lesions while 234 (54%) were inactive cases. Twenty-five of 202 active cases (12.4%) were recurrent cases. While the overall prevalence of active and inactive BU in the surveyed area was 0.44%, the highest prevalence for active cases found in a particular settlement was 8%. Disease prevalence for active and/or inactive cases was higher in villages closer to the Nyong River (Figure 2). It was principally the rural, impoverished part of the population with limited geographic and economic access to health facilities that was affected. Sixty-six (28.2%) of the 234 cases with healed ulcers had severe chronic functional disabilities as a result of contraction deformities. In one case, amputation of a finger was observed.

The age and sex distribution of the 202 cases with clinically diagnosed active BU are shown in Figure 3. A total of 115 (56.9%) of these were males and 87 (43.1%) were females, resulting in a male:female sex ratio of 1.3:1. The age of patients with active BU ranged from 2 to 90 years with a median age of 14.5 years. Fifty percent of the cases were less than 15 years of age, but no case was observed in children less than two years of age. The rate of illness did not differ significantly between the sexes or between different age groups.

A total of 243 active BU lesions were found in the 202 active cases since some patients presented with multiple active lesions. The distribution of lesions according to where they were located on the body is shown in Figure 4. Most lesions (93%) were located on the extremities, with the lower limbs being involved twice as often as the upper limbs. The distribution of lesions by site of localization on the body did not show any significant differences with respect to age or sex. The right or left distribution of lesions was similar both in the upper and the lower limbs ($P = 0.20$ and $P = 0.12$, respectively).

The mean duration of the lesions was 10.5 months (range = 1 week to 8 years, median = 5.5 months). The clinical spectrum of lesions observed was as follows: 187 (92.8%) of the cases presented with ulcers while 15 (7.4%) cases had non-ulcerative lesions (four cases with nodules, seven cases with plaques, and four cases with edema). The average diameter of ulcerative lesions was 10.3 cm (range = 1–107 cm). Bone involvement was clinically suspected in 30 (14.9%) of the 202 cases. Involvement of the eyes and genitalia was seen in two cases each. The extent of the lesion or its proximity to a joint (elbow, wrist, knee, ankle) made severe complications after spontaneous healing probable in 58 (28.7%) of the cases.

A BCG vaccination scar was observed in 105 (52.0%) of the 202 active cases. Multiple lesions defined as lesions present simultaneously on different parts of the body were observed more often in children ($\leq$15 years old) without a BCG scar compared with those with the scar (7 of 20 versus 1 of 30; $P < 0.01$) suggesting a protective effect of this vaccination against more severe forms of BU in this age group. No association was found for bone involvement in BU even after stratifying patients by age groups.

One hundred seventeen cases (57.9%) said that a family member or relative had previously had or currently had BU. In one family, three children had active BU at the same time. This also occurred in two children each in three other families. Relatives of patients with active BU, or members of their households who had had BU, were asked when the illness had...
occurred. Seventy-five percent reported that it was within the last three years, and 5% reported that it occurred more than 10 years ago. The different types of sources of (drinking) water (water tap, river, well, spring) were not significantly associated with the presence of BU. Patients probably went to the swamps for other domestic activities.

An analysis of the health-seeking behavior of the cases revealed that more than two-thirds (67.3%) of them were exclusively under treatment given by traditional healers and less than 10% of them were being treated exclusively by trained health personnel.

Samples for laboratory confirmation of the presence of *M. ulcerans* in the lesions were taken from 164 (81.1%) of the 202 clinically diagnosed active cases. Samples could not be taken from the other 38 cases either because they presented with non-ulcerative lesions such as nodules, plaques, or edema (n = 15) or because they presented with ulcers in an advanced stage of healing (n = 23). Direct microscopic examination of the 164 samples stained by the Ziehl-Neelsen technique revealed acid-fast bacilli in 38 (23.2%) cases. A total of 162 samples were analyzed by the *IS2404* PCR technique (two PCR samples were lost). One hundred thirty-five (83.3%) of these samples were positive for *M. ulcerans* DNA. In 34 cases, both Ziehl-Neelsen staining and the PCR were positive.

**DISCUSSION**

During the last decade, the existence of BU disease in Cameroon has occasionally been suspected, but none of the cases notified on the basis of clinical symptoms was confirmed by laboratory diagnosis. In our study, we identified on clinical grounds 436 persons with active (n = 202) or inactive (n = 234) BU in an endemic area situated in the Nyong River basin in the Center Province of Cameroon, where *M. ulcerans* disease was described for the first time more than 30 years ago. *Mycobacterium ulcerans* DNA was identified by the diagnostic *IS2404* PCR assay in 135 (83.3%) of the 162 analyzed active cases with ulcerative lesions. However, confirmation by two independent laboratory methods as required according to WHO guidelines to positively diagnose BU was made in only 34 (17%) of these patients. Because of logistical reasons, we had to stop systematically obtaining biopsy specimens. If we had been able to continue obtaining these specimens, more extensive analyses by culture of *M. ulcerans*, staining for acid-fast bacilli, PCR analysis, and histopathologic examination of excisional biopsy specimens would probably have yielded more double-positive laboratory results.

The differential diagnosis of the clinically identified 15 pre-ulcerative lesions (i.e., four cases with nodules, seven with plaques, and four with edema) was most problematic since it had to be done without any laboratory reconfirmation. All cases with inactive (healed) BU also had to be diagnosed exclusively clinically. Thus, diagnostic misclassification may have overestimated the overall prevalence of BU. Conversely, it is generally admitted that the clinical diagnosis of ulcerating BU by an experienced clinician is relatively straightforward in a known endemic area. Other common chronic ulcerative lesions encountered in Cameroon (tropical and venous ulcers, ulcers due to burns) can be differentiated from BU by their clinical aspects and case history. We therefore included all the 202 clinically diagnosed cases with active lesions in the analyses of the clinical presentation and of possible risk and protective factors.

