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BURULI ULCER

Mycobacterium ulcerans infection

Edited by

Dr Kingsley Asiedu
Dr Robert Scherpbier
Dr Mario Raviglione

World Health Organization

Global Buruli Ulcer Initiative

Buruli ulcer commonly affects poor people who live near rivers or wetlands.
(Photo: Augustin Guedenon)

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Illness and death from many infectious diseases can be avoided at an affordable cost. Buruli ulcer is one such disease. Yet, over the past few years, this disease has spread to new populations, causing a serious burden in an increasing number of countries and communities.

Since 1998, the WHO Global Buruli Ulcer Initiative has been providing a policy, research and support framework for Buruli ulcer control. We have shown that through early detection and early treatment we can avoid the serious consequences of the disease at an affordable cost. By forging partnerships with other communicable disease control programmes, we aim at utilising existing resources efficiently and strengthening health services in order to reach Buruli ulcer patients early with effective interventions.

The existing information on Buruli ulcer at a global level is summarized in this monograph. Those who have received this report have a unique responsibility, as they are leaders in society who can make a substantial contribution to the prevention and control of this increasingly common disease.

Dr Gro Harlem Brundtland
Director-General
World Health Organization

Executive Summary

The purpose of this book is to provide an overview of Buruli ulcer (*Mycobacterium ulcerans* infection) for the medical and scientific communities and the general public alike.

In recent years, increasing numbers of cases have been reported from West Africa (Benin, Burkina Faso, Côte d'Ivoire, Ghana, Guinea, Liberia, Togo), Australia, French Guyana and Papua New Guinea. Acknowledging the growing spread of this disease, the then Director-General of the World Health Organization (WHO), Dr Hiroshi Nakajima, announced during his visit to Côte d'Ivoire in 1997 that WHO would mobilize international efforts to deal with the disease. As a result, WHO established the Global Buruli Ulcer Initiative (GBUI) in early 1998 with the aim of: helping to recognize Buruli ulcer as a health and developmental problem and to advocate for support to endemic countries; seeking partnerships for control and research; and coordinating global control and research efforts.

What is Buruli ulcer?

It is a disease of the skin caused by *Mycobacterium ulcerans*, a bacterium related to those causing tuberculosis and leprosy. Buruli ulcer usually begins with a painless nodule or papule in the skin, which, is left untreated, leads to massive skin ulceration. The extremities are often involved. As the treatmentwith antibiotics has been disappointing to date, at present surgery is the treatment of choice.

Burden of the disease

In terms of number of cases, Buruli ulcer is probably the third most common mycobacterial disease in immunocompetent humans after tuberculosis and leprosy. However, due to the lack of precise data, the burden of the disease at global and national levels is not entirely known. In some areas of Benin and Côte d'Ivoire, at present, the number of cases may exceed those of tuberculosis and leprosy. In Côte d'Ivoire, over 5000 cases have been recorded since 1995. In some communities in this country, up to 16% of the population has been found to be affected by Buruli ulcer. In one community in Ghana, 22% of the people had the disease. A survey done in one of the endemic districts (population 106 560) in Ghana estimated the prevalence at 3.19 per 1000.

The disease most commonly affects impoverished inhabitants in remote rural areas with limited access to health care. It often occurs in close proximity to slow-flowing or stagnant bodies of waters. All age groups, but particularly children under 15 years of age, are affected. No racial or socioeconomic group is exempt. Interestingly, HIV infection is not a risk factor. As of today, the mode(s) of transmission is not entirely known. The organism probably enters the body through small breaks in the skin from contaminated soil, water or vegetation. Recent evidence suggests that in some cases insects may be involved in the transmission of the disease. There is also anecdotal evidence to support person-to-person transmission.

Control strategy

The current control strategy promoted by GBUI consists of:

- health education in the communities most affected;
- adoption of educational materials adapted to the needs of the countries;
- community-based surveillance through training of health care workers to increase early detection and rapid referral for treatment, in collaboration with disease control programmes such as those for leprosy and Guinea worm;
- strengthening the health care capacity in endemic areas by upgrading surgical facilities and improving laboratories; and
- rehabilitation of those already deformed by the disease.

The work of WHO on Buruli ulcer

Following a number of consultations with world experts, WHO has taken the leadership in coordinating Buruli ulcer control and research efforts worldwide. This is necessary if such efforts are to be effective and focused.

The following has happened since early 1998, when the WHO's GBUI was established.

1. A preliminary meeting of an ad hoc Task Force was held in February 1998. Later, an Advisory Committee with full membership of 16 experts was established. This Committee includes world authorities on Buruli ulcer and representatives from endemic countries. Most of the members of the Committee, and other selected experts, contributed to this monograph.
2. The first International Conference on Buruli Ulcer Control and Research was held in Yamoussoukro, Côte d'Ivoire, on 6–8 July 1998, and resulted in an increased awareness of the disease. At this Conference, the *Yamoussoukro Declaration on Buruli ulcer* was signed by three heads of state and the Director-General of WHO. The report on this Conference is shown in Annex 2.
3. Progress has been made in raising awareness of the significance of Buruli ulcer. However, more work needs to be done in this area, as the disease is still unknown to many. In this regard a newly established website (www.who.int/gtb-buruli) will assist in supplying this much needed information. The first WHO educational leaflets in English and in French, targeting community workers at district and village levels, have been printed and are now being distributed in endemic countries.
4. Country assessments in Benin, Côte d'Ivoire, Ghana and Togo were conducted between March and July 1998 with the aim of understanding the problem of Buruli ulcer and discussing the importance of this disease with the various

government authorities. This has energized those countries to make the necessary efforts to address the disease. As a result, focused programmes have been established in Benin, Côte d'Ivoire and Ghana.

5. Standard case definitions and forms for surveillance and clinical management of patients, and standard guidelines for treatment and referral of patients have been developed by the WHO Advisory Committee in consultation with other experts worldwide.
6. A WHO scientific working group, consisting of some 40 world experts on the disease, known as the International *Mycobacterium ulcerans* Study Team (IMuST), has been established in collaboration with Dr John Hayman of Box Hill Hospital, Australia. The IMuST seeks to develop control and research activities, and help coordinate the world's efforts against Buruli ulcer.
7. Collaborating centres will soon be established in some of the international research institutions to support research by facilitating exchange of materials and assisting in training activities.
8. Research in the following areas with potential impact on control of the disease has been identified as a priority by the WHO Advisory Group and IMuST:
   - operational steps in the implementation of adequate control measures;
   - mode(s) of transmission;
   - environmental changes that favour emergence of the disease;
   - surveys to determine the burden of the disease;
   - rapid methods of diagnosis, so that when effective drugs become available the presence of the infection can be determined before the disease appears; and
   - activities of known antimicrobial drugs on *M. ulcerans*, starting with animal models and continuing to clinical trials.

Country activities

One of the main objectives for Buruli ulcer control is to strengthen the health services in general, and surgical facilities in particular, so that patients needing surgical care have access to quality care. Appropriate training of health care professionals in areas such as surgery, physiotherapy, pathology, microbiology and epidemiology is needed. These will help build capacity in the endemic countries, which will go far beyond the treatment of Buruli ulcer patients.

Activities of nongovernmental organizations (NGOs) and other agencies

Advocacy by GBUI for support to endemic countries has yielded concrete results. NGOs such as ANESVAD, Spain; the Association Française Raoul Follereau, France; the Associazione Italiana Amici di Raoul Follereau, Italy; the Catriona Hargreaves Charitable Trust, London, UK; the Fondation Luxembourgeoise Raoul Follereau, Luxembourg; the Humanitarian Aid Relief Team, Provo, Utah, USA; Médecins Sans Frontières, Luxembourg; and the Sasakawa Memorial Health Foundation, Japan; are providing direct support to some endemic countries such as Benin, Côte d'Ivoire and Ghana. The Belgium Cooperation is supporting control activities in Benin. The Damien Foundation, Belgium, supports research activities on Buruli ulcer at the Institute of

Tropical Medicine in Antwerp, Belgium. The Japanese government, through its embassies in Côte d'Ivoire and Ghana, financed the contruction of a surgical block for the Saint Michael Health Center in Zoukougbeu and the construction of wards for the Amasaman Health Center, respectively. Pharmaciens Sans Frontières, Paris, France, provides equipment and supplies to the Saint Michael Health Center in Zoukougbeu.

Conclusions

1. Early detection and rapid surgical treatment are the best interventions currently available until other means of preventing or treating the disease are identified.
2. Strengthening the general health services in deprived areas is key to Buruli ulcer control. This consists in improving the health education of communities in endemic areas, in training health care workers in early recognition, and in upgrading surgical facilities for rapid treatment.
3. Research in areas that could provide better understanding on the mode of transmission, prevention and treatment is urgently needed.

Chapter 1

Introduction

Dr Kingsley Asiedu & Prof. Françoise Portaels

In December 1997, on the occasion of his visit to Côte d'Ivoire, the then Director-General of the World Health Organization (WHO), Dr Hiroshi Nakajima, announced the deployment of a coalition of international efforts against Buruli ulcer (*1*). As a result, an ad hoc Task Force on Buruli ulcer specially set up by the WHO met for the first time in February 1998 to review the current knowledge of the disease and propose a plan of action for the effective treatment and control of and research on Buruli ulcer. The recommendations of the Task Force and its proposed plan of work are set out in Annex 1.

Buruli ulcer has emerged in recent times as an increasingly important cause of human morbidity around the world, partly due to environmental changes. In Australia, the disease is commonly referred to as Bairnsdale ulcer. The name Buruli ulcer originated from the district of Buruli in Uganda, where the first large numbers of cases were reported in the late 1960s and early 1970s. In recent years, increasing numbers of cases have been reported from West Africa [(Benin (*2–4*), Burkina Faso (*5*), Côte d'Ivoire (*6,7*, Kanga JM, unpublished data, 1998), Ghana (*8–10*), Guinea (Sagno M, Portaels F, unpublished data, 1995), Liberia (*11,12*) and Togo (*13*, Tignokpa N, Priuli GB, unpublished data, 1998)] and from Australia (*14,15*). In addition to actual numbers of cases, there has also been increasing geographical spread of the disease within these countries.

Clinical features and treatment

Buruli ulcer starts as a painless, often itchy, nodule or papule in the skin, which is often ignored by the patient. Because of the indolent nature of the disease, there is substantial delay in seeking care. This nodule, if left untreated, often leads to massive skin ulceration followed by debilitating complications, including contracture deformities, amputation of limbs and loss of organs such as the eye, breast and genitalia. A few cases of death from sepsis, tetanus and haemorrhage have been reported. Increasing numbers of bone infections are also being reported and these could complicate the management of cases. These bone infections may be the results of direct spread from the overlying skin lesion or may support the hypothesis of a haematogenous spread. The destruction of the tissues is caused by a toxin produced by the organism, and the exact chemical nature of putative toxins has recently been determined (*16,17*). Surgery is the current treatment of choice, but adequate surgical facilities are rarely available in most endemic areas in the developing world. Consequently, hospitalization is usually prolonged, averaging 3 months per patient, but may be as long as 18 months or more. Unfortunately, treatment with antibiotics has been disappointing. Further research on drug efficacy is needed, especially in view of the problems of infections associated with surgical treatment.

Implications for society and the health services

With an increasing number of cases and the complications currently associated with the disease, the long-term socioeconomic impact of Buruli ulcer on the rural economy could be substantial (*10*). Because treatment is surgical, the disease could seriously undermine the efficient use of scarce health care resources in the endemic countries. Complicated cases require prolonged hospitalization and consume a large measure of resources compared with some other diseases. Clearly, early diagnosis and better treatment reduces some of the complications of the disease.

Historical overview

After tuberculosis and leprosy, Buruli ulcer is the third most common mycobacterial disease in immunocompetent humans (*13*). The causative organism was first described by MacCallum, who discovered acid-fast bacilli in a biopsy from a leg ulcer in a young child from Bairnsdale, Australia in 1940, and published the first clinical description of this new mycobacterial infection in 1948 (*18*). Before 1948, the disease was already known in Africa. Large ulcers, almost certainly caused by *M. ulcerans*, were described by Sir Robert Cook in 1897. During the years 1923 to 1935, Kleinschmidt, a missionary physician in north-east Congo, observed undermined skin lesions rich in acid-fast bacilli (*19*).

In Africa, the history of Buruli ulcer can be divided into two main periods: before 1980 and after 1980 (see Fig. 1). There were many important publications before 1980 on the disease in several African countries: Cameroon, the Democratic Republic of the Congo, Gabon, Ghana, Nigeria and Uganda. In the Central African Republic, Kenya, Sudan, and the United Republic of Tanzania, cases were suspected but never confirmed. The most significant contributions came from the Democratic Republic of the Congo and Uganda. The Uganda Buruli Group studied the clinicopathological and epidemiological aspects of the disease extensively, and opted for the term "Buruli ulcer" because large numbers of cases were first detected in the district of Buruli near lake Kyoga (*20*). The data were extensively described in several review articles (*21–23*). The information on Buruli ulcer in the Democratic Republic of Congo was summarized by Janssens in 1972 (*21*) and by Meyers in 1974 (*19*).

After 1980, new foci of Buruli ulcer emerged in West Africa. A dramatic increase in the incidence of the disease is now reported in several West African countries, especially in Benin, Côte d'Ivoire and Ghana. New foci were recently discovered in Angola (*24*), Burkina Faso (*5*), Guinea (Sagno, M, Portaels F, unpublished data, 1995) and Togo (*13*, Tignokpa N, Priuli GB, unpublished data, 1998).

The Yamoussoukro Conference and the future of Buruli ulcer control and research

In view of the growing spread and impact and the general lack of awareness of Buruli ulcer, WHO in collaboration with the Government of Côte d'Ivoire, the Sasakawa Memorial Health Foundation in Japan, the Association Française Raoul Follereau, the Damien Foundation, Belgium, and the Humanitarian Aid Relief Team, Provo, Utah, USA organized the first International Conference on Buruli Ulcer Control and Research in Yamoussoukro, Côte d'Ivoire in July 1998 (*25*). More information on the Conference is given in Annex 2.

The signing of *The Yamoussoukro Declaration* on Buruli Ulcer (see Annex 3) by the Presidents of Benin, Côte d'Ivoire, and Ghana and by the Director-General of WHO served to stimulate all the participants and to spawn hope that such proclamations will be followed by meaningful action. The declaration also served to back WHO's efforts to address the disease. The final resolution of the Conference (see Annex 4) calls on the endemic countries to provide free treatment to those afflicted by the disease, as is done for those suffering from tuberculosis and leprosy, and on the international community to assist endemic countries to deal with the disease.

Chapter 2

Epidemiology

Dr John Hayman & Dr Kingsley Asiedu

Environmental changes

In many areas, *M. ulcerans* infection has occurred only after significant environmental disturbance. In the original paper describing the disease, published in 1948, the first patients from the Bairnsdale district in Australia presented in 1939 (*18*). In December 1935, there had been the worst floods on record in the district, when all road and rail links had been cut and much property destroyed. In Uganda, Barker examined cases of *M. ulcerans* in the Busoga district on the east side of the Victoria Nile, north of Lake Victoria (*26*). Although cases were known in other parts of the country, there had been no known cases in that district before 1965. Barker postulated that the outbreak was related to unprecedented flooding of the lakes of Uganda between 1962 and 1964 as a result of heavy rainfall.

In Nigeria, infections have occurred among Caucasians living on the campus of the University of Ibadan after 1965 (*27*), when a small stream flowing through the campus was dammed to make an artificial lake. The first case reported in Côte d'Ivoire was a 7-year-old French boy who lived with his parents beside Lake Kossou (*28*), an artificial lake in the centre of the country. In Liberia, cases have been reported in the north of the country following the introduction of a swamp rice field to replace an upland one (*11*). This agricultural change was accompanied by the construction of dams on the Mayor river to extend the wetlands. In Papua New Guinea, the infection occurs mainly near the Sepik and Kumusi rivers; in the latter areas the disease is known as the "Kumusi ulcer". The disease in Papua New Guinea spread after the flooding and devastation that followed the eruption of Mount Lamington in 1951. Reid described how older people living in the villages blamed the volcano for the disease (*29*).

The recent outbreak of the disease on Phillip Island, Victoria, Australia was temporally associated with the formation of a small swamp that backed up behind a newly constructed vehicle track (*14*). Improved drainage of this area was followed by a cessation of cases in the immediate vicinity of the marsh. The following year, cases continued to occur approximately one kilometer to the west of the swamp, and were centred around a golf course spray irrigation system that used a mixture of recycled sewage and groundwater. In retrospect, the two foci at Phillip Island were probably interlinked because some of the water that collected in the swamp the preceding year is likely to have originated on the golf course. A marked decline in cases followed modifications to this irrigation system. These observations suggested that *M. ulcerans* may be present in groundwater, that a nutrient-rich environment may favour its survival and growth, that *M. ulcerans* is able to colonize man-made reticulation systems and that it is likely to be spread by aerosol.

The epidemiology of Buruli ulcer is poorly understood. The source(s) of *M. ulcerans* in nature is becoming more clear from epidemiological data and from molecular biological findings. Because all major endemic foci are in wetlands of tropical or subtropical countries, environmental factors must play an essential role in the survival of the etiological agent. Focal outbreaks have followed flooding, human migration *(30)* and man-made topographical modifications such as dams and resorts. Deforestation and increased basic agricultural activities may have significantly contributed to the recent marked increases in the incidence of *M. ulcerans* infections, especially in West Africa, where the disease is rapidly emerging. In Benin, for example, the disease prevalence in areas with environmental changes is about 180 per 100 000 population, whereas in those without environmental changes it is about 20 per 100 000 (Portaels F, personal communication, 1998).

Geographical distribution

Buruli ulcer has been reported from at least 27 countries around the world (see Fig. 2 and Table 1), mostly in tropical areas. In several of these countries the disease is not considered to be a public health problem, hence the current distribution and the number of cases are not known. Possible reasons include: (a) that the distribution of the disease is often localized in certain parts of endemic countries; (b) that Buruli ulcer is a not a notifiable disease; and (c) that in most places where the disease occurs, patients receive care from private sources such as voluntary mission hospitals and traditional healers. Hence the existence of the disease may not come to the attention of the ministries of health.

Table 1. Regions and countries with reported cases of Buruli ulcer worldwide

Region	Countries
West Africa	Benin, Burkina Faso, Cameroon, Côte d'Ivoire, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, Togo
Other parts of Africa	Angola, Congo, Democratic Republic of Congo, Gabon, Sudan, Uganda
Western Pacific	Australia, Kiribati?, Papua New Guinea
Asia	China, India, Indonesia, Japan, Malaysia,
Americas	Bolivia, French Guiana, Mexico, Peru, Suriname

Figure 1. Map of Africa showing the distribution of Buruli ulcer before (dark blue) and after (light blue) 1980

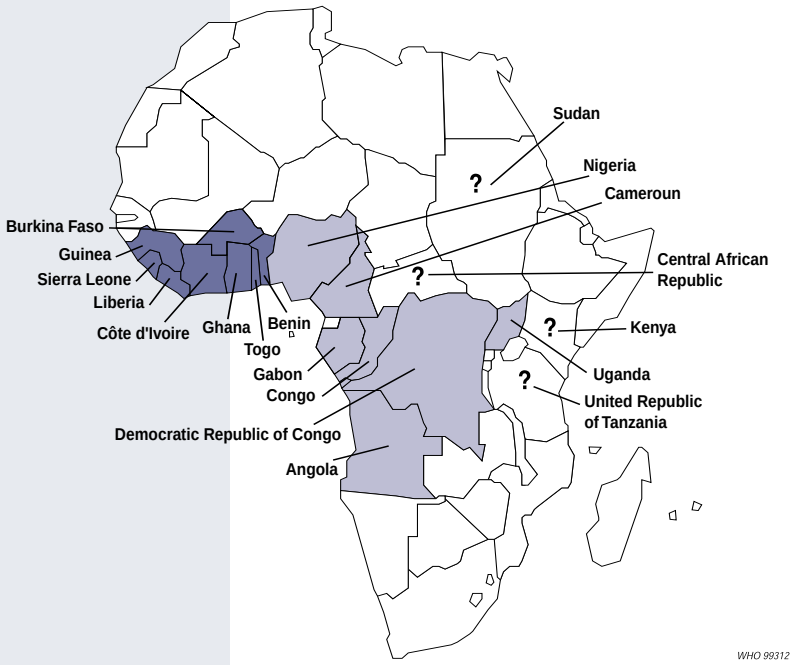
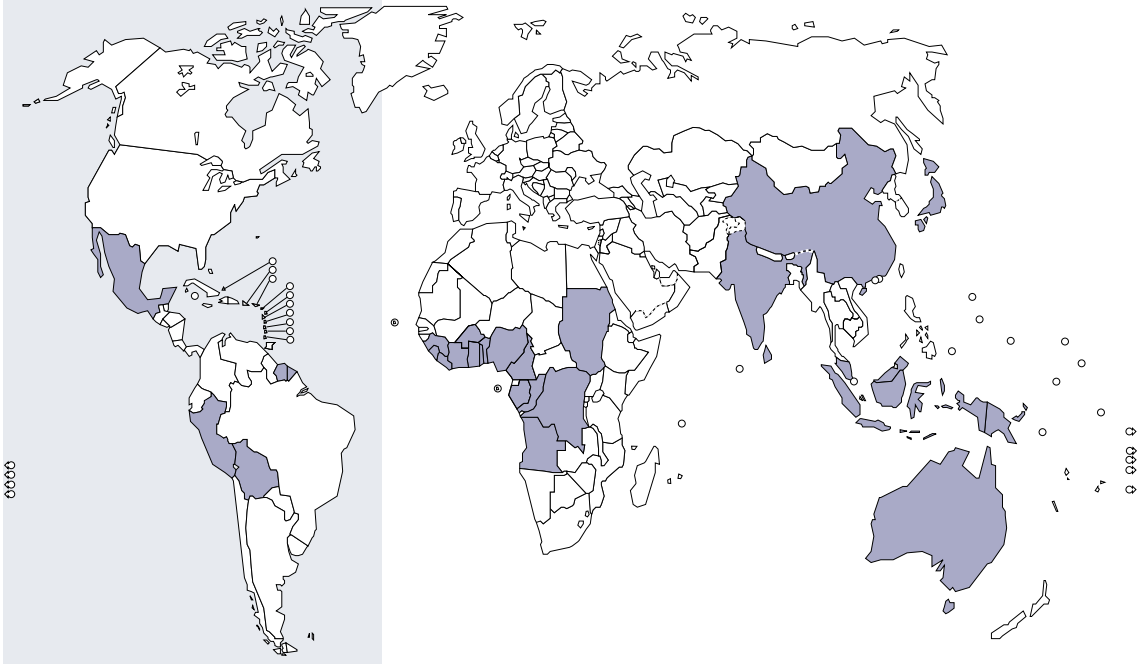


Figure 2. Map of the world showing the geographical distribution of Buruli ulcer



The designations employed and the presentation of material on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines represent approximate border lines for which there may not yet be full agreement.

Burden of the disease

The global burden of Buruli ulcer is not known. Even at country level, such information is scanty. Most reports on the disease are based on passive presentation by patients to a health facility. Given the difficult access to health care in endemic areas, as well as the social, economic and cultural circumstances of those most affected, these numbers could be just the tip of the iceberg. In a few communities where the prevalence has been estimated, however, the disease burden is high. Disease rates in Uganda have been estimated at 2–5% of the population (*31*). In Côte d'Ivoire, some villages have rates as high as 16% (*7*). In Ghana, a rate of 22% has been reported in a community (*32*). In Côte d'Ivoire, over 10 000 cases were reported between 1978 and 1997, with more than half of these reported between 1995 and 1997 (Kanga JM, unpublished data, 1998). In Benin, more than 2300 cases were recorded between 1988 and 1997 (Guedenon A, unpublished data, 1998). In Ghana, nearly 2000 cases were recorded between 1993 and 1997 (Brookman-Amisah E, unpublished data, 1998). Nearly all of these data come from passive case finding and maybe, therefore, an underestimate of the real burden.

Mode of transmission

The disease often occurs in close proximity to water bodies, but no specific activities that bring people into contact with water have been identified (i.e. fetching of water, fishing, washing, bathing, etc). The mode of transmission of Buruli ulcer is not entirely known. Recent evidence suggests that insects may be involved in the transmission of the infection (*33*). These insects are aquatic bugs belonging to the genus *Naucoris* (family Naucoridae) and *Diplonychus* (family Belostomatidae). Trauma is probably the most frequent means by which *M. ulcerans* is introduced into the skin from surface contamination. The initial trauma can be as slight as a hypodermic needle puncture or as severe as gunshot or exploding land mine wounds (*34*). Other studies have suggested aerosol spread but these are not proven (*14*). In Australia, animals such as koalas and opossums are naturally infected (*35*, Flood P, unpublished data, 1998). Epidemiological evidence has not clearly supported person-to-person transmission. However, Muelder & Nourou found that 10 out of 28 patients had relatives who had also had the disease, and cautioned against the dismissal of person-to-person transmission (*36*). Given the number of patients who shed large numbers of bacilli from their wounds and live in very close contact with relatives, more cases should have been observed. The cases reported by Muelder & Nourou could perhaps have been exposed to a common source of infection.

Age and sex of patients and site of the lesion

Buruli ulcer commonly affects poor people in remote rural areas with limited access to health care. The disease can affect all age groups, although children under the age of 15 years (range 2–14 years) are predominantly affected. There are no sex differences in the distribution of cases among children. Among adults, some studies have reported higher rates among women than males. No racial or socioeconomic group is exempt from the disease. Most ulcers occur on the extremities; lesions on the lower extremities are almost twice as common as those on the upper extremities. Ulcers on the head and trunk accounted for less than 8% of cases in one large series (*7*).

Co-infection of Buruli ulcer with other diseases

Human immunodeficiency virus (HIV)

A few isolated cases of dual infection by *M. ulcerans* and HIV have been documented: a 16-year-old Nigerian boy (*37*), and a pregnant woman (*38*) and three other patients in Zaire (*39*). In a study in Côte d'Ivoire, 20 Buruli ulcer patients tested for HIV were negative (*7*); and in study in Ghana, 2 out of 60 patients (3.3%) tested positive compared to 6.1% in the antenatal population over an 18-month period (*40*). Buruli ulcer was most common in children under 16 years of age and there were no HIV-positive cases in this age group. These reports so far indicate that HIV-infected people are not at increased risk for Buruli ulcer, and that HIV infection does not appear to affect the treatment outcome for Buruli ulcer (*10*).

Other mycobacterial diseases (tuberculosis and leprosy)

The associations of Buruli ulcer with tuberculosis and leprosy have not been extensively investigated. While, in general, there is no association between Buruli ulcer and leprosy, Meyers & Connor found six cases of dual infection with *M. ulcerans* and *M. leprae* among 1061 leprosy patients and 180 Buruli ulcer patients. It was noted that these six patients had tuberculoid and borderline forms of leprosy (*41*). There has been no report of co-infection with *M. ulcerans* and *M. tuberculosis*.

Chapter 3

Basic microbiology

Prof. Françoise Portaels

M. ulcerans belongs to a group of mycobacteria that are potentially pathogenic for humans or animals. These are sometimes called "opportunistic mycobacteria" or "occasional pathogens". Most species belonging to this group are found almost everywhere in nature, and may become pathogenic under special circumstances. Some of them have rarely (e.g. *M. malmoense*) or never (e.g. *M. ulcerans*) been isolated from the environment. The epidemiological profiles of the diseases they cause, however, suggest that they are present in nature (*23*).

Recently, *M. ulcerans* was detected by molecular biological techniques in water samples collected in Australia (*42, 43*) and in bugs collected from roots of aquatic plants in swamps in endemic regions of Benin and Ghana (*33*). *M. ulcerans* was, however, not recovered by culture from these environmental samples. Isolation of *M. ulcerans* in primary culture from clinical specimens is possible but not easy.

Isolation in primary culture

Several authors have discussed the difficulties of isolating *M. ulcerans* in primary culture from clinical specimens (*44*,*45*). Despite numerous attempts, *M. ulcerans* has never been cultivated from environmental samples, although a large variety of other mycobacterial species have been isolated (*43*). Several reasons may explain the difficulty or the inability to cultivate *M. ulcerans* from clinical or environmental specimens.

Sampling

Sampling is often inadequate. Clinical specimens should be collected from sites usually rich in bacilli, e.g. the necrotic base of the lesion and the undermined edges of the ulcer, including subcutaneous tissue. Environmental specimens should be collected from sites where *M. ulcerans* is suspected to be concentrated, e.g. by filtering organisms, and from sites where *M. ulcerans* is demonstrably best able to survive. In tropical regions, surface samples are subjected to high temperatures and ultraviolet light, which in the laboratory have been shown to affect the viability of the bacilli. In deeper sites, such as at the bottom of swamps, ultraviolet rays do not penetrate and the temperature is lower and more stable. Moreover, in deeper parts of swamps oxygen concentration is reduced: *in vitro*, microaerophilic conditions favour the multiplication of *M. ulcerans* (*46*).

Transportation to the laboratory

M. ulcerans grows optimally on conventional mycobacteriological media at 32 °C, and is very sensitive to higher temperature. One day at 41 °C kills more than 90% of the bacilli and, for some strains, one day at 37 °C also kills more than 90% of the bacilli (*33*). Meyers et al. also observed that growth at 32 °C was retarded after exposure to 37°C for one day, and was completely inhibited after exposure to 40 °C for 10 days (*47*). The temperature of transportation to the laboratory is therefore critical, especially

for specimens collected in tropical countries where the temperature may exceed 37 °C for long periods.

In many studies, primary cultures were set up days or weeks after collection of the specimens. Ideally, the specimens should be processed on the day of collection to obtain a maximum of positive primary cultures. When this is not feasible, specimens may be kept at +4 °C or in transport media. Freezing is not advisable because *M. ulcerans*, like other mycobacteria (e.g. *M. leprae, M. lepraemurium*), is highly sensitive to freezing–thawing cycles (*48*).

Transport media

Three transport media (S, P and P5) have been developed in the Mycobacteriology Unit of the Institute of Medicine, Antwerp, Belgium. Transport medium S is a selective Dubos medium supplemented with antibiotics, as described by Saxegaard for the isolation of *M. paratuberculosis* from the intestinal tissues of goats (*49*). Transport medium P is a Dubos medium supplemented with PANTA[a] as used for the isolation of *M. tuberculosis* in the BACTEC system. Culture rates from specimens transported in S or P media are identical (*50*). Given the preference of *M. ulcerans* for low oxygen concentrations, a new semi-solid transport medium (P5) was developed by the addition of 0.5% agar to transport medium P. The three transport media produce comparable results (about 40% positive primary cultures). Successful primary culture is not related to the period of elapsed time in the transport medium but is dependent on the number of viable acid-fast bacilli present in the inoculum. Transport medium P5 is, however, superior to S and P because positive cultures can be obtained even after 7 weeks of storage compared to 3 weeks with S and P. Some 90% of primary cultures are positive after less than 3 months' incubation at 32 °C. These results do not depend on the type of transport medium used.

Decontamination methods

M. ulcerans is susceptible to decontamination methods. All of the decontamination methods currently used for the isolation of *M. ulcerans* from clinical specimens (Petroff, NALC-NaOH) or for the isolation of mycobacteria from environmental specimens (Petroff, oxalic acid) (*51*) have a detrimental impact on the viability of *M. ulcerans* (*52*). This explains, at least in part, the difficulty often experienced in cultivating this organism from clinical specimens and the failure to cultivate *M. ulcerans* from environmental specimens that, by definition, are heavily contaminated with other microorganisms and thus require drastic methods for decontamination. Moreover, it is likely that environmental samples are less rich in bacilli than clinical specimens. Indeed, smears from some clinical specimens stained by the Ziehl-Neelsen method usually reveal clumps of acid-fast bacilli, (4+ according to the scale of the American Thoracic Society) (*53*), while environmental specimens are infrequently smear-positive (Portaels F, unpublished data, 1998). The application of drastic methods on scanty positive specimens may therefore be detrimental to the successful culture of *M. ulcerans*.

[a] *A mixture of five antibiotics (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin).*

Culture media

Löwenstein-Jensen medium is the most appropriate of the common conventional solid media for mycobacterial cultivation. Ogawa and Middlebrook media are less appropriate. The optimal pH for growth of *M. ulcerans* lies between 5.4 and 7.4 (*54*).

Incubation conditions

Incubation at 32 °C is essential for the isolation of *M. ulcerans* in primary culture. Another important factor is the oxygen concentration. It has recently been demonstrated that reduced oxygen concentration enhances the growth of *M. ulcerans,* suggesting a preference of this organism for microaerophilic environments (*46*).

In vitro characteristics of M. ulcerans

M. ulcerans is a slow-growing mycobacterium. Its generation time is about 20 hours, similar to that of other slow-growing mycobacterial species (*55*). Primary cultures may take between 6 and 8 weeks, similar to tubercle bacilli, but subcultures are generally positive within 2 weeks depending on the number of acid-fast bacilli in the inoculum.

M. ulcerans can be easily identified by classical identification schemes (*56)*. Several phenotypic characteristics differentiate *M. ulcerans* from the other slow-growing mycobacterial species. Very few strains grow at 37 °C. The organism is resistant to isoniazid, but most of the strains are inhibited by hydroxylamine and *p*-nitrobenzoate. The other slow-growing species that are susceptible to hydroxilamine and *p*-nitrobenzoate are also susceptible to isoniazid (*54)*. Some phenotypic characteristics seem to differentiate African, Australian and North American strains (*57*). Acid phosphatase activity is in general positive for African strains, but negative for strains from other origins.

Chapter 4

Immunology

Dr Mark Evans & Dr Mark Wansbrough-Jones

The interaction between the host immune response and an infecting organism determines the extent and rate of progression of the disease through the steps of incubation, clinical manifestations, healing and protection against further infection. The susceptibility of a population to the infection is determined by the extent and duration of exposure, the virulence of the organism, the host immune response and susceptibility of the individual to disease, which may be genetically determined.

Infecting organism

M. ulcerans is unique among mycobacteria in that much of the pathology appears to be mediated by toxin production. This toxin, recently characterized as a polyketide called mycolactone (*16*), has profound effects on lymphocytes and macrophages *in vitro*, suggesting that it may cause local immunosuppression in infected tissues *in vivo*. At present, little is known about strain variation among wild strains of *M. ulcerans*, but laboratory strains have been identified that do not produce toxin and that are avirulent in a guinea-pig model of *M. ulcerans* disease (*16*,*58*).

The host immune response

The natural history of *M. ulcerans* disease is a progression from early skin nodules, in which there are abundant extracellular acid-fast bacilli, extensive subcutaneous necrosis and little inflammatory response, through to ulceration with the histological hallmarks of a paucity of acid-fast bacilli and patchy granuloma formation. Skin fibrosis occurs during the healing stage, often leading to severe contractures. What determines the extent of the lesion is unknown, but the host immune response is likely to play an important role and it is of interest that many lesions are thought to heal spontaneously.

The subsequent accumulation (and/or induction) of toxin may be of a sufficient concentration to cause lysis of the macrophage host and to paralyse the cellular functions of infiltrating lymphocytes or macrophages. In turn, this localized immunosuppression may contribute to delaying an early systemic immune response to mycobacterial antigen. This may account for the observation that patients with active lesions are often unresponsive to *M. ulcerans*-derived antigen (burulin) on skin testing (*59*). Later, during the healing phase characterized by the appearance of granulomas, there is conversion to a positive burulin test indicating that a specific cellular response develops. Such a picture is unlike that seen in tuberculosis, where patients are tuberculin-positive regardless of the stage of the disease.

Little is known about the human immune response to *M. ulcerans* infection, but the pathological findings strongly suggest that cell-mediated immune responses have an important role in healing. This is supported by the only studies published concerning the delayed hypersensitivity response of patients with Buruli ulcer to a crude preparation of *M. ulcerans* (burulin) given intradermally. This was an attempt to mimic the tuberculin test used to assess immunity to *M. tuberculosis*. Patients with early *M. ulcerans* disease showed no reaction, whereas a positive response was elicited from patients with healing lesions (*59*). A different test, in which whole blood samples are incubated with PPD and gamma interferon production is measured by an ELISA assay, has shown a good correlation with the tuberculin test in patients with tuberculosis. Since there is considerable antigenic overlap between the mycobacteria, it was thought that this could be used to assess further the immune reactivity of patients with *M. ulcerans* disease. Unpublished observations on Australian patients with healed ulcers, however, have not shown any positive reactions. This is currently being investigated further, using antigen prepared from *M. ulcerans* itself.

Investigation of antibody responses to mycobacteria has been unrewarding because of the antigenic overlap between this group of organisms and their ubiquity in the environment. However an important question about the pathogenesis of *M. ulcerans* disease is that if mycolactone inhibits the development of an effective immune response, how does the host overcome this so that healing can occur in the long term? One explanation would be that the toxin is neutralized by an antibody response that develops slowly. Mycolactone's macrolide structure makes it unlikely to elicit antibodies, and studies of Australian patients with active or healed ulcers have failed to show any neutralizing antibody response (Johnson P, unpublished data, 1998). It is possible, however, that complexes of mycolactone with tissue proteins are antigenic; this has not been studied to date.

Host susceptibility

Anecdotal evidence from observations on families in endemic areas raises the possibility that host factors influence susceptibility to *M. ulcerans* infection. In a village in Benin, for example, there is a group of children all living in the same small compound, and all of whom share the same father but three different mothers. All the children born to one mother have had *M. ulcerans* disease but not those from the other mothers (Portaels F, personal communication, 1998).

Similarly, in one large family in a small village in Ghana, some have Buruli ulcer and some do not. In the same village, within a different family, one (non-identical) twin has the disease while the other does not. Human leukocyte antigen (HLA) typing is being undertaken in these individuals. Preliminary studies from Australian patients are reported to show some increase in HLA DR3 prevalence (Hayman J, unpublished data, 1999).

If the progress of this disease is determined by the ability of the host to mount a cell-mediated immune response, it would be expected that HIV co-infection would influence its course, but this does not appear to be the case. Data from Ghana show no increase in the prevalence of HIV infection among patients with Buruli ulcer and no difference in the presentation or course of the disease in co-infected patients. In a series of 60 Buruli ulcer patients, 2 were HIV infected (3.3%) compared to 6% HIV seropositivity (20 out of 329) in an antenatal population from the same rural hospital (*40*).

Immunization

In the largest study examining the effect of BCG in protecting against Buruli ulcer in Uganda, BCG was given to those with negative, low or middle-grade tuberculin reactions (*60*). It was found to offer an overall protection of 47% against the disease, but the effect was limited to those with tuberculin reactions under 4 mm before vaccination and those in the first year after immunization. Both previous *M. ulcerans* disease and an existing BCG scar at entry into the trial appeared to protect against the disease, the protective effects being 88% and 82% respectively.

In the Uganda Buruli Group studies, both a positive tuberculin skin test and BCG vaccination were associated with a substantially lower incidence of *M. ulcerans* disease (*61*). The protection rate varied with the geographical incidence of infection, from only 18% protection in high-incidence areas to 74% in low-incidence areas. Also, the onset of symptoms was delayed by 2–3 months in those who were tuberculin-positive or who had received BCG, compared with those who were tuberculin-negative.

Both of these studies are encouraging for the prospect of developing a more specific vaccine. Proposals to investigate the potential for a *M. ulcerans* toxoid based on a modified toxin or a mutant non-toxigenic strain are being considered (see above).

Much is still to be learned concerning the immunology of Buruli ulcer and this knowledge could have far-reaching consequences beyond prevention of the disease itself.

Chapter 5

Modern diagnostic techniques

Dr Paul Johnson, Dr Tim Stinear, Prof. Françoise Portaels, Mr Karim Chamal, Dr Karen Dobos & Dr Harold King

Buruli ulcer is often diagnosed late, when treatment can be very difficult and frustrating. Confirmation by culture takes 6–8 weeks. Rapid diagnostic methods for *M. ulcerans* infection (i.e. skin and serological tests), as well as methods of rapid identification of the organism in clinical and environmental specimens would be a significant advance in the management of *M. ulcerans* infection. Screening to detect early infection could guide early intervention; at present, only early excision of the nodule is possible.

Polymerase chain reaction (PCR) in the diagnosis of M. ulcerans infection

All free-living organisms contain DNA molecules that encode the genetic information necessary for the structure, function and replication of that species. DNA usually exists as paired, very long, complementary molecules (double-stranded DNA) made of combinations of four different units or "bases" (A,G,C,T). The order of bases along the molecule encodes the genetic information. When DNA needs to be copied to allow a cell to replicate, double-stranded DNA is first made single-stranded by cellular enzymes. The single strand then acts as a blueprint or template. Because the bases A and T always pair with each other and not with G or C (and vice versa), the new or complementary strand is a perfect copy of the original, but in the reverse direction. When the two strands are again separated and the complementary strand is itself copied, the resultant molecule will contain the same information in the same direction as the original.

PCR is a method that artificially amplifies minute quantities of DNA to levels that can be easily detected in the laboratory. Most importantly, the sequence of the DNA that is amplified is determined by the sequence of the PCR "primers" – short sections of DNA that initiate PCR amplification. Since the primer sequences can be chosen, one can specify exactly which sequence of DNA will be amplified.

PCR has two main advantages over traditional methods that identify microorgansims by culture. Firstly, for slow-growing organisms such as mycobacteria, an accurate etiological diagnosis can be made in hours instead of weeks. Secondly, PCR may be very helpful when bacteria or viruses are not recoverable by culture, as in the case of leprosy.

PCR requires a test-tube, a supply of primer-pairs, an enzyme that is able to copy single-stranded DNA (e.g. *Taq* polymerase) and a supply of free bases that can be used to create the complementary strand (all commercially available). Purified DNA

(e.g. from a swab or tissue biopsy) is then added, and the mixture is "melted" by heating in a machine called a thermal cycler (heat makes double-stranded DNA become single-stranded). Once the primers have attached, *Taq* polymerase can begin to copy the rest of the template strand. The second primer is designed to attach at a different site on the template DNA strand; it initiates the creation of a new strand but in the opposite direction. Exponential amplification is achieved in the thermal cycler, by sequentially heating and cooling the PCR reaction mixture. Heating causes double-stranded DNA to melt and become single-stranded. Cooling allows new primers to bind to the single-stranded template DNA, and *Taq* polymerase can then generate new strands starting at the point where the primers attach (extension). Each cycle of extension and denaturation leads to a doubling of the amount of template DNA. As extension occurs from the two primers in opposite directions, and heating and cooling is repeated many times, the net result is the exponential amplification of the DNA sequence that lies between the two primer sequences. At the end of the reaction, a section of template DNA will have been amplified millions of times and can be readily detected by size using gel electrophoresis (PCR-positive). If DNA of the correct sequence was not present in the original sample, no amplification occurs (PCR-negative).

There are several PCR methods available that could increase the speed of diagnosis of *M. ulcerans* infection (*43*,*45*,*62*). PCR is relatively expensive, however, and is notorious for producing false-positive results in laboratories that lack experience with PCR. In high-prevalence regions such as West Africa, PCR may not be any more rapid than an accurate clinical case definition combined with a smear that shows acid-fast bacilli. In countries such as Australia, where the incidence is low, the great majority of patients who have nodules, papules or skin ulcers do not have *M. ulcerans* infection. In this situation, PCR is a quicker way of making the diagnosis with a high degree of confidence. The main advantage of PCR is that *M. ulcerans* infection can be diagnosed within 24 hours. Culture confirmation takes 6 weeks or more. PCR usefulness for mycobacterial infections is generally limited, however, and at present it is recommended that PCR is used as a rapid ancillary test, not as a replacement for culture and histology.

The PCR method recently developed in Australia targets a newly described DNA insertion sequence in *M. ulcerans*. When genomic *M. ulcerans* DNA is digested with the restriction enzyme *Alu*I, many 1109 base-pair fragments are obtained. These *Alu*I fragments have now been shown to be part of a larger 1293 base-pair repeated sequence that, by chance, happened to contain two *Alu*I restriction sites. This insertion sequence is repeated at least 50 times per genome. It has been identified in all Australian and African isolates of *M. ulcerans* tested to date and has not been found in at least 45 other mycobacterial species, including *M. marinum*, *M. leprae* and *M. tuberculosis*. The sequence has been named IS*2404* (Genbank accession number AF003002) (*62*,*63*).

The diagnostic PCR protocol consists of 3 phases (*62*):

1. heat and alkaline lysis (to release DNA from *M. ulcerans* cells);
2. extraction of total DNA from sample; and
3. PCR reaction to detect *M. ulcerans*-specific DNA in extracted total DNA (primers slightly modified from Ross et al.) (*42*).
   Primer 1: 5′-gat caa gcg ttc acg agt ga-3′
   Primer 2: 5′-ggc agt tac ttc act gca ca-3′

Suitable clinical specimens for PCR include material obtained with a dry swab and fresh tissue. Swabs are rubbed carefully but firmly around the undermined edge of the ulcer and the material obtained is washed off the swab by vortexing the tip of the swab in a small volume of distilled water. Fresh tissue specimens are diced with a sterile blade in a sterile dish and then resuspended in distilled water. Great care must be taken to keep the sample preparation, PCR master-mix preparation and agarose gel areas of the laboratory separate in order to prevent cross-contamination. It is advisable to include multiple negative controls on every PCR run. All results must be discarded if any negative control is positive. To control for inhibition, each PCR is performed in duplicate. The second tube is "spiked" with approximately 100 molecules of purified *M. ulcerans* DNA. If this spiked positive control tests negative, the PCR reaction is being inhibited. Inhibition in clinical specimens can often be overcome by repeating the PCR using a 1:10 dilution of the extracted DNA sample.

In the past, all presumed positive PCR results were checked by Southern blot, using an internal probe based on IS*2404*. However, a PCR product of the correct size that did not hybridize with the probe was rarely identified. Current practice is to rely on comparison of the size of the PCR product from unknown samples, with the size of the product obtained with the positive control. If the two PCR products (positive control and unknown sample) align precisely, and the negative controls are negative, it may be concluded that the unknown sample is positive for *M. ulcerans*. It is recommended that new laboratories use Southern blotting or an equivalent method of verification to establish that the PCR product is the correct sequence.

To date, it has been established that PCR has a specificity of 100% and a sensitivity of 96% compared with culture. As with all microbiological methods, sensitivity depends heavily on the quality and representativeness of the specimen that is received by the laboratory.

Recent studies have shown that swabs taken from a patient with a strongly smear-positive *M. ulcerans* infection remained positive by PCR for up to 3 weeks when the swabs were stored dry in plastic containers out of direct sunlight at room temperature (approximately 22 °C). It is recommended, however, that samples be processed within 48 hours of their arrival in the laboratory. Some samples may contain very few organisms, and a degree of reduction in organism numbers over time is to be expected.

PCR for detecting M. ulcerans in environmental samples

There is strong epidemiological evidence that *M. ulcerans* is an environmental mycobacterium, although it has never been successfully cultured from any environmental site. As PCR is not inhibited by the presence of culturable organisms, it has the potential to overcome this problem. Unfortunately, PCR is exquisitely sensitive to inhibition by many compounds such as humic and fulvic acids, which are ubiquitous in the environment and are not removed by standard DNA extraction protocols. The first confirmation that *M. ulcerans* was present in environmental water samples was obtained in 1997 (*42*), by combining the highly sensitive and specific IS*2404* PCR with a method that separated sample DNA from naturally occurring inhibitors of PCR.

Three different strategies have now been used to overcome inhibition in environmental samples from *M. ulcerans* endemic regions. The first of these is gel chromatography. Environmental water samples are concentrated and subjected to homogenization with glass beads, followed by heat and alkaline lysis to release DNA. Total extracted DNA is then run through gel chromatography columns that separate DNA from contaminants on the basis of size (*62*). Although relatively simple, the method is cumbersome and time-consuming. The second method uses paramagnetic beads linked to *M. ulcerans* antibodies to capture whole cells and separate them from contaminants in a magnetic field (immunomagnetic separation) (*43*). Antibodies are raised in laboratory animals. Captured cells are washed to remove inhibitors and then DNA is released by standard methods prior to PCR. The third approach also uses paramagnetic beads, but here the beads are linked to *M. ulcerans*-specific oligonucleotide probes, which capture IS*2404* DNA that has been released from *M. ulcerans* by homogenization and alkaline lysis. The immobilized DNA is washed to remove inhibitors and used directly as a template for IS*2404* PCR. The latter two methods each have limitations and advantages, but offer superior detection sensitivity and are less time-consuming than gel chromatography.

An important consideration with any of these methods is quality assurance. The high sensitivity of the IS*2404* PCR means that inadvertent contamination may lead to false-positive results. It is essential that appropriate quality assurance measures are implemented if these types of assay are being attempted. Safeguards include physical barriers in the laboratory, such as separate work areas for each stage of the assay, and frequent use of negative controls within a batch so that contamination can be rapidly detected. It is important that negative controls are used all the way through the extraction and PCR process. Even experienced workers have intermittent problems with contamination.

DNA fingerprinting techniques for M. ulcerans

Molecular typing methods may be categorized into three broad groups on the basis of the type of macromolecules targeted for sub-typing, i.e. methods based on fatty acids, proteins and nucleic acids. Actually, the genotypic typing methods (DNA fingerprinting) that evaluate differences at the DNA level are used more commonly and have emerged as revolutionary tools for epidemiological studies.

The use of DNA fingerprinting for the identification of *M. tuberculosis* has greatly improved understanding of the epidemiology of tuberculosis: transmission routes of different strains have been recognized (*64*); outbreaks of multidrug-resistant strains have been detected early; and the relative importance of reinfection versus reactivation can now be elucidated (*65*).

Various molecular methods for fingerprinting of *M. ulcerans* are now being developed to facilitate studies on the epidemiology of Buruli ulcer.

DNA sequencing

Direct comparison of genomic DNA sequences of bacterial strains is the best means of quantitatively determining whether two strains are similar or different. Portaels et al. have analysed the 3′-terminal region of the 16S rRNA gene sequence of 17 strains of *M. ulcerans* from Africa, Australia and America (*57*). This analysis has revealed three subgroups that vary according to the continent of origin. More recently, a fourth subgroup

was discovered in China and Japan confirming the existence of an Asian type (Faber WR et al., unpublished data, 1999).

Restriction fragments length polymorphism (RFLP)

RFLP based on insertion sequences

Insertion sequences (IS) are mobile genetic elements that are usually present in numerous copies within a bacterial genome. These elements can be used as probes, and because the number and location of IS elements vary, each strain will have a unique banding pattern. Recently, molecular analysis of *M. ulcerans* has revealed two new insertion sequences: IS*2404* and IS*2606* (*63*). Southern blot analysis to detect IS*2404* and IS*2606* shows inconclusive RFLP patterns between different strains. Due to the high number of copies of both elements, the banding patterns are difficult to interpret, limiting the value of the Southern blot method to type *M .ulcerans* isolates (*63*).

RFLP based on pTBN12 plasmid.

Jackson et al. have used pTBN12, a well defined plasmid, as a probe with *Alu*I restriction fragments (*66*). The probe was able to distinguish 11 RFLP patterns.

PCR typing methods

PCR is another molecular method that has become increasingly important for epidemiological studies. The technique detects and amplifies small amounts of DNA; 10–100 copies of the templates are enough to perform DNA amplification. Thus, PCR can be used to type organisms that grow slowly on laboratory media, such as *M. tuberculosis* (*67*). PCR also can be used to detect and type pathogens in patients whose cultures are negative because they have been treated. Moreover, PCR can be used to amplify the DNA from organisms that are present in tissues preserved in formalin (*68*).

PCR of repetitive chromosomal elements (Rep-PCR)

Rep-PCR is a modification of the PCR technique that is more suitable for epidemiological purposes than conventional PCR. In this case, the primers are directed towards repetitive chromosomal elements such as IS*6110* in *M. tuberculosis* and the ERIC sequence in other bacteria (*66*). In *M. ulcerans*, the genomic sequence between the IS*2404* elements has been amplified. The profiles produced by this technique categorized the strains into three subgroups related to the three different endemic regions (Africa, Australia and North America).

Ribotyping

This method involves amplification of a known sequence cut by restriction enzymes, and compares restriction fragments of amplified DNA from different strains. Using this technique, the *M. ulcerans* genome has been found to produce three different restriction profiles related to the origin of the strains.

Pulsed field gel electrophoresis (PFGE)

PFGE permits the generation of simplified chromosomal restriction fragment patterns without having to resort to probe hybridization methods. In this method, restriction enzymes that cut DNA infrequently are used to generate large fragments of chromosomal DNA, which are then separated by special electrophoretic procedures. Preliminary results obtained in collaboration with Dr M. Picardeau of the Pasteur Institute, Paris showed that *M. ulcerans* genomes produce three different profiles according to the three geographical origins of the strains (Type I: Africa, Type II: Australia and Type III: North America).

Amplified fragment length polymorphism (AFLP)

The AFLP technique is based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA (*69*). This technique involves three steps: restriction of DNA and ligation of oligonucleotides and adaptors; selective amplification of sets of restriction fragments; and gel analysis of the amplification fragments. Typically 50–100 restriction fragments are amplified and detected on denaturing polyacrylamide gel. Preliminary results using the radioactive method have shown the presence of two clones related to two different regions of Benin. An alternative method using non-radiolabelled components is currently being developed in collaboration with Professor J. Swings at the University of Ghent, Belgium.