If one supposes that clinical diagnosis together with partial confirmation by laboratory procedures was mostly correct, we may ask, alternatively, whether it was likely that we missed a substantial number of cases. The delimitation of the survey area was empirical, depending substantially on the ability of the local population to identify the disease. However, the population and peripheral local health personal appeared to identify BU even in its early, pre-ulcerative forms surprisingly well. Conversely, we may have missed cases because presentation was voluntary and this could have resulted in a selection bias. Therefore, the results of the survey may rather have underestimated, rather than overestimated, prevalence. On various occasions, we even noticed traditional healers forbidding their patients to present themselves to the survey team. People with inactive BU were not included in the analysis of the clinical presentation and possible risk and protective factors for two reasons. First, we assumed that the presentation of cases on a voluntary basis would induce a selection bias towards active cases. Second, case histories from people with lesions were likely to be less accurate when the disease was acute some years ago.

The endemic area had characteristics similar to those found in foci in other African countries. The clinical characteristics of active cases notified resemble what has been found in similar situations. The endemic area is located in the swampy banks along the slow-flowing Nyong River and some of its tributaries, and is isolated. The frequency of identified cases decreased with the distance to the river. Similar to findings in Côte d’Ivoire, more than half of the children were children ≤15 years old, with the highest rate of infection in those 10–14 years old. No preponderance of women in the group of adult (>15 years old) patients was found, as reported in earlier descriptions of this focus and in other studies. The ulcerative forms predominated in prevalent active BU cases; our finding of 92.8% is consistent with the 89.5% or 96.4% found in prevalence studies in Côte d’Ivoire and in Benin, respectively.

However, in a more recent study performed with 1,700 patients from Benin (from 1997 to 2001), there was no statistically significant difference between the percentages of ulcerated and non-ulcerated forms. The increasing number of non-ulcerated forms detected from 1997 to 2001 is a result of increasing activity of public health programs that raised awareness of BU clinical forms (Debacker M and others, unpublished data).

Our study showed a relatively high frequency (14.9%) of bone involvement, although similar frequencies have been reported in other studies. This high frequency may have been due to a selection bias resulting from our case-finding method and use of clinical diagnosis. Lesions on the limbs were predominant. In the first, restricted case series of the Nyong River focus, upper limbs were predominantly affected, but our findings confirm the general finding of lower limbs being most affected. Even after stratification for sex and age, no unequal right-left distribution of lesions on limbs was found, as reported in some other studies. Similarly, in a recent study in Benin, there were no significant differences between the number of lesions on the right or left limbs (Debacker M and others, unpublished data).

In 1969, the Uganda Buruli Group demonstrated in a controlled trial that BCG vaccination conferred partial protection
against BU for at least six months.\textsuperscript{20} This finding was confirmed by Smith and others in 1976.\textsuperscript{21} In Benin, BCG vaccination appeared to partially protect children less than 15 years of age with confirmed BU against the osseous forms of the disease.\textsuperscript{22} In our study population, children not vaccinated with BCG appeared to have a significantly higher risk of multiple lesions, confirming the partial protective effect of BCG vaccination against severe forms of BU in children.

Buruli ulcer tends to affect several members of the same family, which was previously observed in early studies of the disease in Uganda.\textsuperscript{5} Reported figures of the proportion of households with several BU cases are scarce, varying between 0% and 5%.\textsuperscript{13,23} Our figure of approximately 58% of a history of cases in the family or among people living in the same compound appears high. However, it is not comparable with figures reported in other studies because our questionnaire did not differentiate between other family members currently with BU and family members known to have had BU in the past. The data suggest that within an endemic area, scattered disease foci of various importance subsist over time.

Even with only partial laboratory reconfirmation, we conclude on the basis of clinical and epidemiologic evidence that the area surveyed constitutes a focus of emergent endemic BU of considerable magnitude. The accumulation of family cases during the past three years suggests that BU has recently re-emerged on a larger scale in the survey area. However, this focus has probably existed without interruption, especially if one considers the distribution of active and inactive cases without major transposition of the different individual foci, for more than 30 years in a well-circumscribed area without triggering off any intervention by health authorities. Further support was given to the idea that the disease had never really disappeared from the area by the fact that it was evidently well known and described by the local population.

The preference of patients for traditional medicine reflects a situation in which the health system has had nothing to offer to assist these patients and, consequently, where the population has lost confidence in the ability of the health system to provide assistance. Currently, surgery is the only proven effective treatment of M. ulcerans disease.\textsuperscript{9} Limiting factors include inadequate surgical facilities, the need for a prolonged stay in the hospital, and high treatment costs. If one estimates that the mean duration of the disease without medical intervention is one year, the annual incidence could be estimated to be 100–150 new cases of BU in an area largely identical with the survey area. With more than 25% of these patients being at high risk of developing severe long-term disabilities, a comprehensive public health intervention by health authorities and the health districts concerned is urgently needed. Control strategies promoted by the Global Buruli Ulcer Initiative include strengthening of the health care capacity in BU-endemic areas by upgrading surgical facilities, health education, and staff training in the communities to promote early detection and rapid referral, development of motivational strategies, and rehabilitation of those already deformed by this disease.\textsuperscript{8}

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