Conclusion and perspectives

To be of use as an epidemiological tool, a typing system must give an unambiguous result for each isolate (typeability), give the same result each time the same isolate is tested (reproducibility) and differentiate epidemiologically unrelated strains (discriminatory power). A comparison of the different methods used in the molecular epidemiological studies of *M. ulcerans* is summarized in Table 2.

In conclusion, given the promising results obtained with AFLP (excellent typeability and reproducibility as well as good discriminatory power) efforts should be concentrated on concentrate on the AFLP techniques to apply it to strains isolated from the same patient at different periods of time, to strains from patients belonging to the same family, to strains originating from different villages and departments, and to strains from patients with different clinical forms of the disease.

Table 2. Fingerprinting techniques used for *M. ulcerans*

Genotypic method	Typeability	Reproducibility	Discriminatory power
RFLP	Excellent	Unknown	Good: with pTBN12; non-interpretable profiles with IS*2404* and IS*2606*
DNA sequencing	Excellent	Excellent	Four profiles related to the four geographical regions (Africa, Asia, Australia, North America)
Ribotyping	Excellent	Excellent	Limited to three profiles (Africa, Australia, North America)
PFGE	Excellent	Excellent	Same as ribotyping
Rep-PCR	Excellent	Unknown	Same as ribotyping
AFLP	Excellent	Excellent	Good differentiation between strains from the same region

Candidate antigens for the serodiagnosis of Buruli ulcer disease

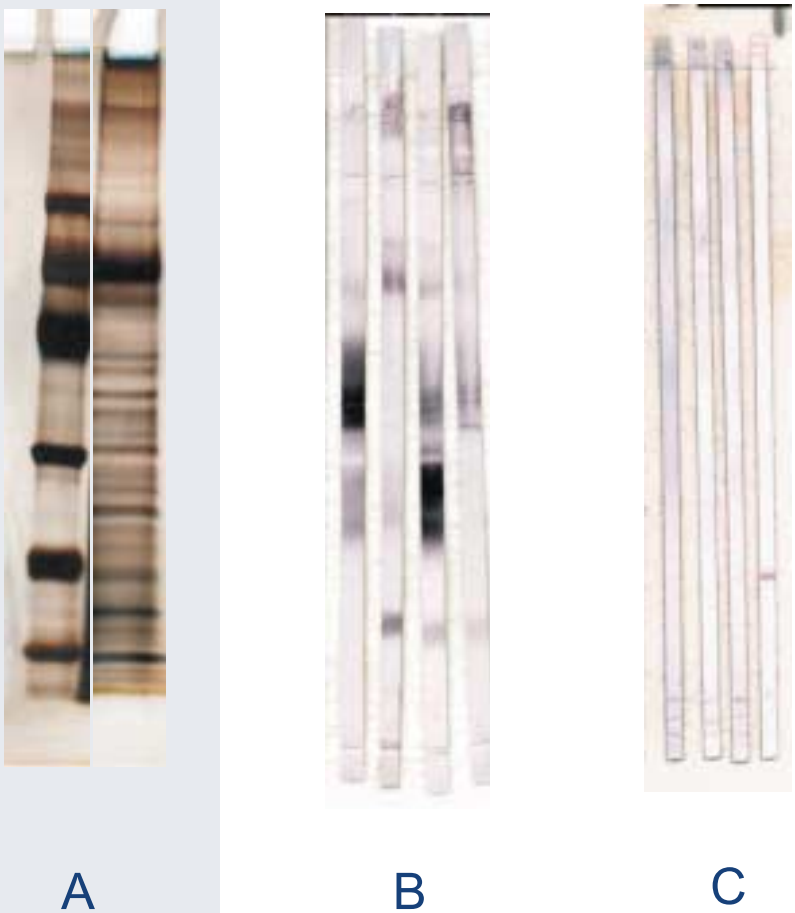
Very little information is known about the host immune response to *M. ulcerans* during infection. Nonetheless, several observations relevant to both humoral and cell-mediated immunity have been reported. Convalescent patients rarely become reinfected with *M. ulcerans*, suggesting that there is a protective immune response from prior disease (*70*). In some cases, a delayed hypersensitivity response has been observed on subcutaneous injection with either *M. ulcerans* or *M. tuberculosis* purified protein derivative, indicating that a cell-mediated immune response can persist during and after infection (*7*,*71*).

In an attempt to more fully characterize the humoral immune response to *M. ulcerans* infection, Dobos et al. tested 62 serum samples from a well characterized case series of Buruli ulcer patients from West Africa for antibodies to *M. ulcerans* culture filtrate (CF) (*7*). For this study, CF was prepared in a serum- and protein-free medium, allowing direct analysis of the constituents actively secreted by *M. ulcerans*. Buruli ulcer patients with active disease were found to produce an antibody response to several different *M. ulcerans* antigens (Fig. 3 A, B). In contrast, serum sample from people

without Buruli ulcer residing in the endemic area produced little to no antibody in response to the CF antigens (Fig. 3 C). Interestingly, three proteins, with apparent molecular masses of 70, 28/26 and 5 kA, were identified that demonstrated strong positive antibody responses in a large number of serum samples from Buruli ulcer patients; these proteins lacked antibody reactivity in control samples. Serum samples from tuberculosis patients from Atlanta, Georgia, USA (where no Buruli ulcer has been reported) demonstrated very low cross-reactivity when tested against the *M. ulcerans* CF, with the exception of a few samples from patients with an antibody response to a common mycobacterial antigen (Fig 3 C). This antigen was found to be the super oxide dismutase (SOD) of *M. ulcerans*, and has been shown to share homology with the characterized *M. tuberculosis* SOD (*43)*. In contrast, samples from Buruli ulcer patients did not react specifically against *M. tuberculosis* CF proteins, suggesting that this response could be diagnostic for *M. ulcerans* infection in areas where tuberculosis is endemic.

These studies suggest that patients with Buruli ulcer generate a humoral immune response specific to *M. ulcerans*. Further studies are now being conducted to examine a broad spectrum of patient sera for antibodies to *M. ulcerans* CF and other subcellular components of *M. ulcerans* to identify specific serodiagnostic antigens. These antigens will then be tested in an experimental ELISA to assess the sensitivity and specificity of this assay using serum samples from Buruli ulcer patients from endemic areas.

Figure 3. Preliminary serological work for the diagnosis of Buruli ulcer.



A B C

Chapter 6

Toxin

Dr Pam Small & Dr Kathleen George

Based on histopathological findings on preulcerative and early ulcerative lesions, Connor and others from the Armed Forces Institute of Pathology postulated that *M. ulcerans* produces a toxin. This was based on the following observations.

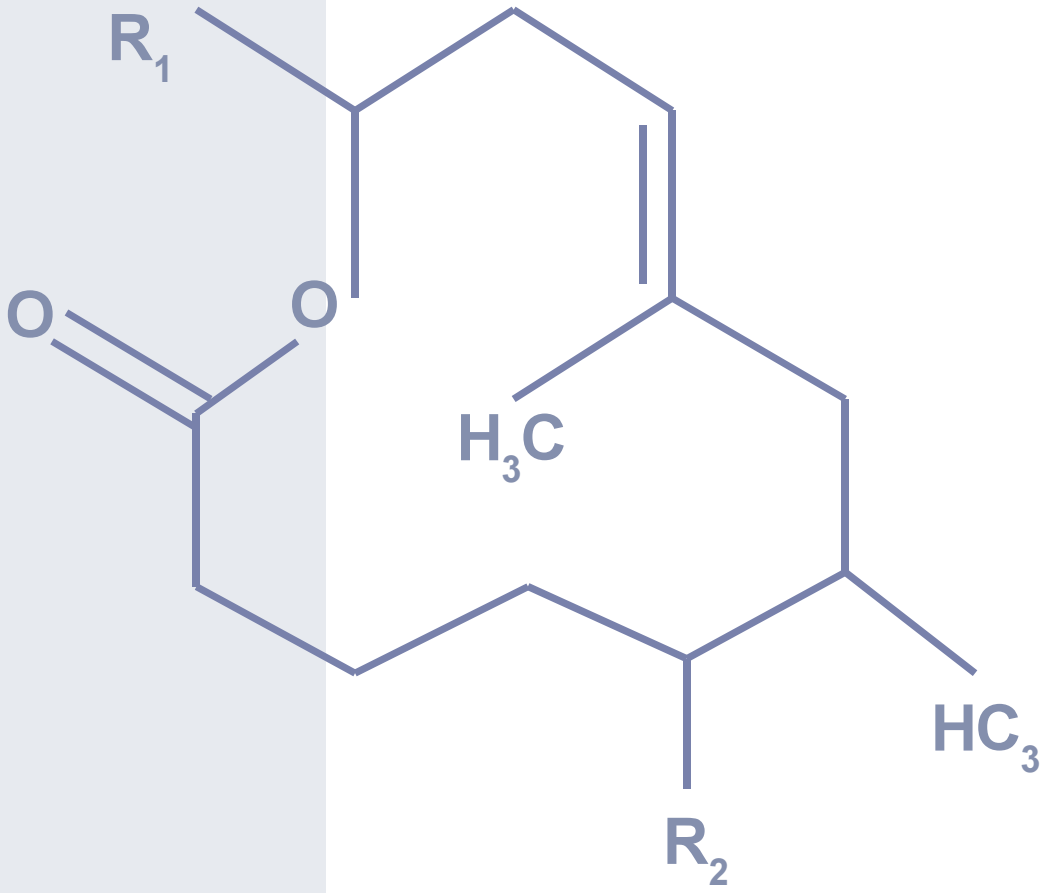
- Necrosis extended far beyond the microcolonies of acid-fast bacilli in the central area of the lesion, and there was an absence of inflammatory exudates.
- Injection of sterile filtrates from *M. ulcerans* into experimental animals produced some of the clinical and histopathological changes of the disease.
- Injection of a 5-fold concentrate of *M. ulcerans* sterile filtrates caused pathology in guinea-pig skin similar to that found in Buruli ulcer patients. Early characterization showed that the active material was heat-resistant, stable over a wide range of pH, and sensitive to pronase.

Isolation of a toxigenic lipid from M. ulcerans

A polyketide-derived macrolide, mycolactone, has been purified from *M. ulcerans*. Mycolactone is also present in sterile filtrate, though in lesser quantities than that associated with the intact bacterium.

Recent work by George et al. has established that a mycolactone was responsible for the cytotoxic phenotype described by earlier investigators (*16*,*17*). More recently, mycolactone has been purified in the laboratory and the structure determined (*16*). High-resolution mass spectrometry established a molecular weight of 743 daltons with a calculated formula of $C_{44}H_{70}O_9$. Two-dimensional NMR spectral analysis identified the molecule as a polyketide-derived, 12-membered macrolide. Although the mechanism underlying the activity of mycolactone is not fully understood, investigations of its effects on L929 cells show that mycolactone arrests cells in G1 of cell cycle. The mycolactone molecule is a 12-membered ring to which two polyketide-derived side-claims (R_1 and R_2) are attached (Fig. 4).

Figure 4. Mycolactone, a polyketide toxin responsible for tissue destruction in Buruli ulcer.*



* Abstracted with permission from George KM, Chatterjee D, Gunawardana G, Welty D, Hayman J, Lee R, Small PL. Mycolactone: a polyketide toxin from *Mycobacterium ulcerans* required for virulence. *Science*, 1999, **283**: 855. Copyright 1999 American Association for the Advancement of Science.

Intradermal injection of purified mycolactone into guinea-pigs produces histopathology markedly similar to that found in human Buruli ulcer. As early as 24 hours after exposure to mycolactone or *M. ulcerans*, abnormal cells with eccentric nuclei were found in the dermis. Within a week, considerable necrosis was present along with a minor influx of mononuclear cells. Although some neutrophils were found, the proportion of neutrophils was much lower than that of mononuclear cells. A number of vascular changes were also found, along with interstitial oedema and microhaemorrhage. Injection of mycolactone resulted in similar pathology to that caused by injection of viable *M. ulcerans*, though the effect was somewhat more rapid. In contrast, intradermal infection with *M. marinum* resulted in a pus-filled lesion. Histopathology was characterized by an enormous number of neutrophils in the first week of infection. Later, a greater proportion of macrophages was present and many giant cells could be seen.

Several investigators have found that virulence in *M. ulcerans*, as in other pathogenic mycobacteria, is lost on passage. George et al. have also found a decline in virulence through subculture, which can be restored by passage through guinea-pig skin (*16*,*17*). There are several characteristics shared by less virulent isolates: they are not cytotoxic for L929 cells; they grow somewhat faster than virulent strains; they are less pigmented; and they differ somewhat in colony morphology. These observations allowed for the isolation of an isogenic tox-variant from *M. ulcerans*. Lipid profiles from this and other tox-isolates examined showed that these strains do not produce mycolactone. These mycolactone isolates are avirulent in guinea-pigs.

In summary, these data show that mycolactone has similar properties to the toxic activity originally described for *M. ulcerans*. Data from animal experiments and genetic evidence from an isogenic tox-mutant show that mycolactone plays a major role in the virulence of Buruli ulcer. Mycolactone is the first polyketide purified from a pathogenic bacterium.

Polyketides are produced as secondary metabolites from a number of soil bacteria in the order *Actinomycetales*. They are "magic molecules" with remarkable biological activity including immunosuppressive (FK406), antibiotic (erythromycin), cytostatic (bafilomycin), antihelmethic (ivermectin) and antifungal (amphotericin). Mycolactone appears to act as both an immunosuppresant and a cytostatin. Although polyketides are not in themselves usually immunogenic, they can be made so. Thus mycolactone may prove to have value either in the treatment or prevention of Buruli ulcer.

Research on Buruli ulcer: impact on other mycobacterial diseases

Basic research on *M. ulcerans* and Buruli ulcer is expected to improve our understanding of other mycobacteria and other mycobacterial diseases, including tuberculosis and leprosy. The genes involved in the synthesis of the Buruli polyketide toxin have not yet been identified in *M. ulcerans*. Nevertheless, a cluster of genes involved in polyketide synthesis has already been identified in both *M. tuberculosis* and *M. leprae*. Although the precise function of these genes remains unknown, it is certain that the identification of Buruli toxin as the first polyketide synthesized by mycobacteria will stimulate research to identify both the presence and function of similar molecules in other mycobacteria, including *M. tuberculosis* and *M. leprae*.

Vaccine development potential for Buruli ulcer

In the absence of effective antibiotic therapy, the development of an effective vaccine to protect against Buruli ulcer is a high priority. Knowledge of the purification and structure of the Buruli toxin suggests that a candidate vaccine (protective and/or therapeutic) based on this molecule could be quickly prepared. Considering both the impact and potential cost savings of this intervention, work towards the development of a vaccine based on a modification of the toxin structure should begin.

Chapter 7

Pathology

Dr Wayne Meyers & Dr John Hayman

Pathogenesis

Based on the histopathological findings and the natural history of a "classic" or "typical" limited ulcerated lesion, and on experimental evidence for the elaboration of a necrotizing and immunosuppressive toxin by *M. ulcerans,* the pathogenesis of Buruli ulcer may take the following course.

The etiological agent is introduced into the dermis or subcutaneous tissue and, following a latent phase of varying duration, the mycobacterium proliferates and elaborates a toxin with an affinity for and cytotoxic effect on adipocytes. The resulting necrosis provides a favorable milieu for enhanced mycobacterial proliferation, accelerating necrosis. During this necrotic phase, there is no or very little cellular host response (the burulin skin test is usually negative). By unknown mechanisms, either the toxin is neutralized or the organisms cease to proliferate or produce toxin. Healing seems to begin when the host develops cell-mediated immunity to components of *M. ulcerans* (the burulin test becomes positive). The granulomas then destroy the etiological agent and the disease subsides by scarring. Metastatic bone lesions probably arise from *M. ulcerans* bacteraemia.

Histopathology

Biopsy specimens from ulcerated lesions should be taken from the edge of the ulcer, but must include the necrotic base and deep tissue. Excisional specimens are advised and, if possible, should extend a short distance beyond the undermined area. Punch biopsy specimens may be unsatisfactory because diagnostic features are often not included. Specimens from a plaque and oedematous lesions should come from the estimated centre of the lesion and include all layers of the skin and subcutaneous tissue. For routine studies, fixation in neutral or buffered formalin is satisfactory.

Histopathological changes

Nonulcerated lesions

Microscopically, preulcerative lesions in the skin are symmetrical circumscribed areas of contiguous coagulation necrosis in the panniculus and dermis, and sometimes the fascia. Ziehl-Neelsen staining reveals many extracellular acid-fast bacilli (Plate 1), usually in the centre of the necrotic zone. Necrosis extends far beyond the foci of acid-fast bacilli. Adjacent to the necrosis there is oedema, but remarkably few inflammatory cells. Fat cells enlarge, die and lose their nuclei, but retain cell membranes (ghost cells) (Plate 2). Many specimens show mineralization in the necrotic tissue. Interlobular septae

in the panniculus are thickened and necrotic, and usually there is marked vasculitis with frequent occlusion of small and medium-sized vessels (Plate 3). Continuing necrosis of the dermis and adjacent epidermis usually leads to ulceration; however, spread of necrosis and proliferation of acid-fast bacilli may extend laterally in the panniculus and fascia, with relative sparing of the dermis and no ulceration. This leads to a plaque and oedematous forms of the disease.

Ulcerated lesions

In the ulcerated lesion, the edges of the ulcer are undermined by destruction of the panniculus (Plate 4, 5). The ulcer base is made up of necrotic debris and fibrin. In the overlying flap of skin there is re-epithelialization at the edge and hyperplasia of the epidermis, and partial sparing of the dermis. Acid-fast bacilli are confined largely to the necrotic base of the ulcer with lateral spread to the panniculus, especially the thickened interlobular septae and fascia. Underlying muscle is rarely invaded.

Healing lesions

In the early healing stage, a poorly organized hypersensitivity granulomatous response develops in the dermis and panniculus. These infiltrations are most prominent just beyond the limit of the undermined area. This reaction eventually organizes into tuberculoid granulomas. Healing and scarring follow the granulomatous phase.

Lymphadenitis

Although clinical lymphadenopathy is not a prominent feature, lymph nodes near lesions are invaded by acid-fast bacilli, apparently via afferent lymphatic drainage, and become necrotic. Regional lymph nodes may contain a few acid-fast bacilli (Plate 6) and show reactional changes with histiocytosis and little or no necrosis. Granulomatous reactions in lymph nodes do not seem to be common.

Osteomyelitis

Although the pathogenesis of osteomyelitis is not yet well understood, bone is probably affected by contiguous spread of the disease, or by *M. ulcerans* bacteraemia. Microscopically, the marrow is necrotic and the trabeculae are eroded. Acid-fast bacilli and frequently secondary bacteria are seen in varying numbers. Bone may be completely destroyed (Plate 7).

Chapter 8

Clinical Features and Treatment

Dr Kingsley Asiedu, Dr Wayne Meyers & Dr Pius Agbenorku

Geographical differences

In Australia, patients present early with their illness, and are treated with relative ease due to ready access to medical care. Complications and sequelae of the disease are rare. It has been hypothesized that the Australian strains of *M. ulcerans* may be less virulent than the West African strain.

By contrast, in Africa, the majority of cases are seen in their late stages (large ulcers often with superinfection) mainly due to difficult access to health care. Hospitalization is therefore prolonged and complications (e.g. contracture deformities and amputations) are frequent and devastating. Traditional therapy is common in Africa (*73*) and, while this is often effective for appropriately selected lesions, if the lesion crosses articulations, severe contractures often result. Furthermore, skin grafting is not done and hypopigmented scars often result. Such scars are sensitive to actinic rays, which can produce skin cancer.

Case definition

This definition is based on the recommendations of the WHO Task Force on Buruli Ulcer at its first meeting in February 1998 (see Annex 1) and the International Conference on Buruli Ulcer Control and Research, Yamoussoukro, Côte d'Ivoire, 6–8 July 1998.

Buruli ulcer is an infectious disease involving the skin, caused by *Mycobacterium ulcerans*, characterised by a painless nodule, papule, plaque or oedema, evolving into a painless ulcer with undermined edges, often leading to invalidating sequelae. Sometimes, bones are destroyed.

Patient classification

Patients are classified as new and recurrent.
New case: a patient with no previous history or treatment for Buruli ulcer.
Recurrent case: a patient with previous surgical treatment for Buruli ulcer who is now presenting with another lesion(s) at the same or different site(s) within one year from the end of the last treatment.

Clinical forms

Two clinical forms of the disease are recognized: active and inactive.

Active form: This is an ongoing infection. The active form can further be divided into nonulcerative and ulcerative disease.

Nonulcerative disease (Plates 8–12)
Papule: Painless and raised skin lesions less than 1 cm in diameter.
Nodule: Painless, palpable, often pruritic, firm lesion 1–2 cm in diameter, situated in the subcutaneous tissue and usually attached to the skin. The skin over the lesion is often hypopigmented.
Plaque: Painless, well demarcated, elevated, firm, indurated lesion more than 2 cm in diameter with irregular edges. The skin around the lesion is often hypopigmented in dark-skinned people.
Oedematous: Diffuse, extensive, non-pitting swelling, ill-defined margin, firm, may be painful with or without colour change over the affected skin, involving part of the limb or an entire limb and associated with constitutional disturbances (fever).

Ulcerative disease (Plates 13–25)
Painless skin lesion characterized by necrotic centre, undermined edges and oedematous skin. In the absence of superinfection, ulcers are painless or minimally painful, and not significantly malodorous.

Inactive form: Characterized by previous infection with a depressed stellate (star-shaped) scar with or without sequelae.

A sequela (Plates 26–33) of Buruli ulcer is defined, for the purpose of this document, as a complication resulting directly from the disease (e.g. contracture deformities, loss of sight) or as a result of treatment (e.g. amputation of limbs). Rarely, carcinoma may develop as a complication of a long-standing ulcer.

Diagnosis

Clinical
Because *M. ulcerans* infection is associated with nonspecific clinical manifestations and an indolent course, every nodule or ulcer in an endemic area should be suspected as *M. ulcerans* infection until proved otherwise. About 70% of cases are in children under 15 years of age. Males and females are almost equally affected. Most of the lesions are on the extremities – more on the lower than on the upper extremities. Nodules are painless swellings in the skin and ulcers have characteristic undermined edges with "cottonwool-like" necrotic slough at the base (Plate 5). Previous residence in an endemic area should raise the suspicion of Buruli ulcer. History and physical examination are often sufficient to make a reasonably accurate diagnosis. The burulin skin test is not useful as a diagnostic tool because early active cases are generally burulin-negative. However, the skin is generally positive in the healing stages and following recovery from the active disease. In the absence of other infections, ulcers are usually painless, constitutional symptoms are uncommon and there is no clinical lymphadenopathy.

COLOUR PLATES

BURULI ULCER

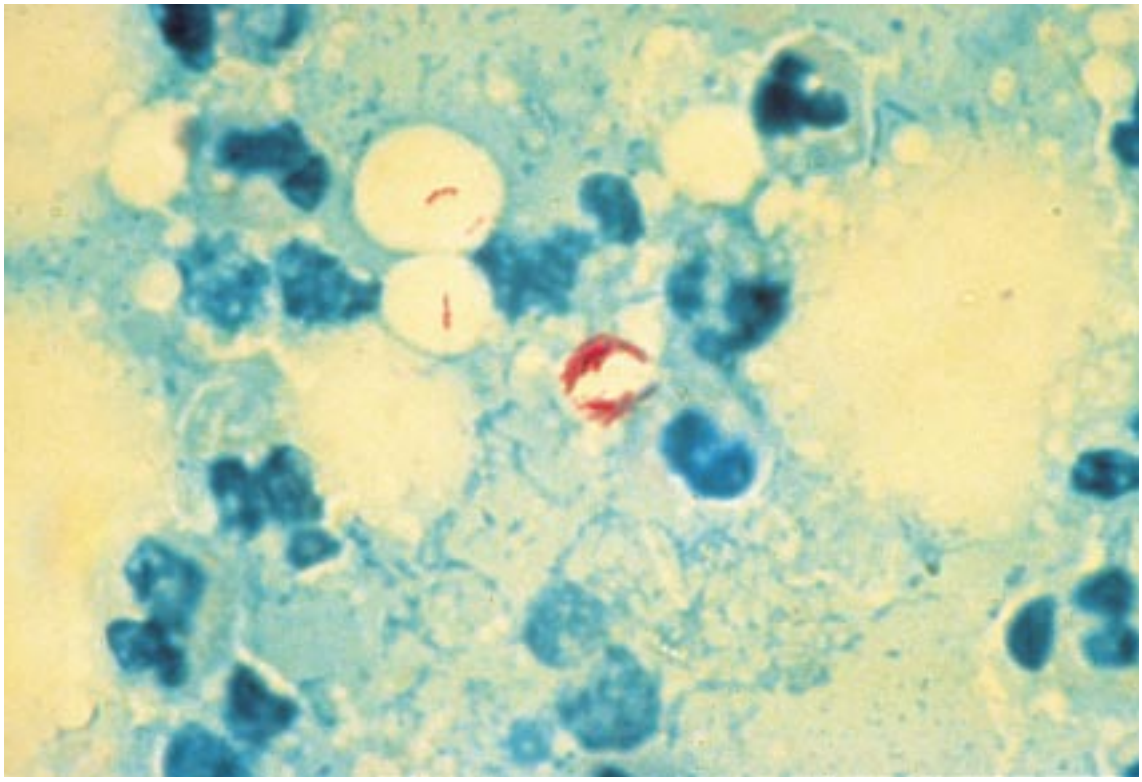


Plate 1. Ziehl-Neelsen stained smear from a Buruli ulcer showing extracellular acid-fast bacilli. (Photo: Wayne Meyers)

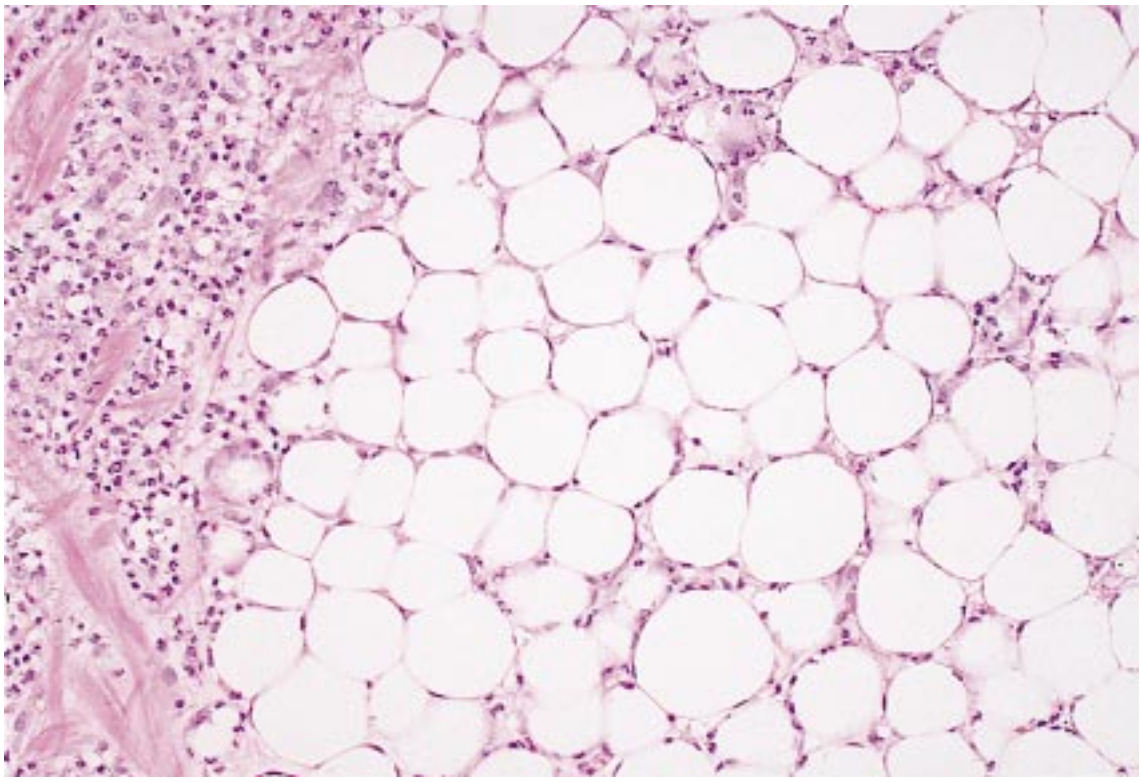


Plate 2. Ghost cells. (Photo: Wayne Meyers)

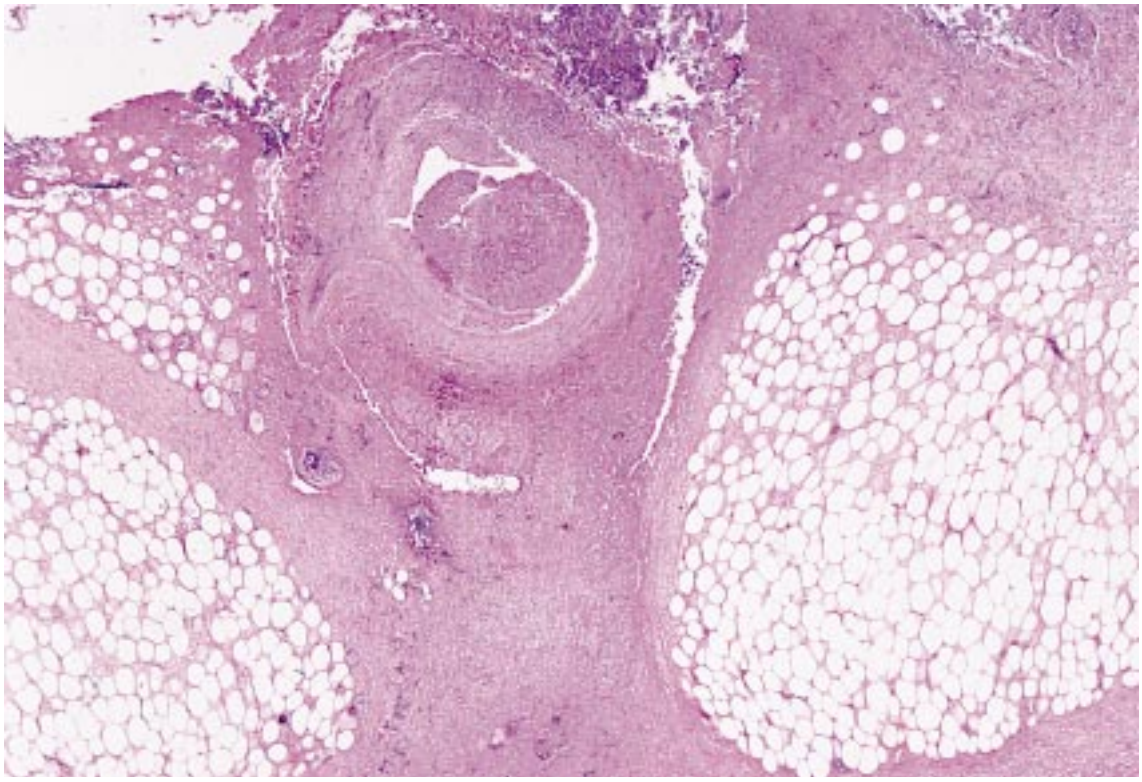


Plate 3. Ghost cells and vasculitis. (Photo: Wayne Meyers)

Plate 4. Biopsy specimen from the edge of an ulcer showing undermining of the dermis and massive necrosis of the skin, dermis, subcutis and the fascia. (Photo: Wayne Meyers)

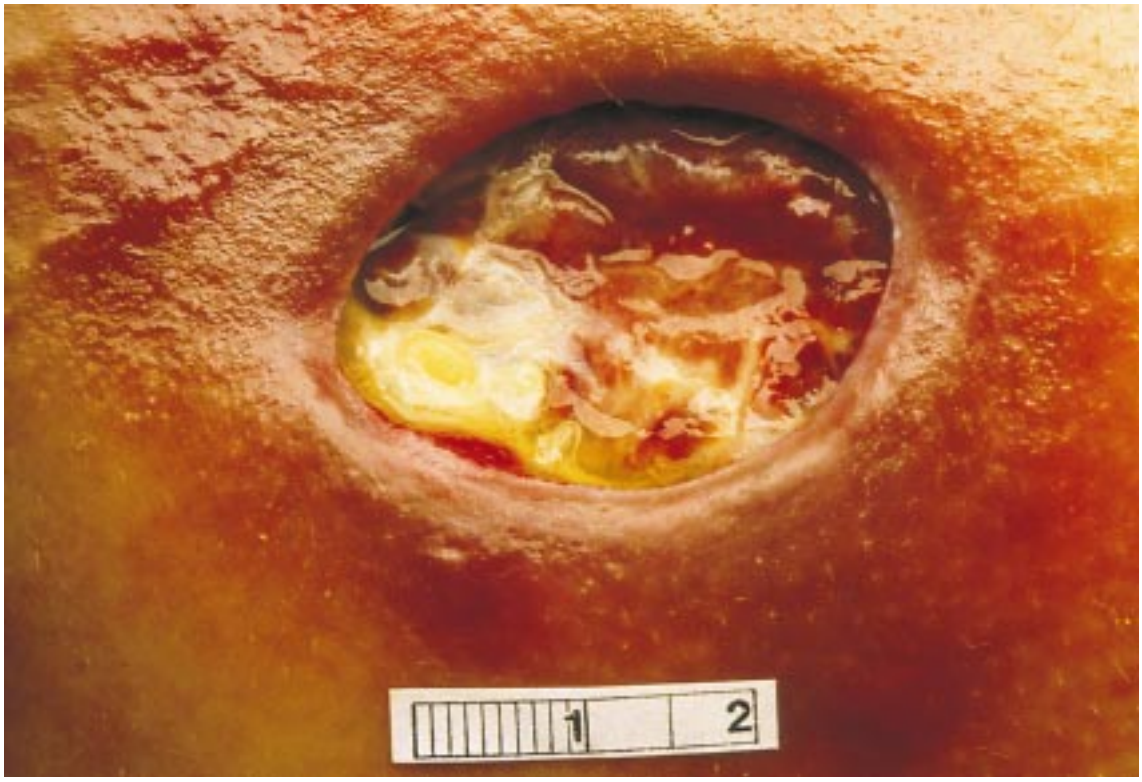


Plate 5. Undermined edges of Buruli ulcer. Note the characteristic yellowish-white necrotic base (cotton wool-like appearance) (Photo: John Hayman)

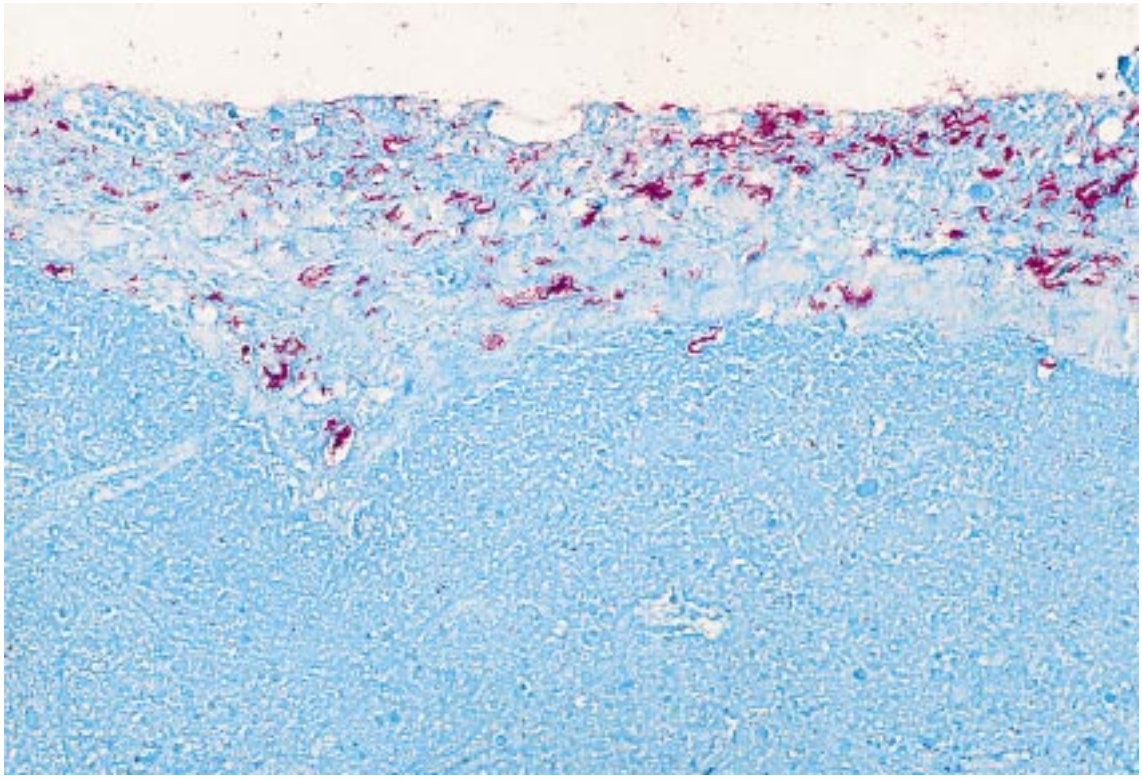


Plate 6. Lymphadenopathy in Buruli ulcer. The parenchyma of the node is necrotic and the capsule is heavily infiltrated by acid -fast bacilli (Photo: Wayne Meyers)

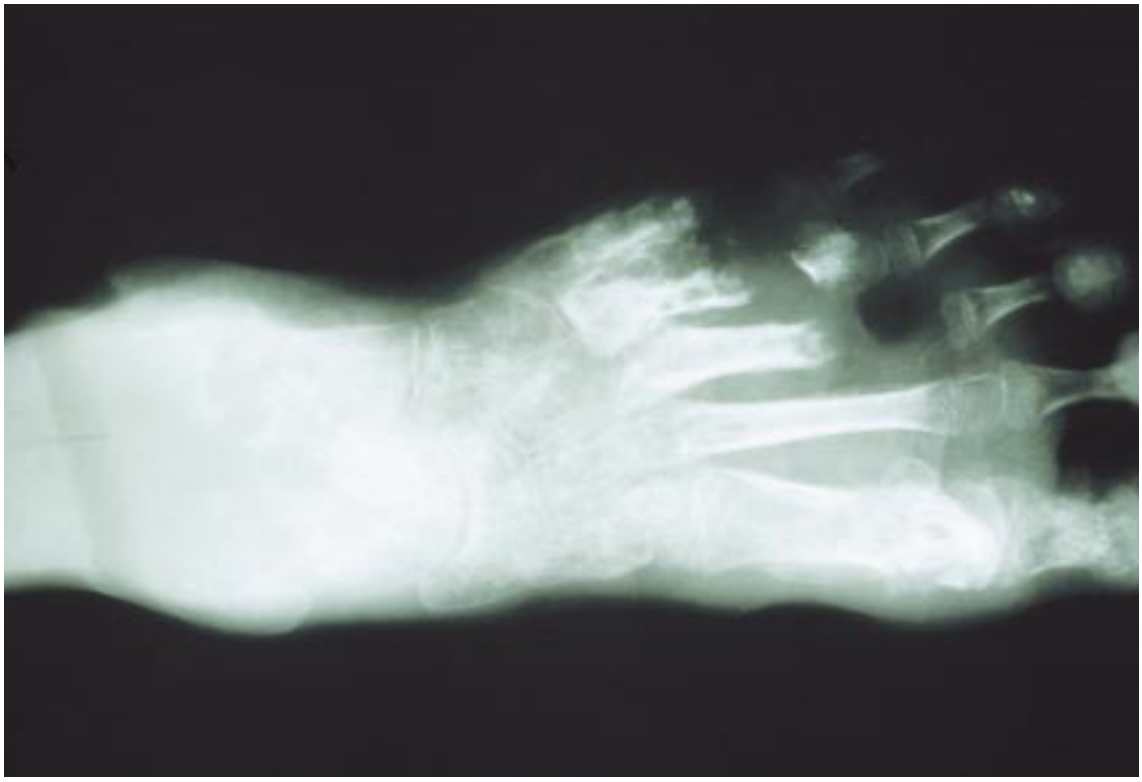


Plate 7. X-ray of the foot showing destruction of the bone. Note the patient had Buruli ulcer on the dorsum of the foot. (Photo: Wayne Meyers & Battista Priuli)

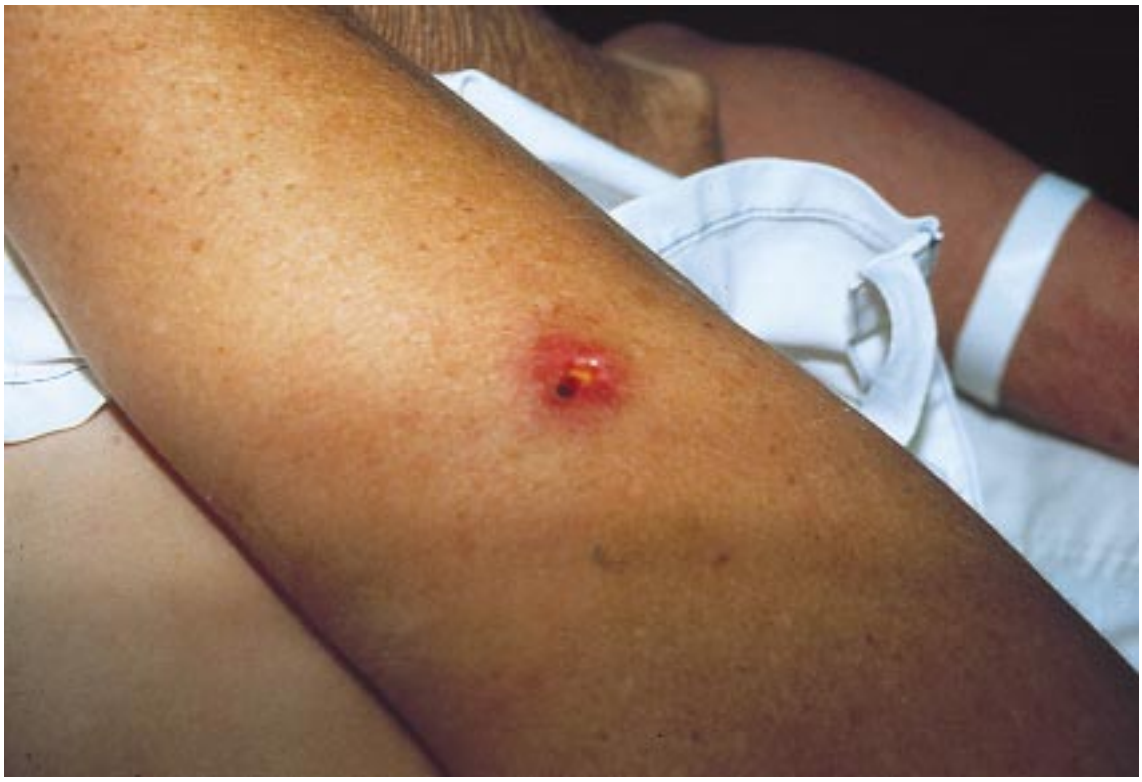


Plate 8. A papule. (Photo: John Hayman)

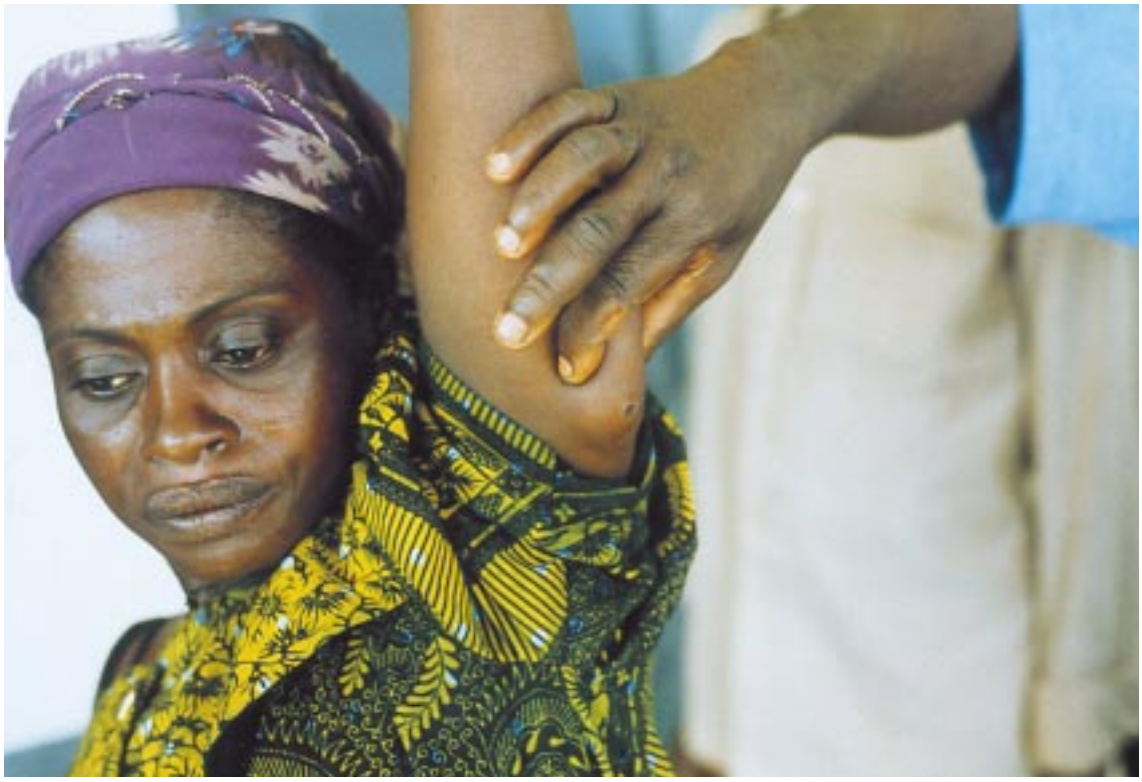


Plate 9. A nodule. (Photo: Mark Evans)

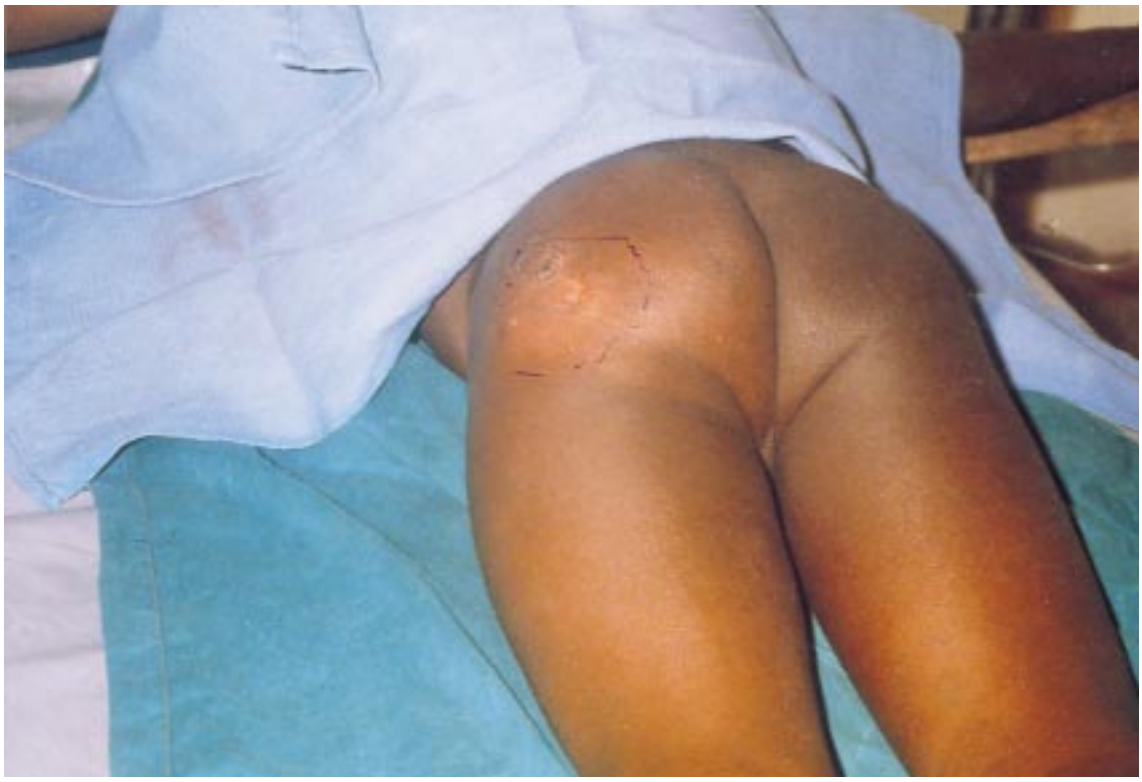


Plate 10. Plaque. (Photo: Mark Evans)

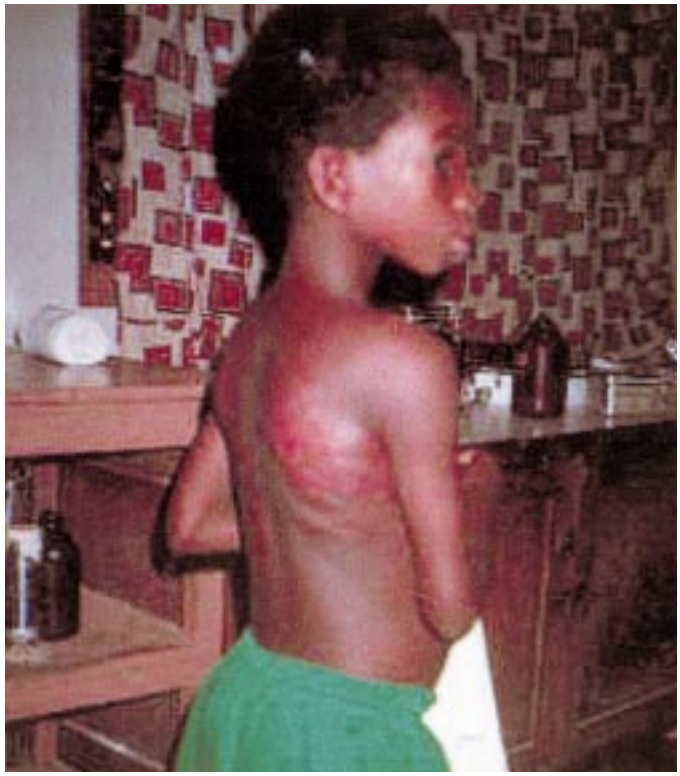


Plate 11. Plaque. (Photo: Kingsley Asiedu)

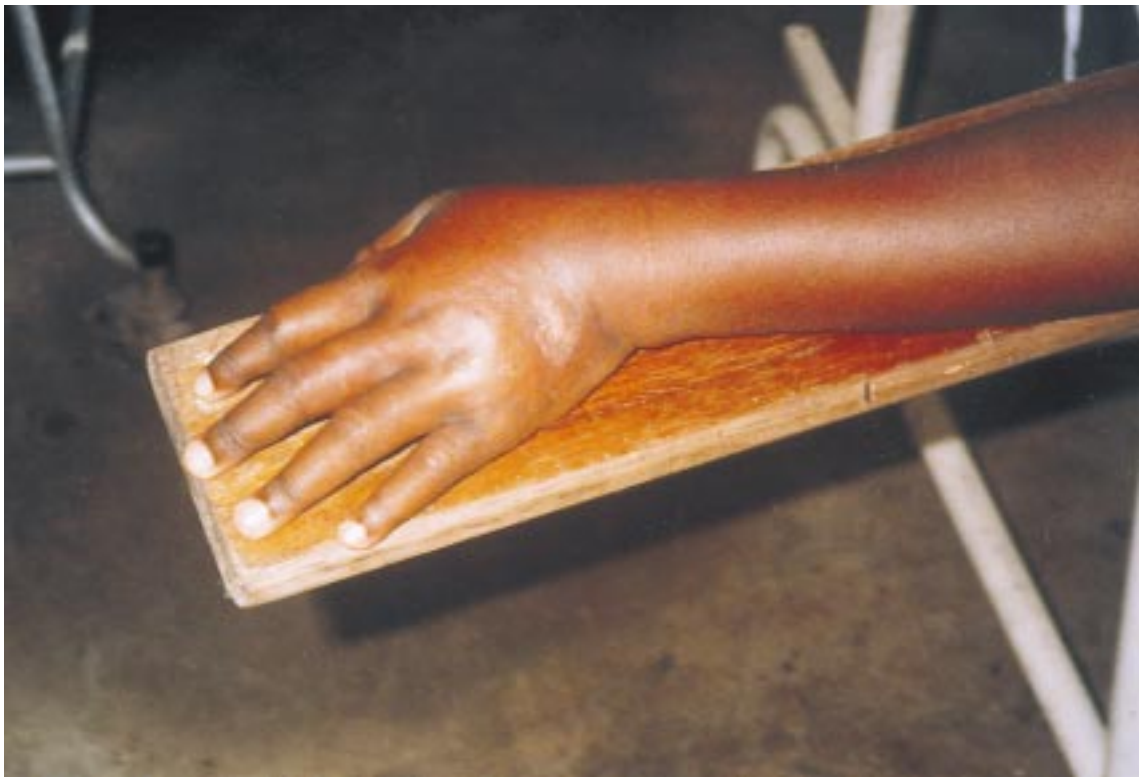


Plate 12. Nonulcerative œdema. (Photo: Samuel Etuaful)

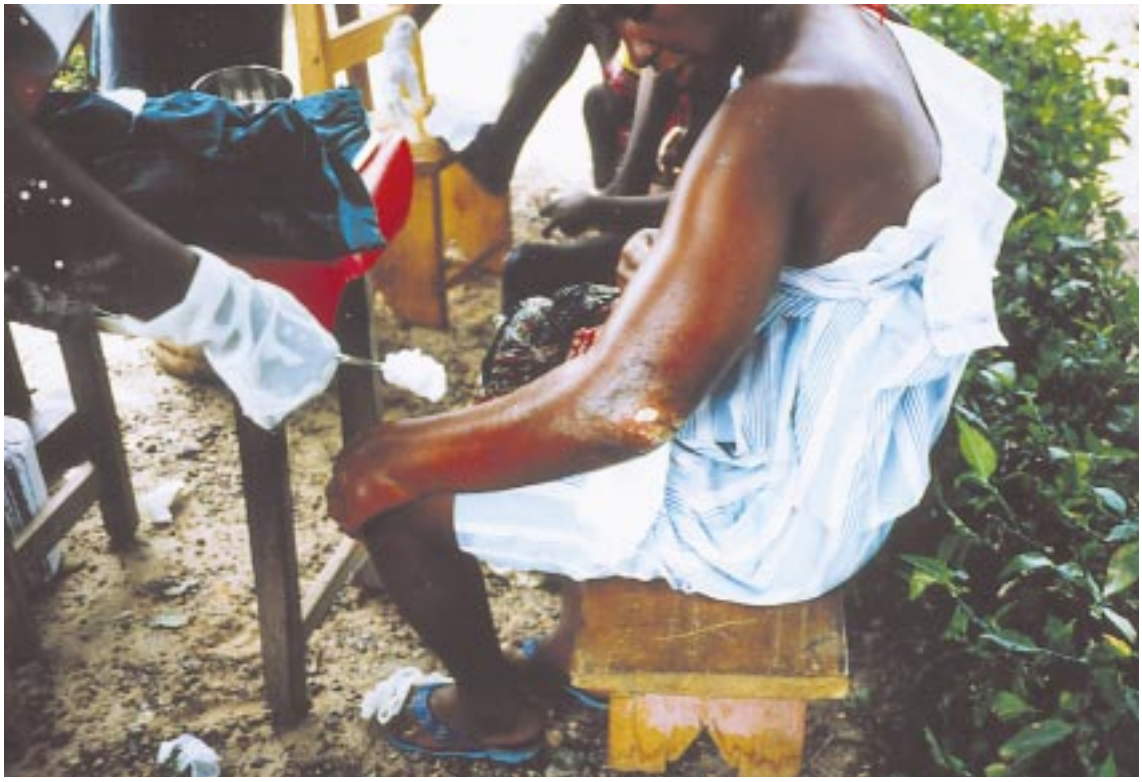


Plate 13. Ulcerative œdema. (Photo: Kingsley Asiedu)

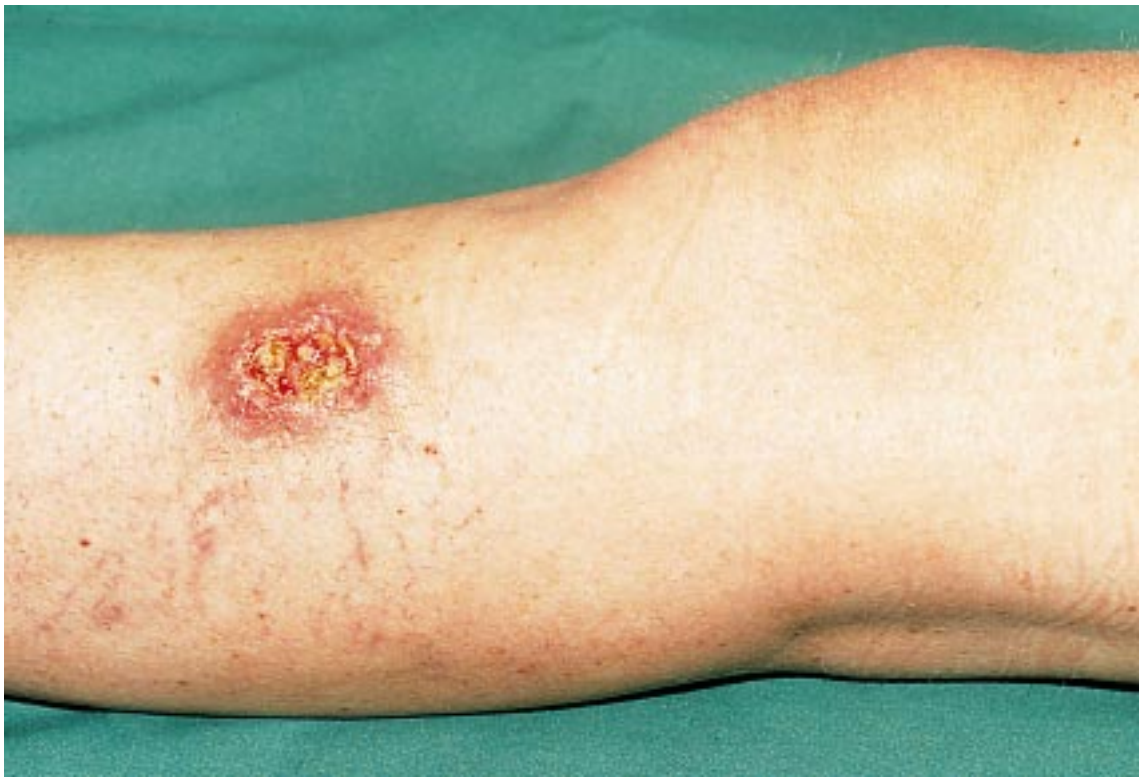


Plate 14. Ulcerative lesion. (Photo: John Hayman)

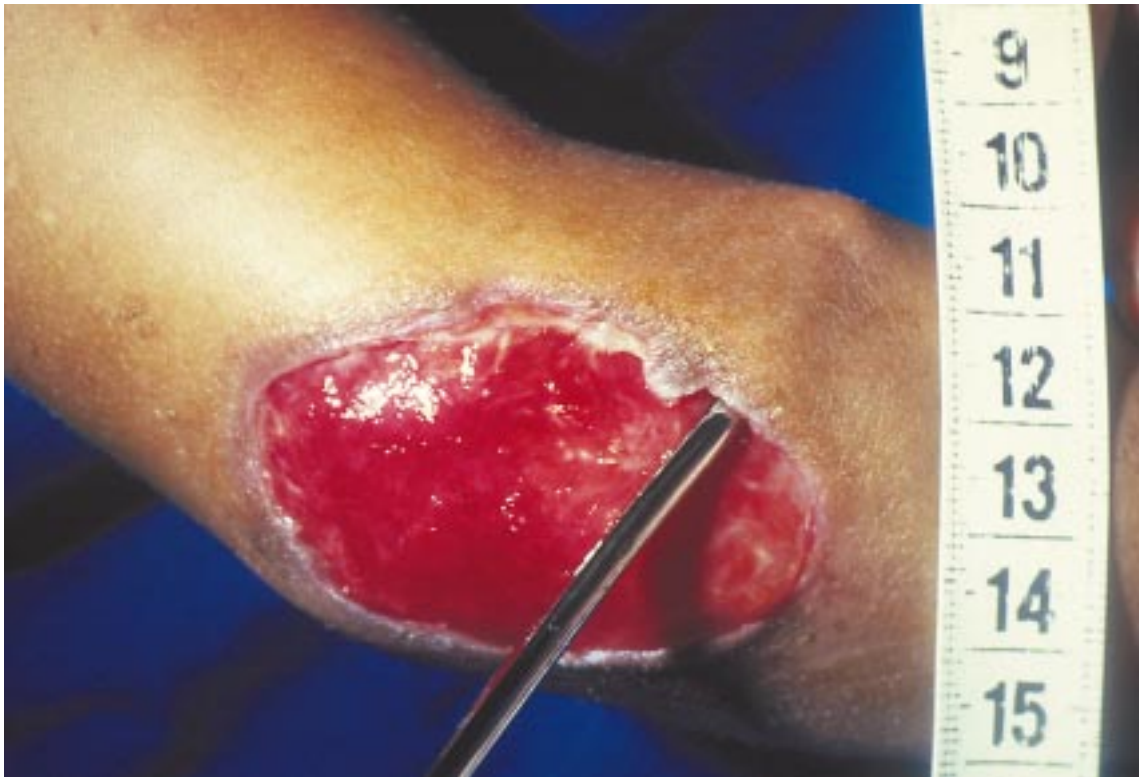


Plate 15. Ulcerative lesion. (Photo: Roger Pradinaud)

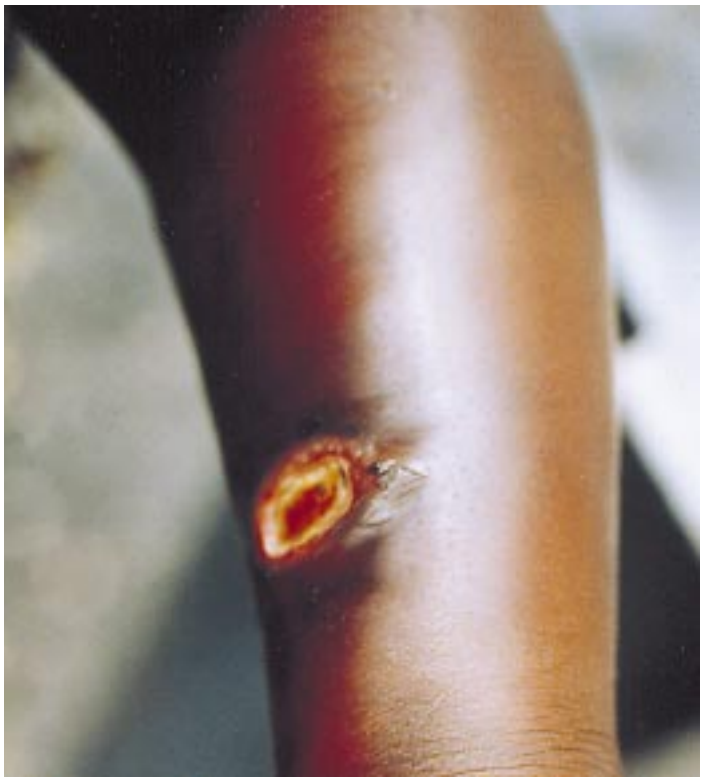


Plate 16. Ulcerative lesion. (Photo: Augustin Guedenon)

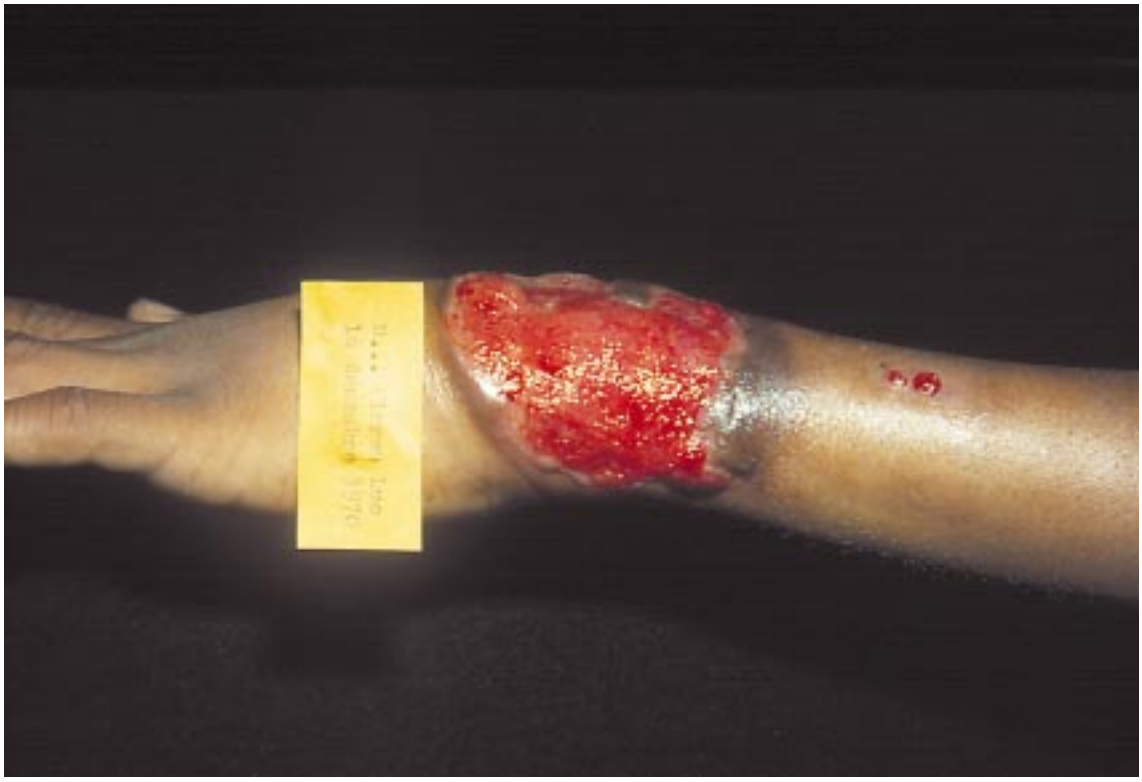


Plate 17. Ulcerative lesion. (Photo: Roger Pradinaud)

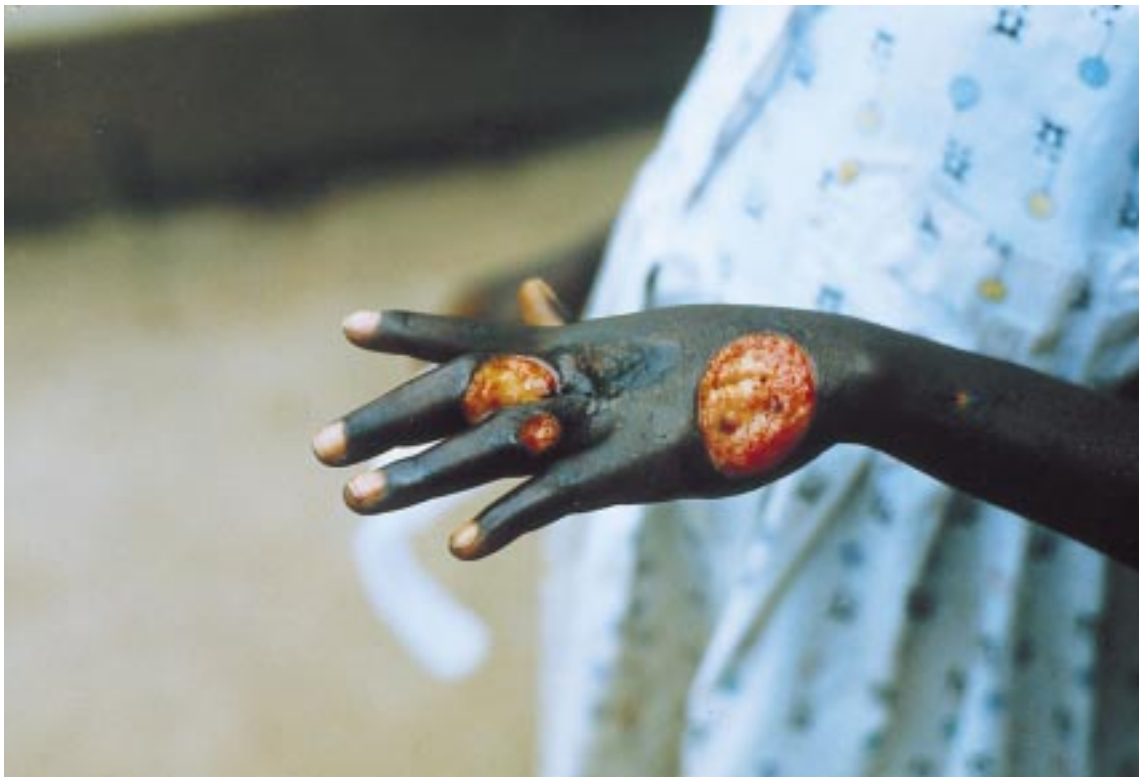


Plate 18. Ulcerative lesion. (Photo: Mark Evans)

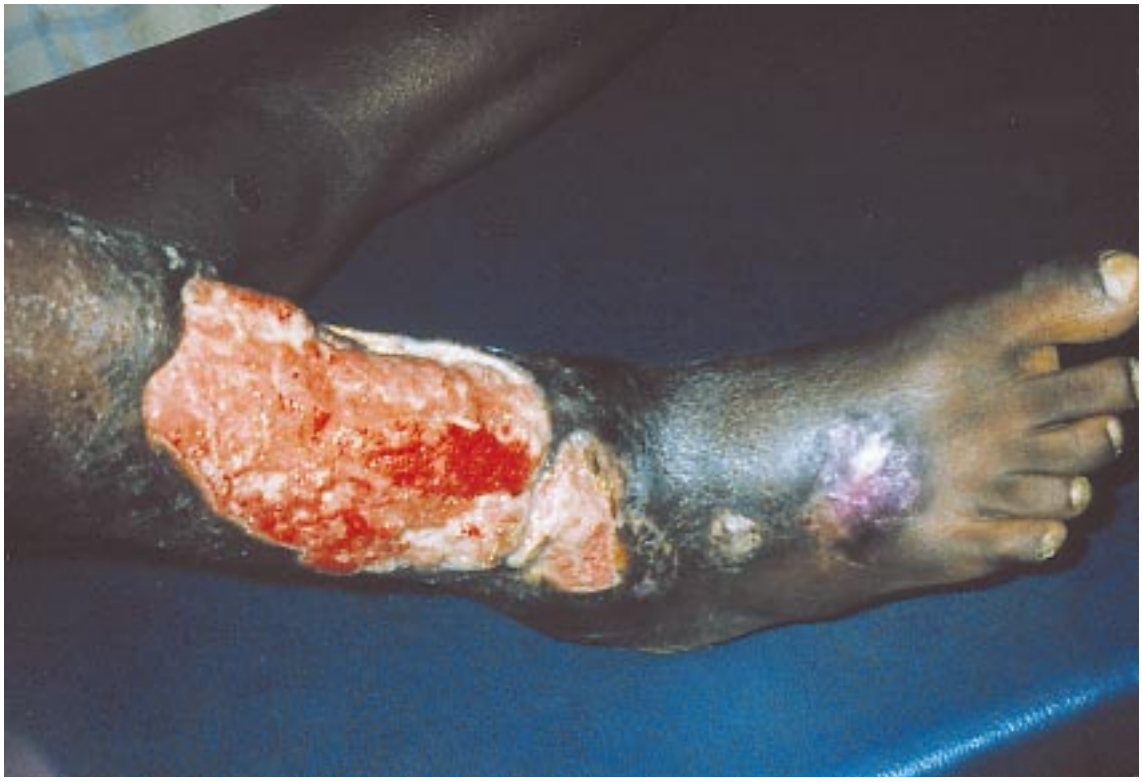


Plate 19. Ulcerative lesion. (Photo: Mark Evans)

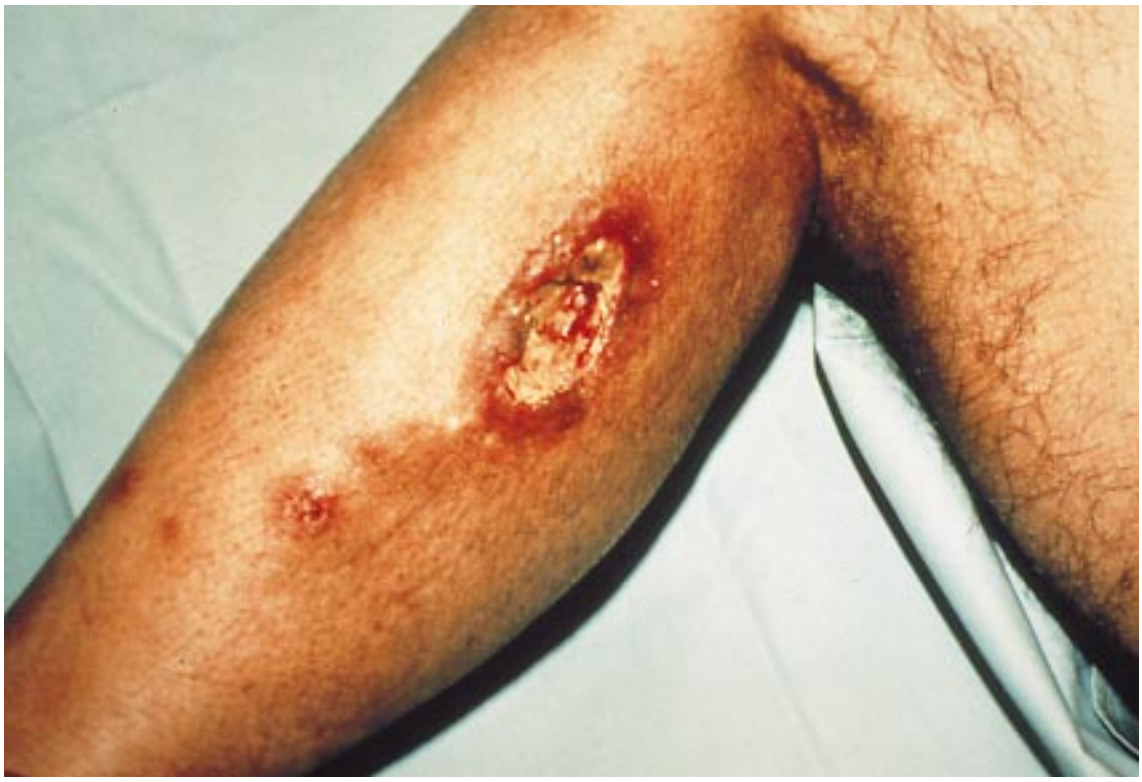


Plate 20. Ulcerative lesion. (Photo: John Hayman)

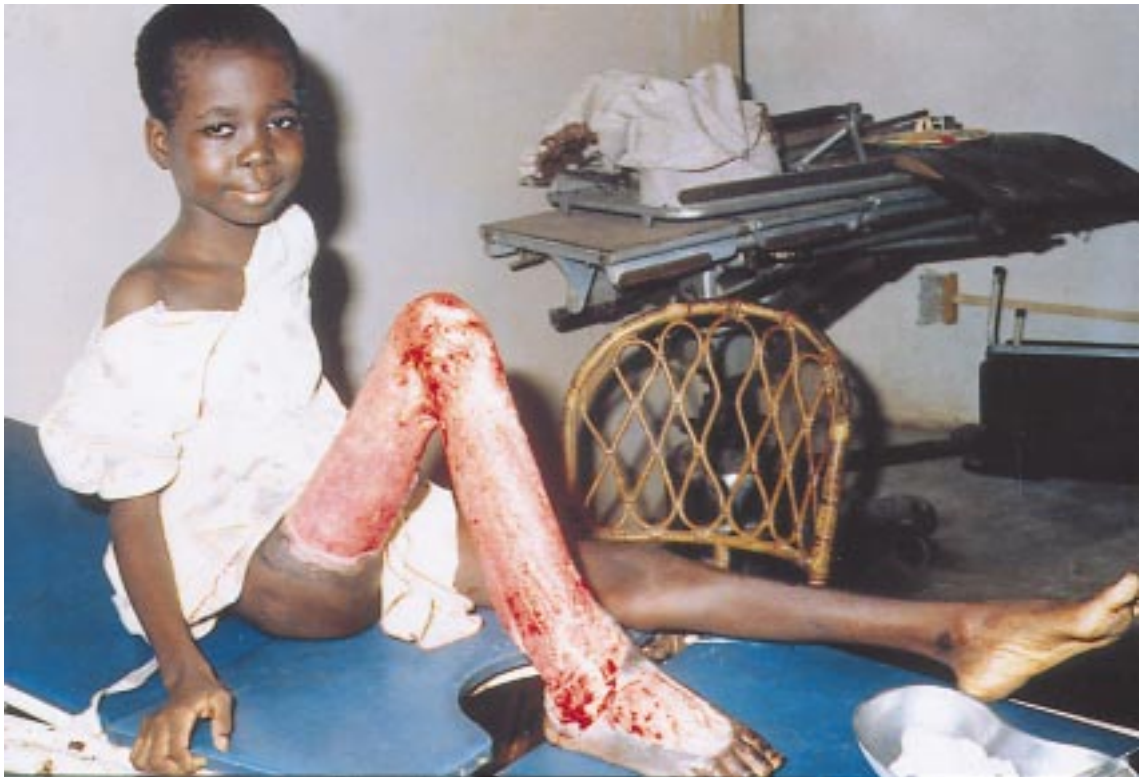


Plate 21. Ulcerative lesion. (Photo: Kingsley Asiedu)

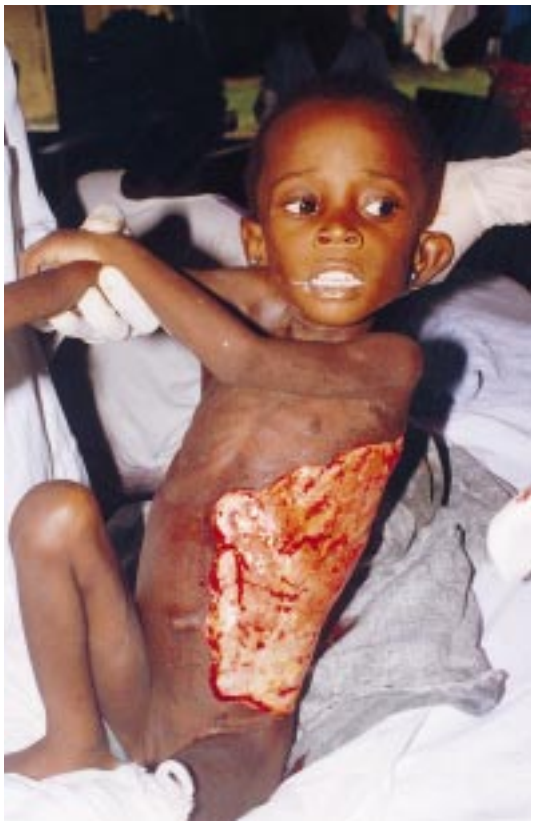


Plate 22. Ulcerative lesion (Photo: Marco Pirovano)

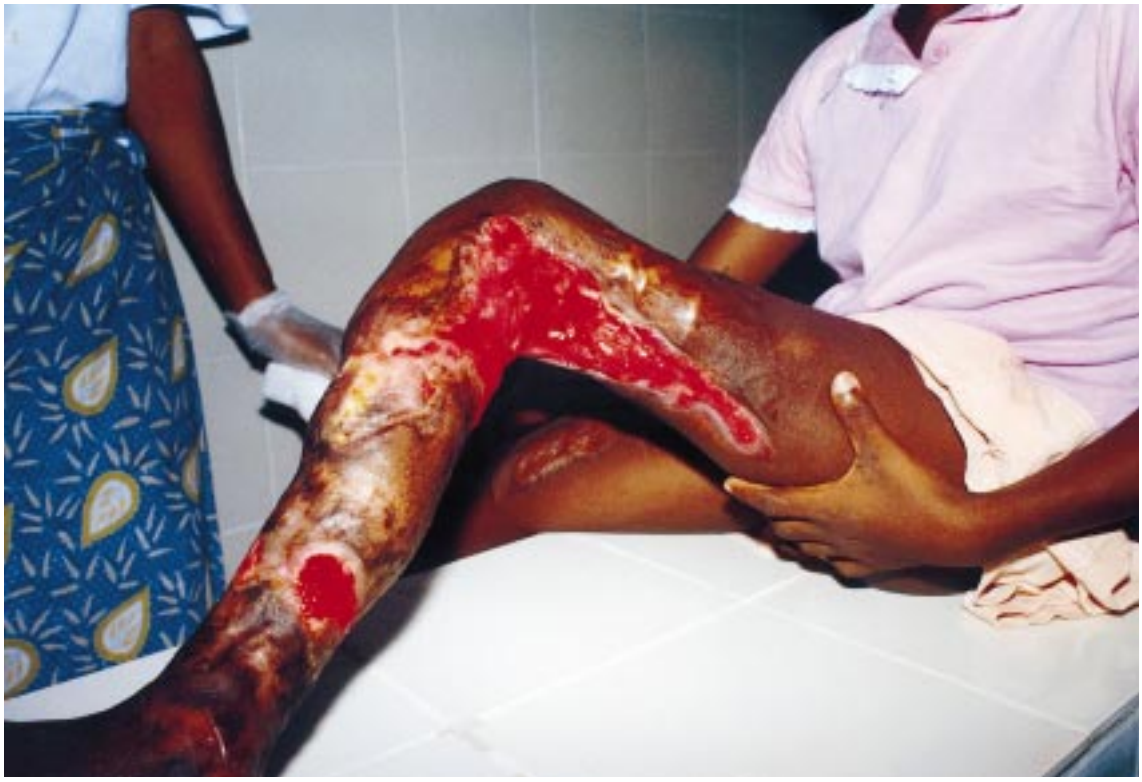


Plate 23. Ulcerative lesion (Photo: Marco Pirovano)

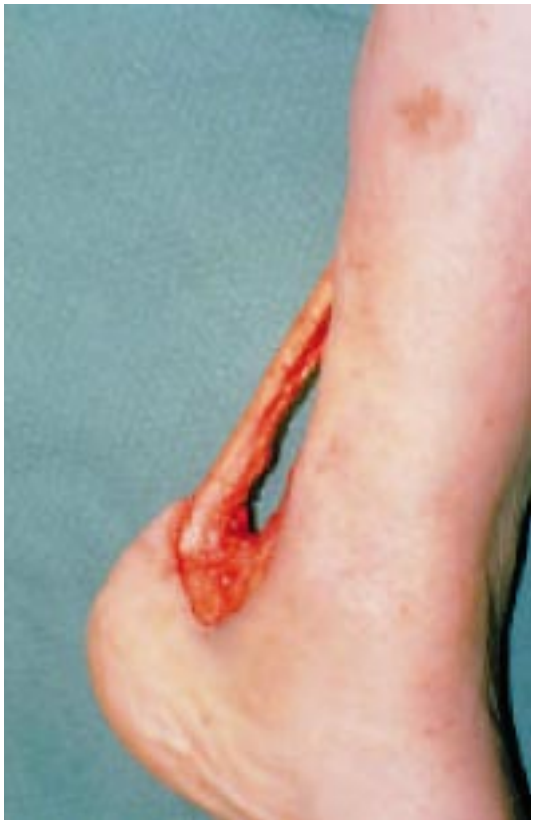


Plate 24. Ulcerative lesion (Photo: John Hayman)

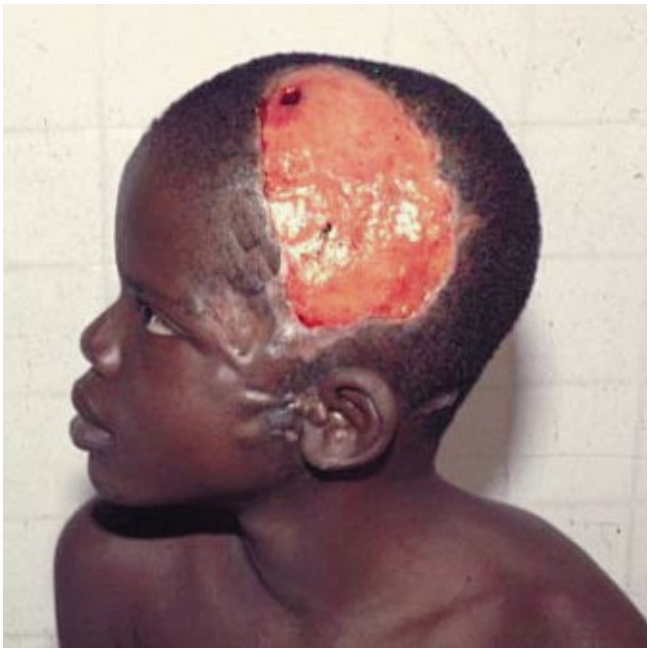


Plate 25. Ulcerative lesion (Photo: National Buruli Ulcer Control Programme, Côte d'Ivoire)

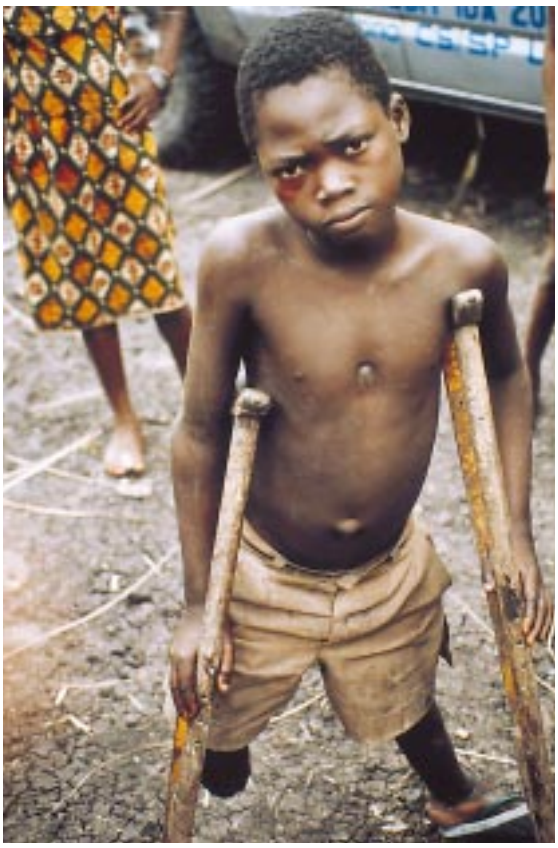


Plate 26. Postulcerative sequelae: amputation of the right leg of a young boy due to Buruli ulcer (Photo: Augustin Guedenon)

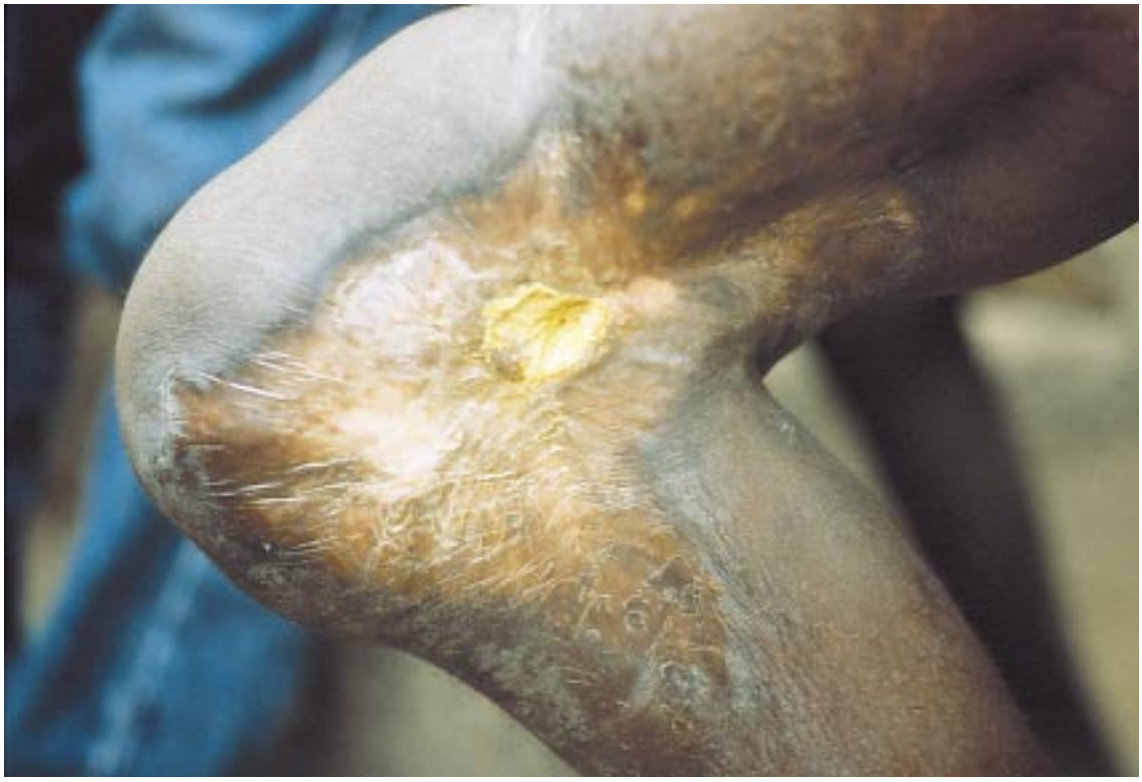


Plate 27. Postulcerative sequelae: contracture deformity of knee joint (Photo: Wayne Meyers)

Plate 28. Postulcerative sequelae: contracture deformity of left upper limb (Photo: Wayne Meyers)

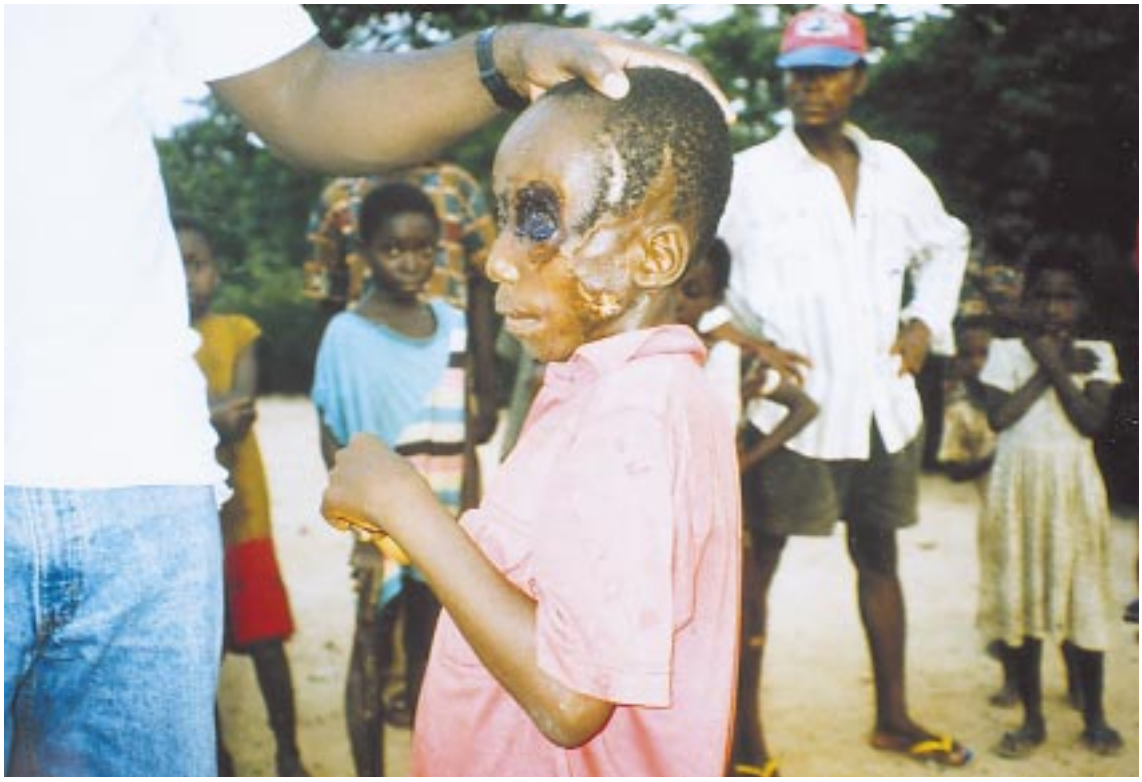


Plate 29. Postulcerative sequelae: an eye complication as a result of Buruli ulcer. The patient lost the left eye (Photo: Kingsley Asiedu)

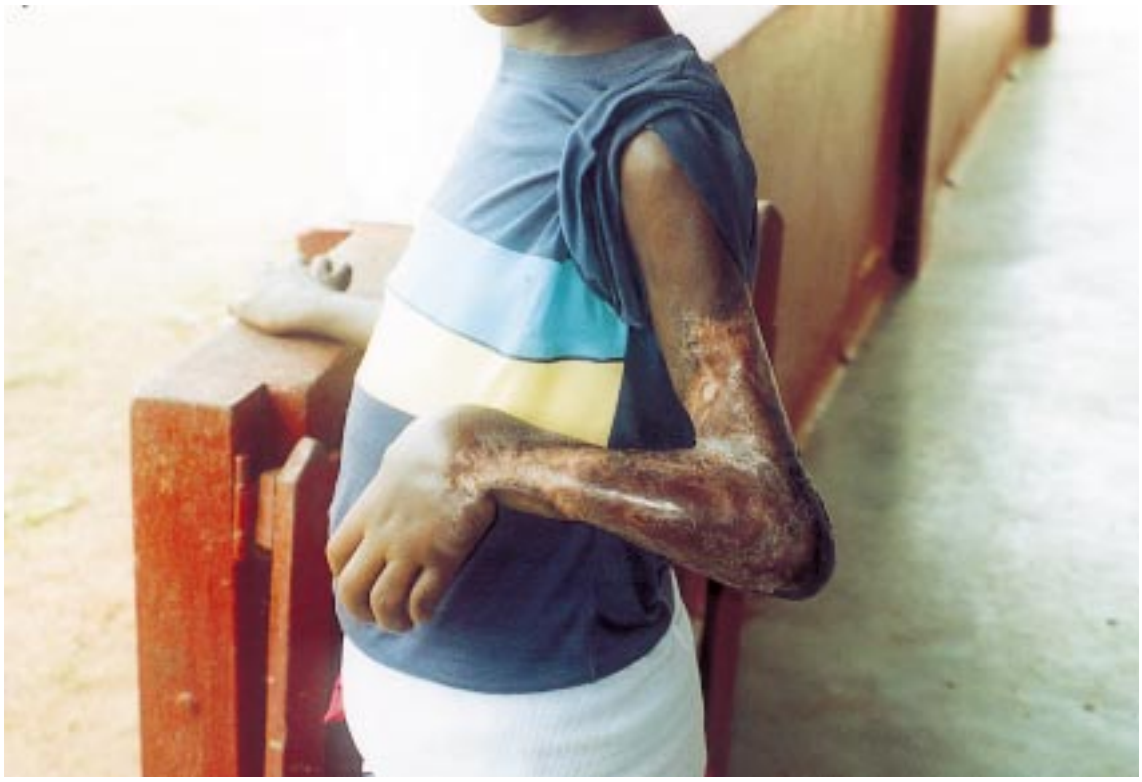


Plate 30. Postulcerative sequelae: contracture deformity of left upper limb (Photo: Kingsley Asiedu)

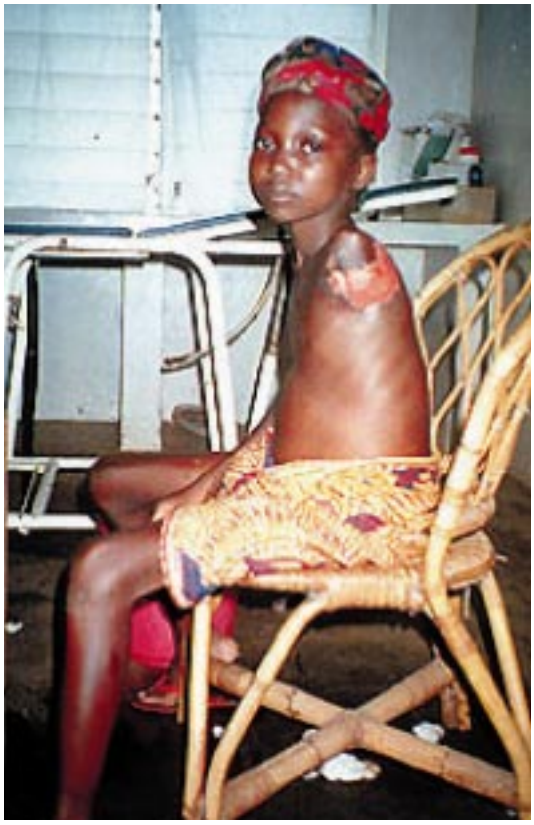


Plate 31. Postulcerative sequelae: disarticulation of the left upper of a young girl as a result of Buruli ulcer (Photo: Kingsley Asiedu)

Plate 32. A group of Buruli ulcer patients in Papua New Guinea. Note the age and sex distribution, typical adherent scars after excision and skin grafting, and the distribution of lesions. (Photo: John Hayman)

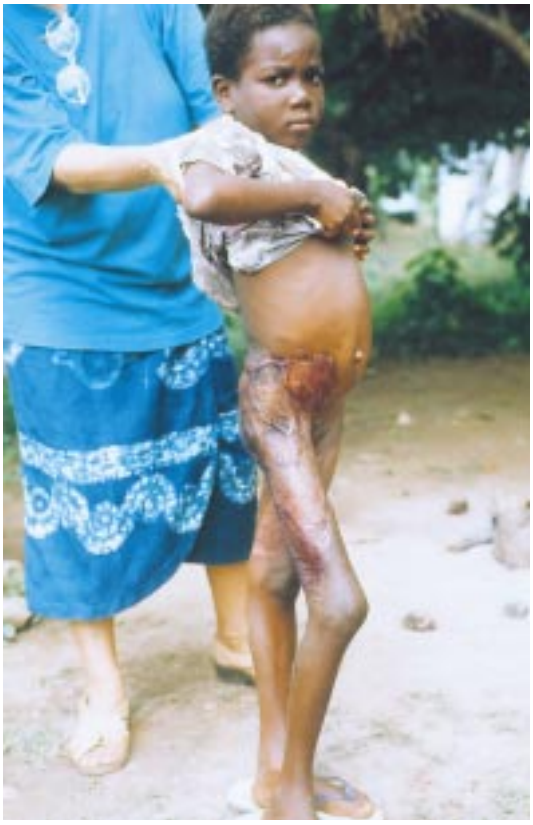


Plate 33. Healed Buruli ulcer after extensive excision and skin graft (Photo: Mark Evans)

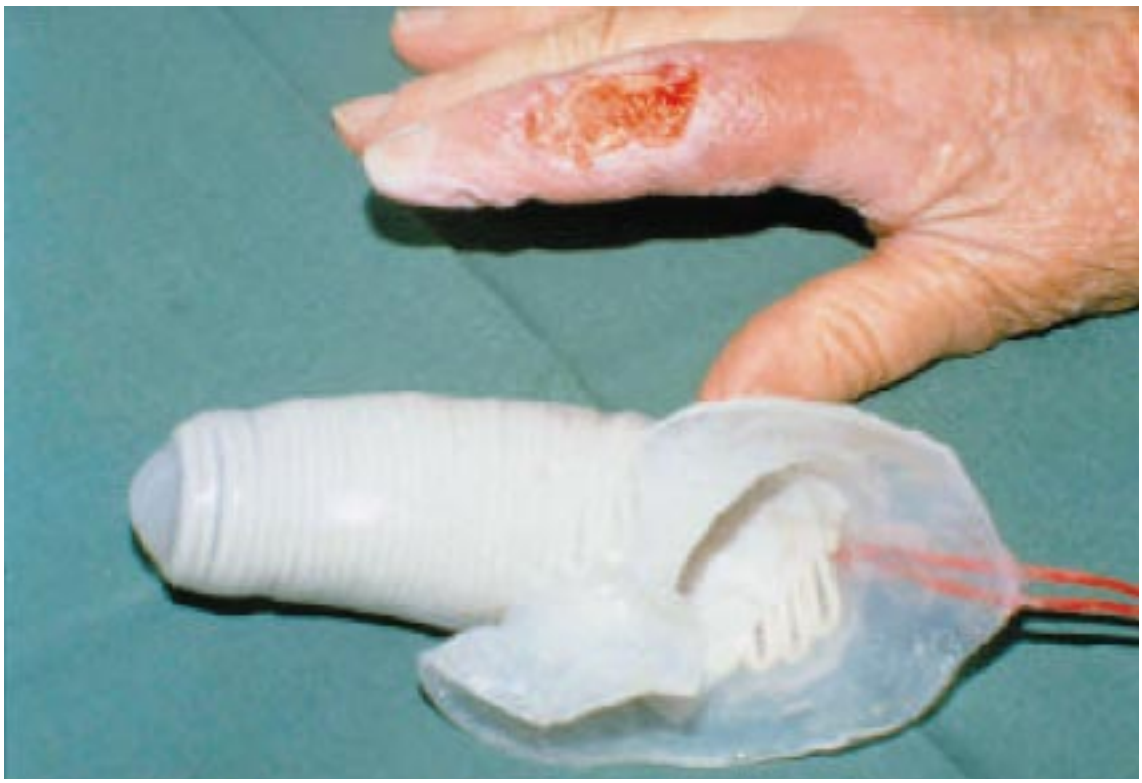


Plate 34. Application of heat in the treatment of Buruli ulcer (Photo: John Hayman)

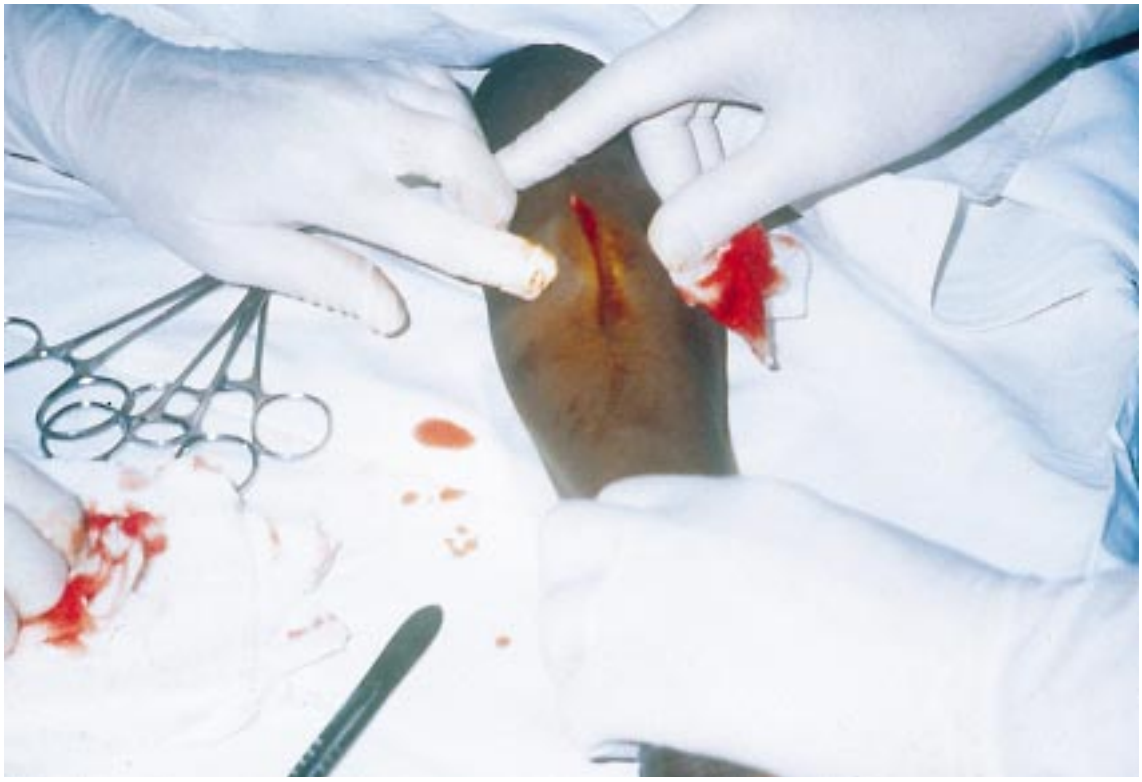


Plate 35. Excision of a nodule (Photo: Françoise Portaels)

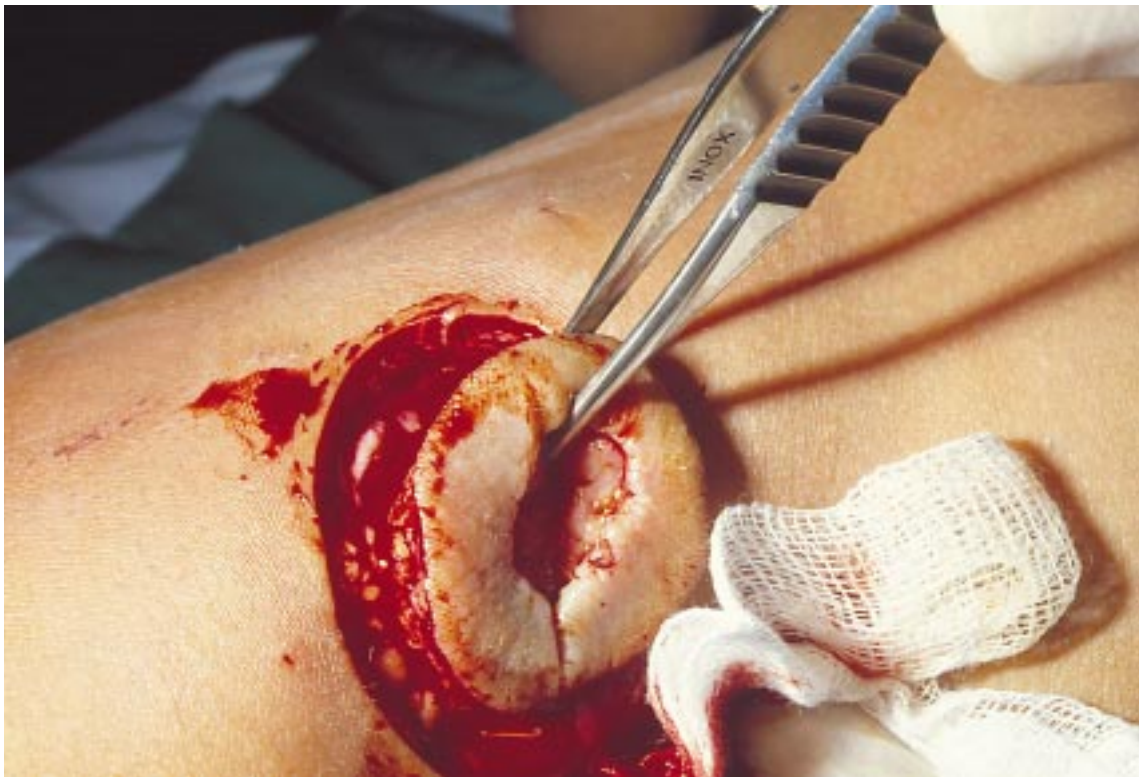


Plate 36. Excision of an early ulcerative lesion (Photo: Roger Pradinaud)

DIFFERENTIAL DIAGNOSIS

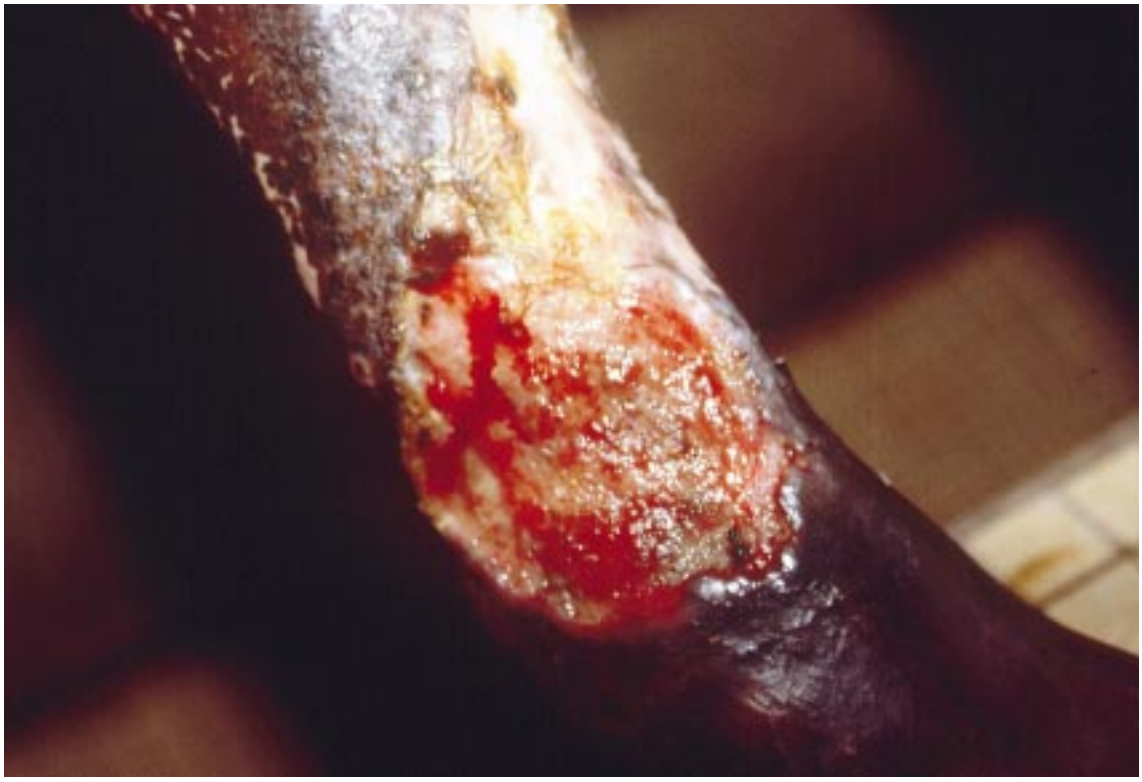


Plate 37. Tropical phagedenic ulcer. Lesions typically are painful, are malodourous and are located on the foot or lower leg. Ulcer margins are raised and firm, but not undermined. (Photo: Wayne Meyers)

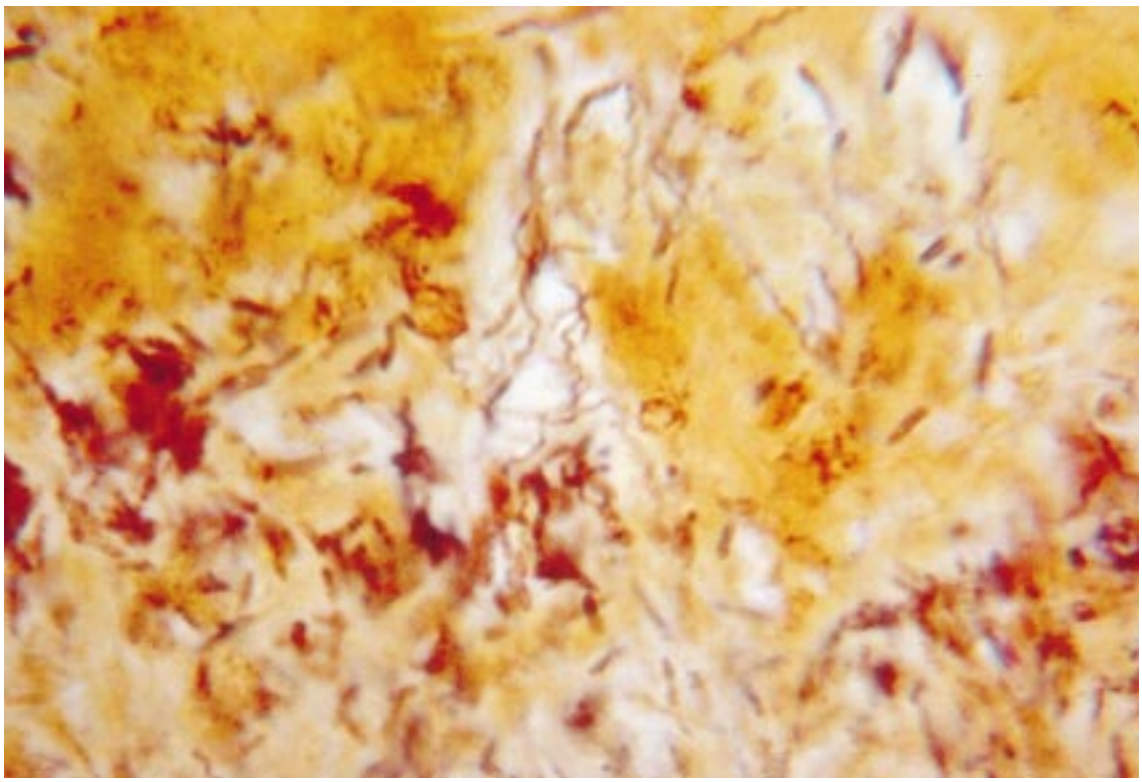


Plate 38. Warthin-Starry stained exudate of a tropical phagedenic ulcer showing the *fusospirochetal flora.* X 1260. (Photo: AFIP)

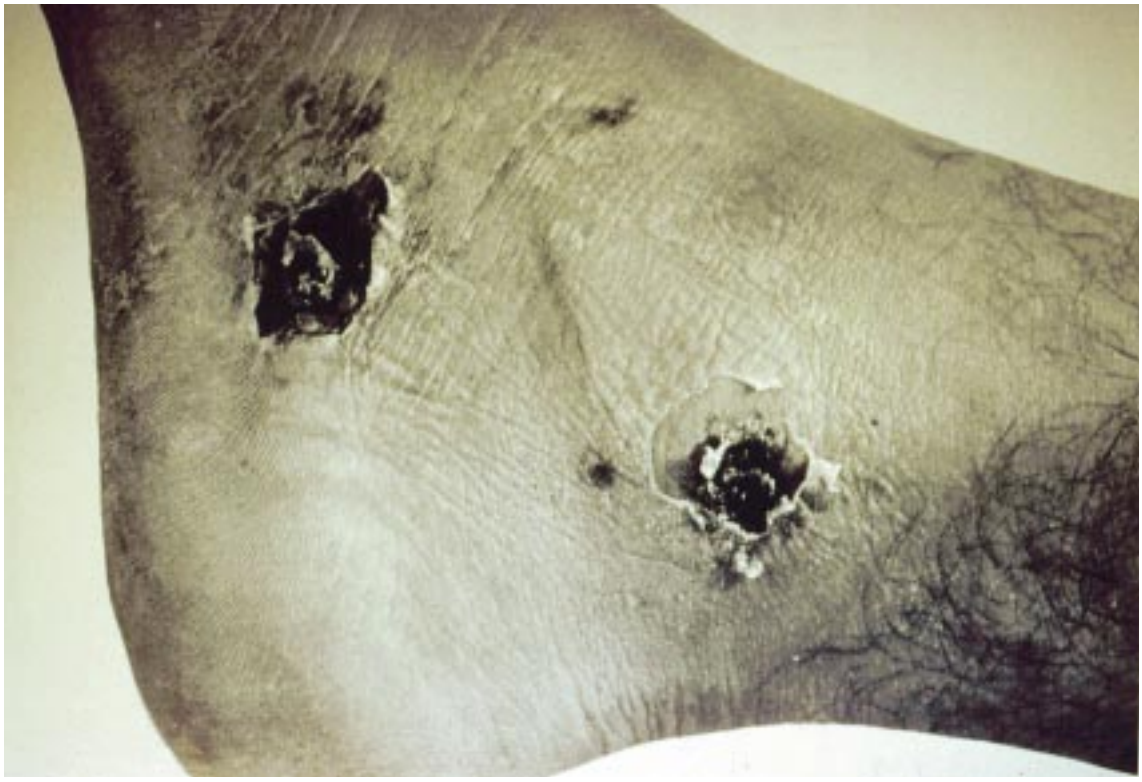


Plate 39. Ulcers of cutaneaous diphteria are shallow, angular shaped, and punched out. Edges slope inward and are often rolled. The etiologic agent, *Corynebacterium diphteria,* is demonstrable in tissue and exudates. (Photo: AFIP)

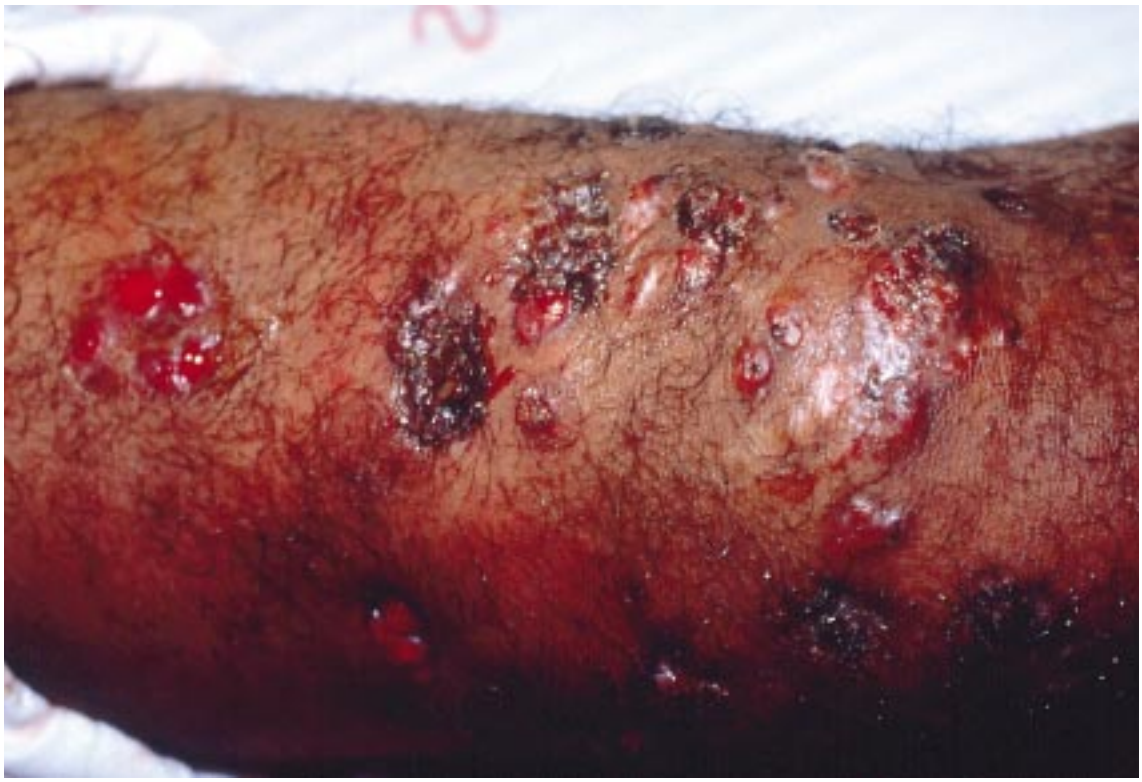


Plate 40. Actinomycosis of leg, most often caused by *Actinomyces israelii.* Infected area is indurated with scattered suppurative ulcers of fistulas. May be confused with disseminated or plaque form of Buruli ulcer. (Photo: Ken Wagner)

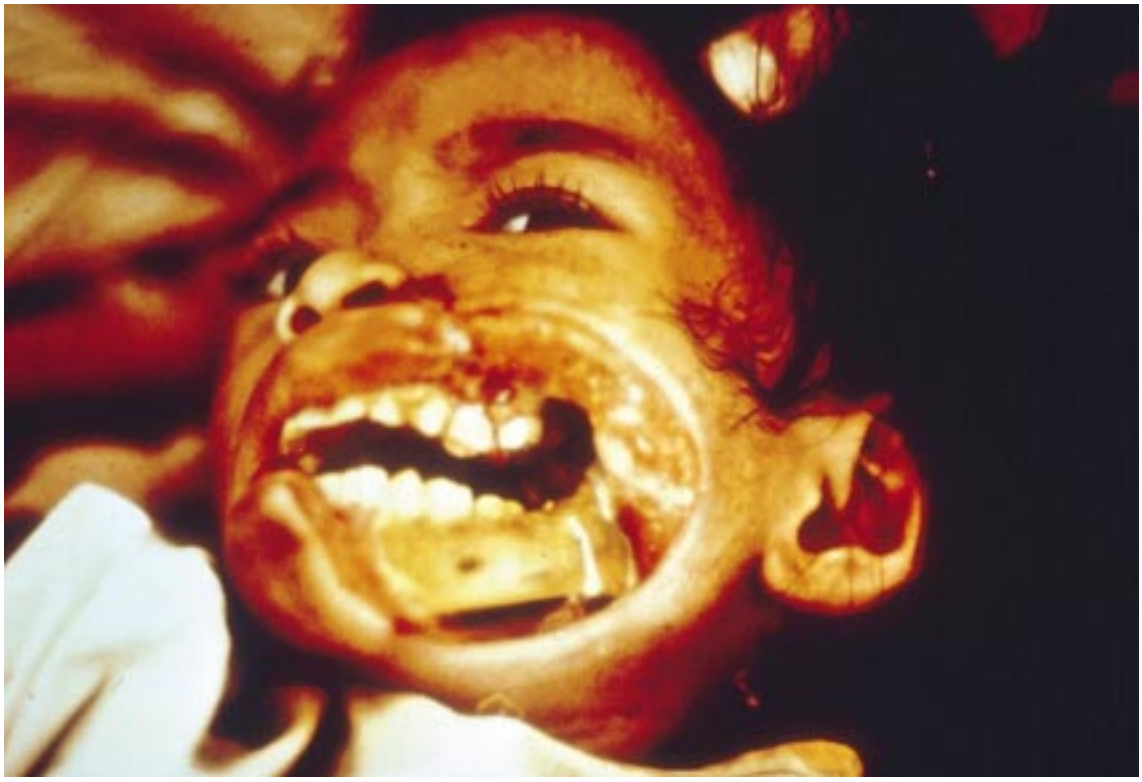


Plate 41. Noma (cancrum oris) in a child. Most noma lesions are on the cheeks and lips of malnourished individuals. Etiology is uncertain but fusobacillary organisms most likely play a role. (Photo: AFIP)

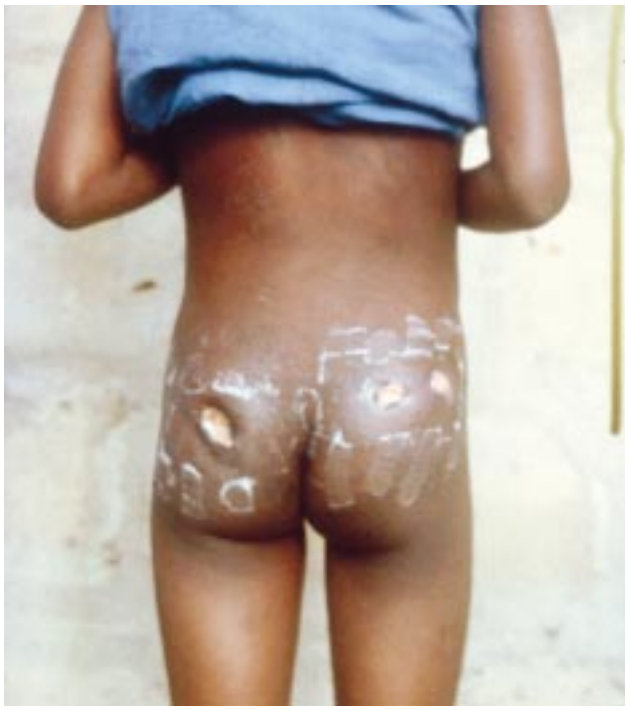


Plate 42. Ulcerated "cold abscesses" caused by *Mycobacterium abscessus*. Note that the lesions are seen at typical sites of intramuscular injections, and result from contaminated needles or syringes. (Photo: Daniel Fountain)

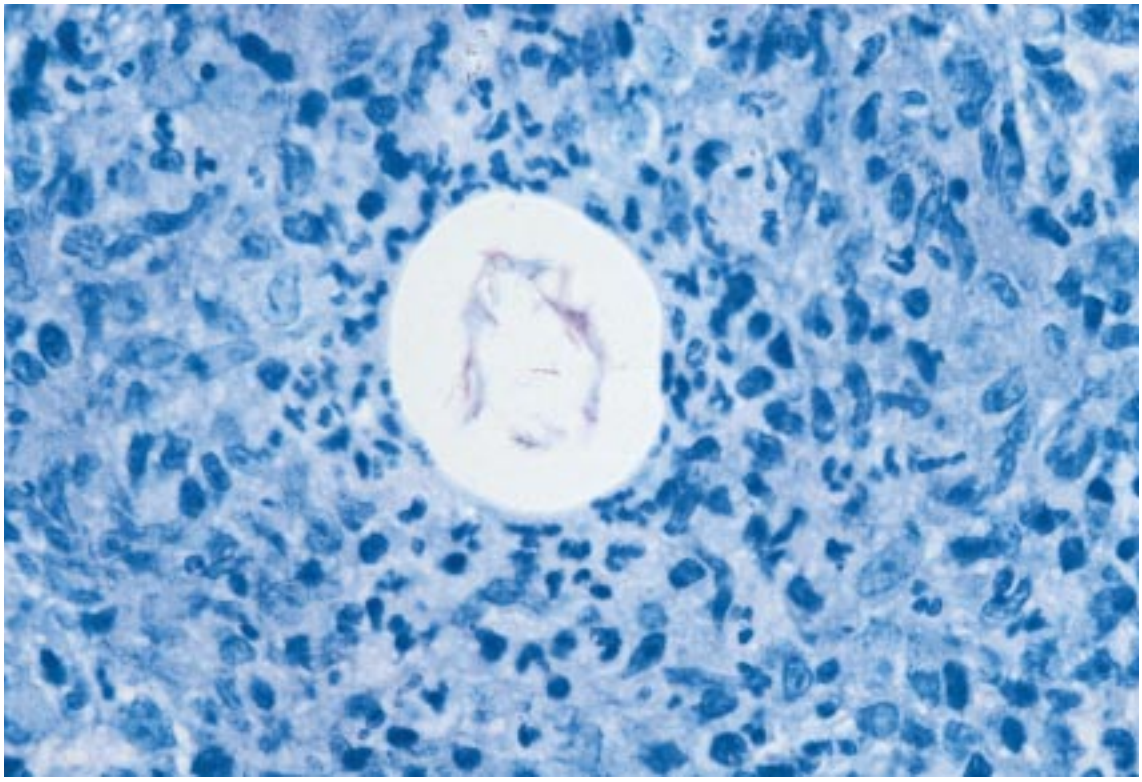


Plate 43. Microscopically, in *M. abscessus* lesions, acid-fast bacilli usually appear within vacuoles in a pyogranulomatous reaction. Ziehl-Neelsen stain, X 180. (Photo: AFIP)

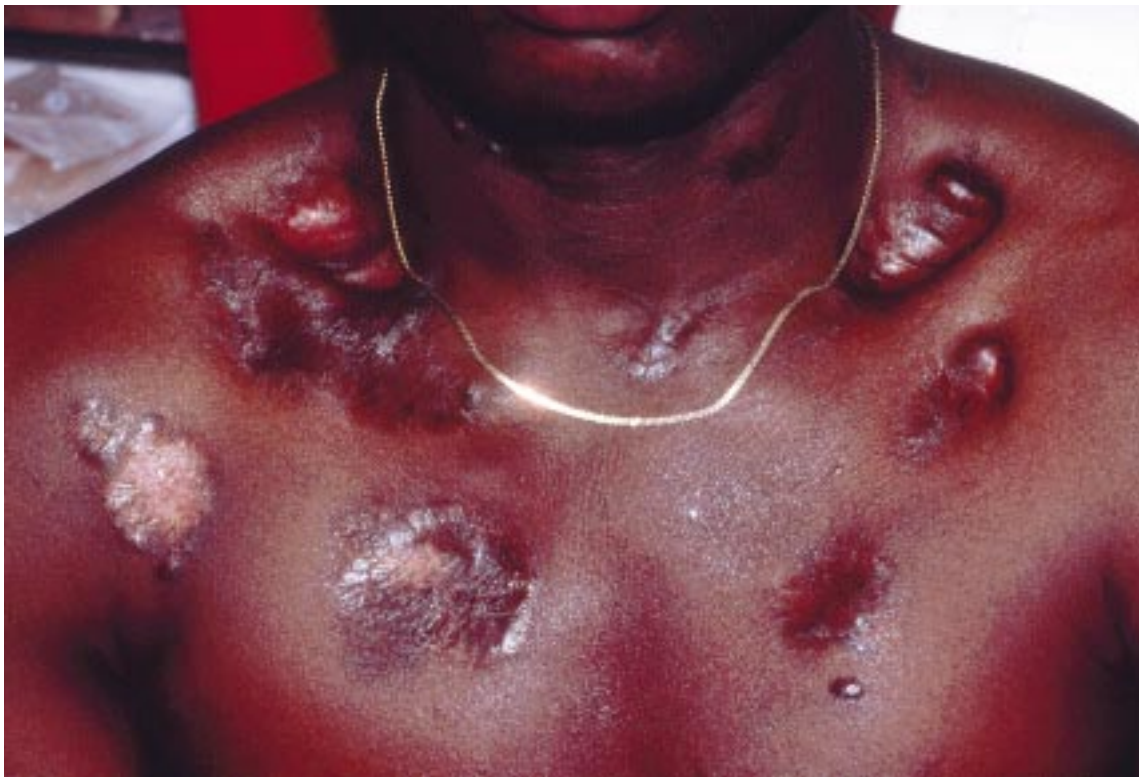


Plate 44. Scrofuloderma in a patient with tuberculosis. There are multiple sinuses in various stages of activity. (Photo: Peter Kern)

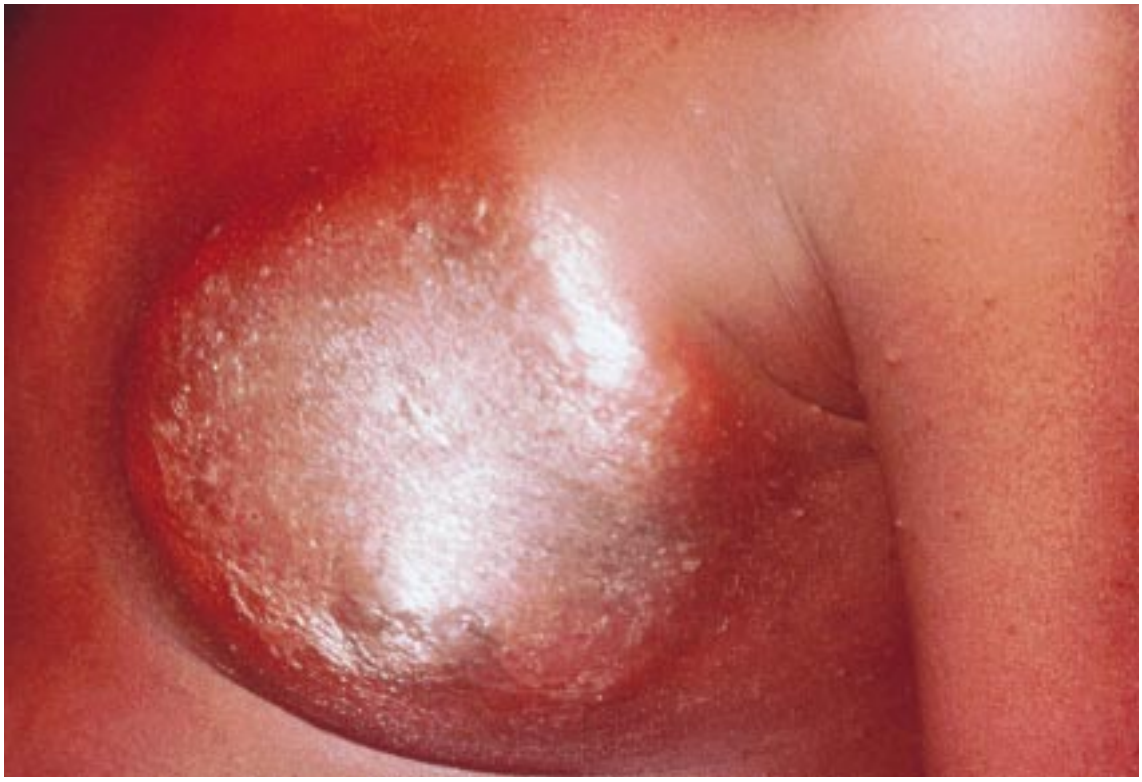


Plate 45. Subcutaneous phycomycosis in the skin of the chest of a patient. (Photo: Leo Lanoie)

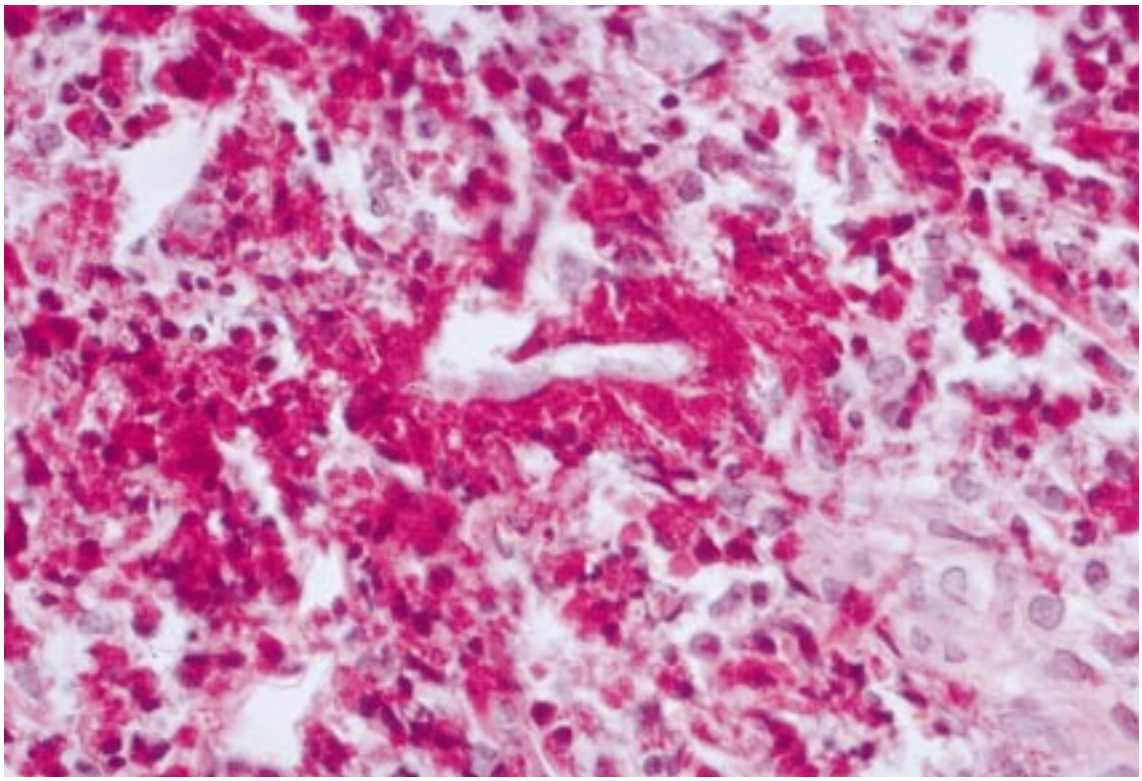


Plate 46. Microscopic view of subcutaneous phocomycosis (see Plate 45), showing massive infiltration of eosinophils and hyphae of the etiologic fungus, *Basidiobolus haptosporus.* Hyphae appear as empty spaces in the centre of the photo. (Photo: AFIP)

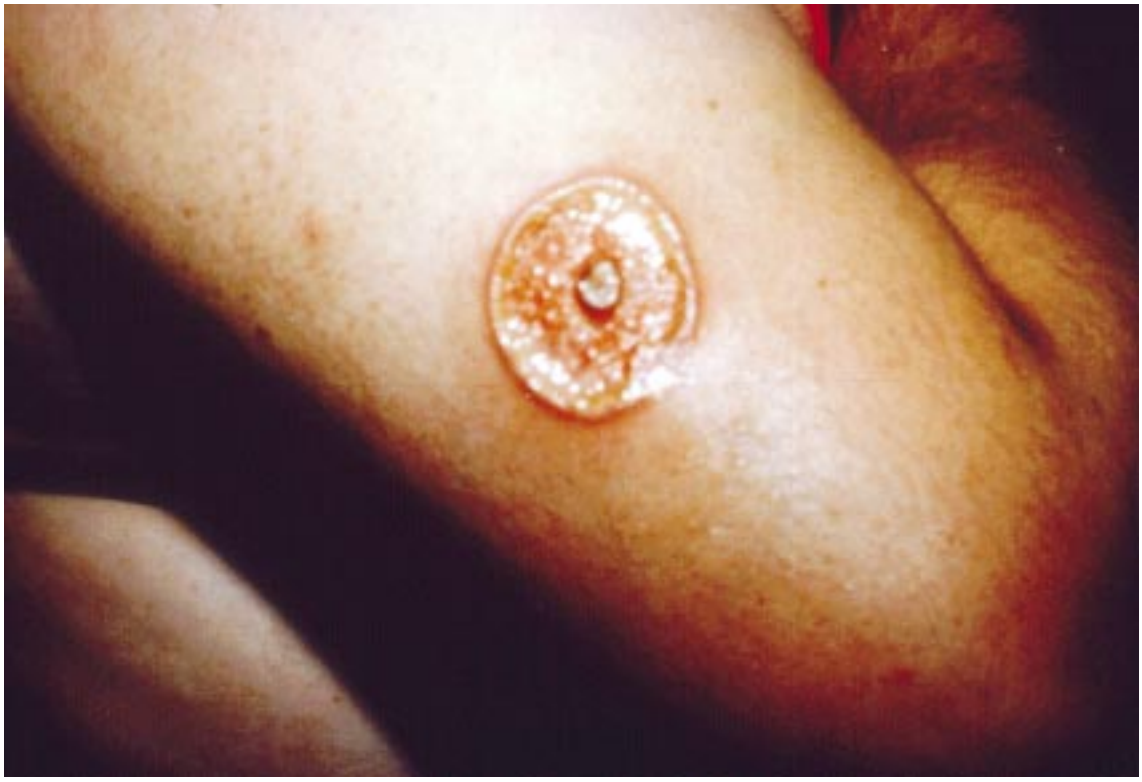


Plate 47. Chronic ulcer of cutaneous leishmaniasis. (Photo: Antonio Lauro Coscina)

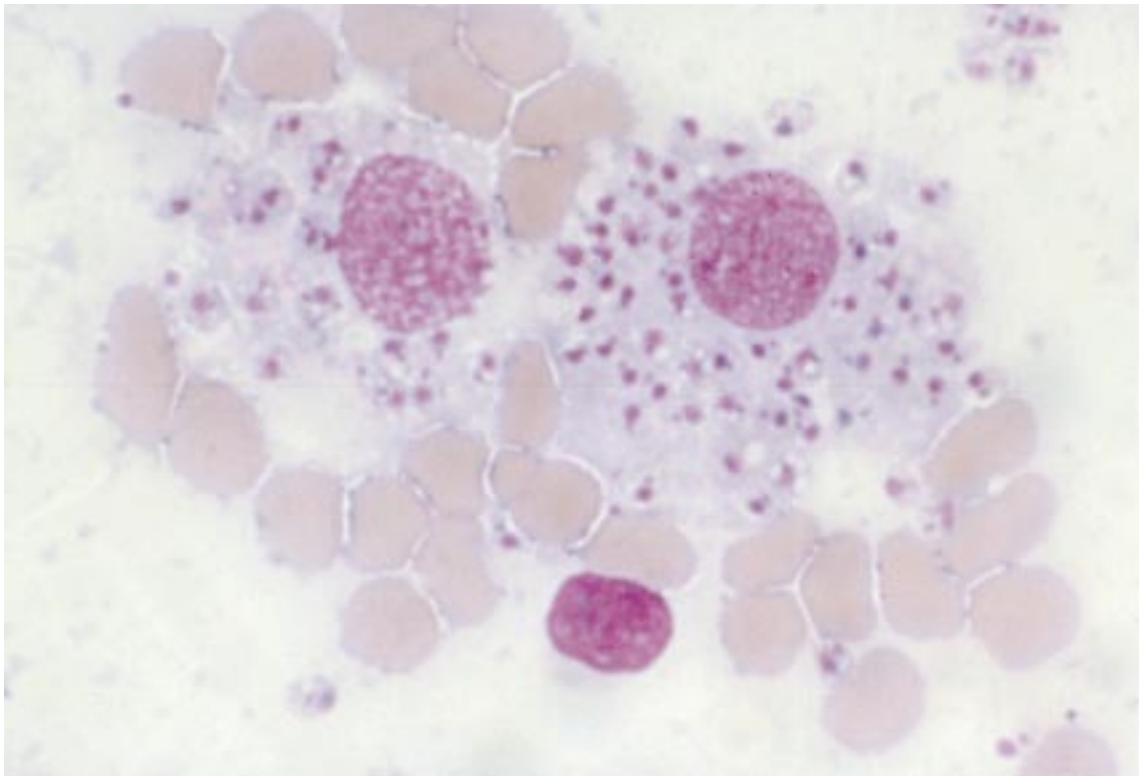


Plate 48. Smear from skin of lesion of leishmaniasis showing typical intracellular amastigotes of *Leishmania.* Giemsa stain, X 330. (Photo: AFIP)

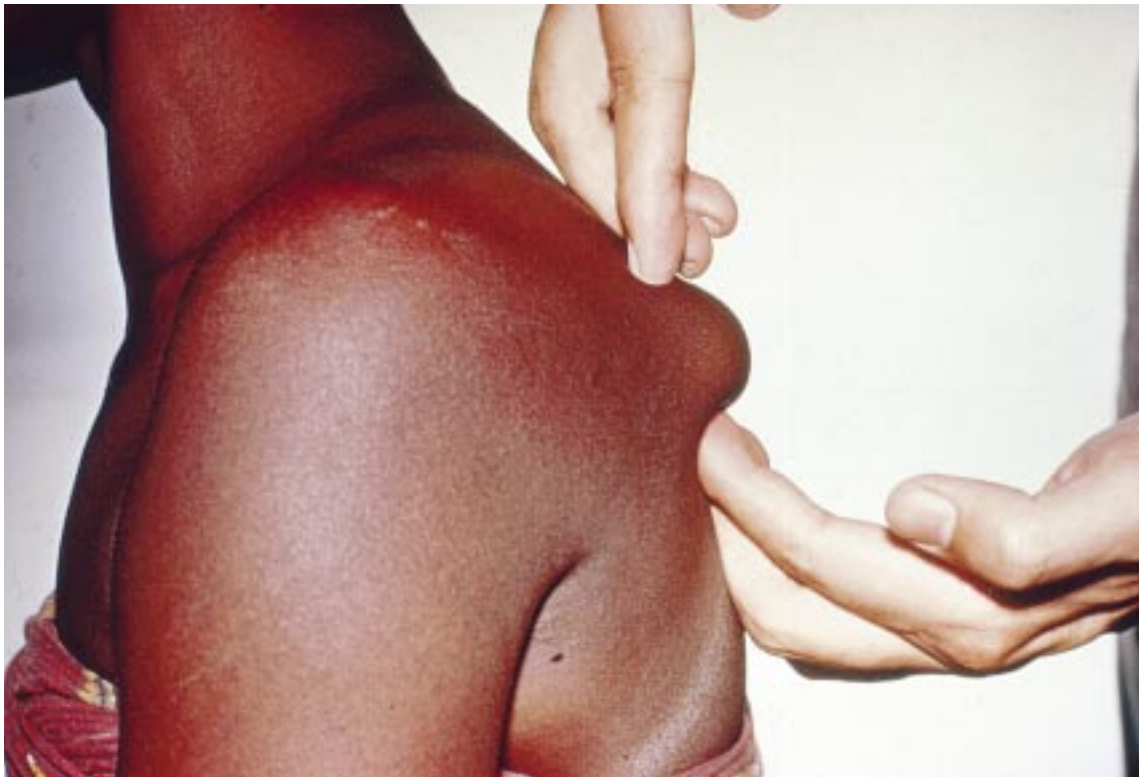


Plate 49. Nodule in skin and subcutaneous tissue over scapula a patient with onchocerciasis. This nonpainful movable nodule contains adult worms of *Onchocerca volvulus.* (Photo: Dan Connor)

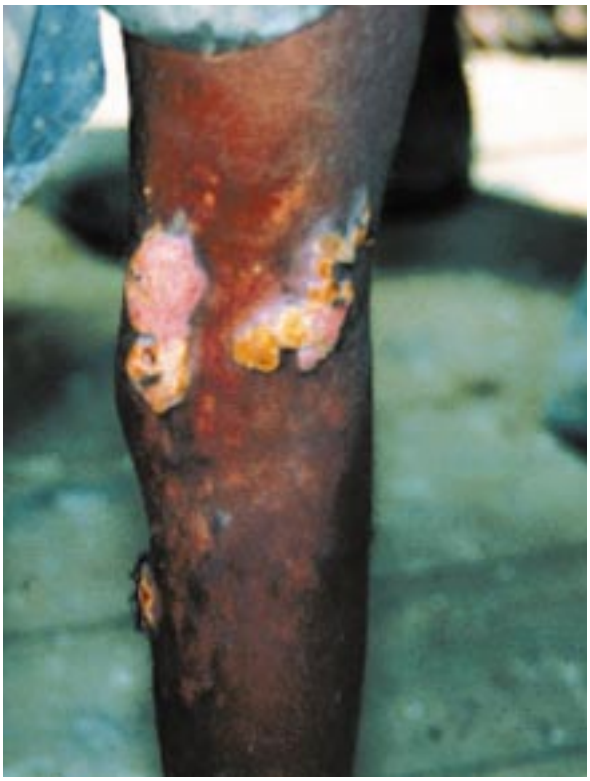


Plate 50. Yaws on the leg of a patient. Causative organism is *Treponema pertenue.* Lesions typically "grow out" compared to Buruli ulcer where the lesions "grow in". Treatment with penicillin is very effective (Photo: John Hayman).

Laboratory

Smears from the necrotic base of ulcers stained by the Ziehl-Neelsen method usually reveal clumps of acid-fast bacilli (Plate 1). Appropriately selected biopsy specimens that include the necrotic base and the undermined edge of lesions with subcutaneous tissue are nearly always diagnostic (Plate 4). *M. ulcerans* can be cultured from many lesions, either from exudates or biopsy specimens, but visible growth often requires 6–8 weeks of incubation at 32 °C. Molecular biological techniques, such as polymerase chain reaction (PCR), are often useful in establishing the diagnosis, especially when culture and histopathological analyses are negative for *M. ulcerans*.

Radiological

Radiology will frequently demonstrate calcification of subcuataneous fat, which may occur in association with a long-standing primary lesion. Osteomyelitis is becoming an increasingly common complication of the disease, especially in West Africa. Appropriate radiological studies will help confirm the diagnosis (Plate 7).

Organization of laboratory support

Four levels of laboratory support are needed to facilitate the diagnosis of Buruli ulcer. These laboratories must therefore be equipped accordingly to achieve their functions. These four levels and their minimum functions and resources are presented in Table 3.

Table 3. Organization of laboratory services

Level of laboratory support	Functions	Resources
1. International reference laboratories for mycobacteriology	●Ziehl-Neelsen staining ●Culture and sensitivity ●Histopathology ●PCR ●Immunology ●Training, and supervision ●Research	●Microbiologist ●Histopathologist ●Immunologist ●Technicians ●Equipment to carry out the designated functions
2. National reference laboratories for mycobacteriology	●Ziehl-Neelsen staining ●Culture and sensitivity ●Histopathology ●PCR ●Training and supervision ●Research	●Microbiologist ●Histopathologist ●Immunologist ●Technicians ●Equipment to carry out the designated functions
3. Regional laboratories	●Ziehl-Neelsen staining ●Culture ●Histopathology ●PCR ●Training and supervision ●Research	●Microbiologist ●Histopathologist ●Technicians ●Equipment to carry out the designated functions
4. District laboratories	●Ziehl-Neelsen staining ●Specimen collection	●Technicians ●Equipment to carry out the designated functions

Differential diagnosis (Plates 37–50)

In experienced hands and in endemic areas, the diagnosis of Buruli ulcer is usually clinically straightforward. Other conditions that must be considered in the differential diagnosis include the following.

- Tropical phagedenic ulcer
- Cutaneous diphtheria
- Actinomycosis
- Cancrum oris (Noma)
- *Mycobacterium abscess*
- Yaws
- Cutaneous leishmaniasis
- Subcutaneous phycomycosis
- Onchocerciasis nodule
- Scrofuloderma
- Lymph node
- Staphylococcal abscess
- Streptococcal infection of the skin
- Phaeomycotic cyst
- Spider or insect bite
- Vascular, diabetic or varicose ulcers
- Malignancy
- Burns
- Leprosy

Clinical management

Classification of the disease

The first classification of the disease was proposed by Muelder and Nourou (*36*). Stage I was the nodule, stage II cellulitis, stage III the ulcer, and stage IV scarring. This classification, though a good beginning, was considered incomplete because it did not capture other forms of the disease. In Australia, for example, papules rather than nodules are the norm. Osteomyelitis and the disseminated forms were not captured in this classification.

In view of these problems, another classification was proposed at the Yamoussoukro Conference (Table 4). Although final agreement on this proposed classification has yet to be reached, it provides a broader classification of the disease.

Table 4. Proposed clinical classification (CC) for the management of Buruli ulcer

CC1. Nonulcerative	CC2. Ulcerative	CC3. Postulcerative
1.1 Nodule/papule	2.1 Early ulcerative*	1.1 Scar without a sequela
1.2 Plaque	2.2 Late ulcerative*	1.2 Scar with a sequela such as contracture deformity, amputation, loss of organs, e.g. eye
1.3 Oedema	2.3 Involvement of organs, e.g. eye	1.3 Mixed
1.4 Disseminated	2.4 Osteomyelitis	
1.5 Mixed	2.5 Mixed	

**An early ulcerative lesion has a diameter of 2 cm including undermined edges and a late ulcerative lesion has a diameter of more than 2 cm including undermined edges.*

Antibiotic treatment

Treatment with antibiotics has usually been unsuccessful so far. Recent evidence suggests that rifampicin, amikacin and clarithromycin may promote healing in preulcerative and early ulcerative lesions, but they are often not effective in extensive lesions (*74*). Further evaluation of the effectiveness of these drugs and newer antimycobacterial antibiotics, especially in the nonulcerative and early ulcerative forms of the disease, is needed in view of problems that may be associated with surgical treatment (see below).

Heat treatment

Continuous local heating to 40 °C (e.g. by circulating water jackets) promotes healing even without excision, but must be applied constantly for 4–6 weeks (Plate 34). In addition, it is believed heat treatment improves blood flow, antibiotic penetration and phagocytosis (*47*,*75*). However, this type of treatment is often not practicable in many endemic areas.

Hyperbaric oxygen therapy

M. ulcerans grows best at lower oxygen tension. Hyperbaric oxygen treatment alone has inhibited lesions in a murine model of *M. ulcerans* infection, but not in a patient in which it was used as an adjunct to rifampicin and heat treatment (*75*,*76*).

Surgical treatment

Currently, surgery is the treatment of choice. However, several factors mitigate against this mode of treatment. These include inadequate surgical facilities and expertise in most endemic areas, the risks associated with surgery and problems with infections, including HIV. In general, surgery should be performed under general or regional anaesthesia. Excision of lesions should include a minimal amount of normal tissue according to the degree of induration. Preulcerative lesions are excised *en bloc*, and the skin is closed primarily (Plate 35). Ulcers are widely excised, and skin grafts applied (Plate 36). The use of a tourniquet, when available, is recommended. This

allows complete excision of the ulceration without bleeding. Tourniquet is released, and hemostasis checked, before dressing. On appropriate patients, excision and skin grafting can be done immediately, thus avoiding repeated surgery and anaesthesia and reducing superinfection and the duration of hospitalization. Most often, however, optimal results are obtained when grafting is performed after granulation has developed. Appropriate physiotherapy is mandatory when contracture deformities are likely to develop.

Recommendations for specific treatment of different forms of Buruli ulcer

Nodule. Depending on the location, the lesion should be excised and primarily sutured by a qualified medical doctor[b] or qualified health personnel[c].

Nonulcerated plaque. This should be surgically excised and a skin graft applied by a surgical specialist or a qualified medical doctor.

Oedematous. It is first advisable to rule out other bacterial infections, e.g. cellulitis. If the diagnosis is not certain, broad-spectrum antibiotics should be given for 7–10 days. Once the diagnosis is certain and the lesion can be defined by palpation, excision and skin grafting should be carried out by a surgical specialist or a qualified medical doctor. In some cases, incision with irrigation of necrotic tissues is possible.

Active ulcer. An ulcer up to 2 cm in diameter, including the undermining areas, and not superinfected should be excised and primarily closed, if feasible, by a qualified medical doctor or a qualified health personnel. An ulcer more than 2 cm in diameter, including the undermined areas, and superinfected should be excised by a qualified medical doctor without primary closure. Broad-spectrum antibiotic coverage is recommended, and skin grafting should be carried out after granulation tissue has been formed. An active ulcer close to an articulation or natural orifice must be referred to a surgeon.

Sequelae. Referral to appropriate specialist (e.g. plastic, orthopaedic, eye) for further management. Additional referral for provision of prosthesis, physiotherapy and rehabilitation is recommended.

Detection and management

Because about 70% of those affected by Buruli ulcer are children below 15 years, the detection of cases could be increased if the school system is involved in the control of the disease. In this context, six levels of detection and management are proposed.

- Community
- School
- Health centre
- District hospital
- Regional hospital
- Tertiary hospital

Some guidelines and minimum resources for the management of Buruli ulcer are given in Table 5.

[b] A qualified medical doctor is an non-surgical specialist who has substantial postgraduate experience in surgery.

[c] A qualified health personnel is a non-physician (e.g. medical assistant, nurse) who has been trained in minor surgical procedures including excision of nodules.

Table 5a. Detection & Management of Buruli ulcer

	Community	School	Health centre
Nodules & Papules	●Recognition and appropriate referral ● IEC**	●Recognition and appropriate referral ●IEC**	●Excision with primary suturing* ●Appropriate referral ●IEC**
Plaque	●Recognition and appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**
Oedema	●Recognition and appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**
Ulcer <2 cm	●Recognition ●Wound dressing* ●Appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**	●Wound dressing ●Antibiotic and anti-tetanus coverage ●Excision* ●Appropriate referral ●IEC**
Ulcer >2 cm	●Recognition ●Wound dressing* ●Appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**	●Wound dressing ●Antibiotic and anti-tetanus coverage ●Appropriate referral ●IEC**
Others including osteomyelitis and involvement of other organs e.g. eye, genitalia	●Recognition and appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**
Sequelae	●Recognition and appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**	●Recognition and appropriate referral ●IEC**

District hospital	Regional hospital	Tertiary hospital
●Excision with primary suturing ●Appropriate referral ●IEC**	●Appropriate management of referred cases ●IEC**	●Appropriate management of referred cases ●IEC**
●Excision & skin graft ●Physiotherapy ●Appropriate referral ●IEC**	●Appropriate management of referred cases ●IEC**	●Appropriate management of referred cases ●IEC**
●Excision & skin graft ●Physiotherapy ●Appropriate referral ●IEC**	●Appropriate management of referred cases ●IEC**	●Appropriate management of referred cases ●IEC**
●Wound dressing ●Antibiotic and anti-tetanus coverage ●Excision ●Appropriate referral ●IEC**	●Appropriate management of referred cases ●IEC**	●Appropriate management of referred cases ●IEC**
●Excision & skin graft ●Antibiotic and anti-tetanus coverage ●Appropriate referral ●IEC**	●Appropriate management of referred cases ●IEC**	●Appropriate management of referred cases ●IEC**
●Appropriate treatment* ●Appropriate referral ●IEC**	●Appropriate management of referred cases ●IEC**	●Appropriate management of referred cases ●IEC**
●Appropriate treatment* ●Referral for rehabilitation ●IEC**	●Appropriate management of referred cases ●Referral for rehabilitation ●IEC**	●Appropriate management of referred cases ●Referral for rehabilitation ●IEC**

** If capacity exists*
*** Information, Education, and Communication*

Table 5b. Some minimum resources needed for case management

Level/ Resources	Community	School	Health Centre
Personnel	●Trained community/ village health worker	●Teachers ●Students	●Qualified medical doctor ●Qualified health personnel e.g. medical assistant, nurse
Infrastructure	●Space provided by the community	●Existing school	●Existing health centre. May need to be upgraded to allow for minor surgical procedures, e.g. excision of nodules
Equipment	●Simple wound dressing set	●Nil	●Basic surgical set (see Annex 9)
Logistics	●Dressing materials ●Simple record-keeping books ●IEC materials	●Simple record-keeping books ●IEC materials	●Medical &surgical supplies ●Laboratory reagents ●Registration books ●IEC materials

	District Hospital	Regional Hospital	Tertiary Hospital
	●Qualified medical doctor ●Surgical specialist (s) ●Qualified health personnel e.g. medical assistant, nurse ●Physiotherapist	●Same as a district hospital	●Same as a regional hospital
	●Existing district hospitalwith a surgical unit equipped to do skin grafting. May need to be upgraded if such facilities do not exist ●Physiotherapy ●Rehabilitation	●Existing regional hospital with surgical unit equipped to do plastic and reconstructive surgery. May need to be upgraded if such facilities do not exist ●Physiotherapy ●Rehabilitation	●Existing tertiary hospital with surgical unit equipped to do plastic and reconstructive surgery. May need to be upgraded if such facilities do not exist ●Physiotherapy ●Rehabilitation
	●Dermatome & meshgraft	●Dermatome & meshgraft	●Dermatome & meshgraft
	●Medical & surgical supplies ●Laboratory reagents ●Register ●IEC materials	●Medical & surgical supplies ●Laboratory reagents ●Register ●EC materials	●Medical & surgical supplies ●Laboratory reagents ●Register ●IEC materials

Chapter 9

Prevention, Surveillance and Control

Dr George Amofah, Dr Mark Evans, Dr Jordan Tappero & Dr Kingsley Asiedu

The management of Buruli ulcer is recognized to be frustrating and often unrewarding. The chronic and often recurrent nature of the ulcer makes it expensive to manage both for the patient and for the health service providers. In the absence of an effective drug treatment of the disease, the need for the development of preventive and control strategies becomes even more paramount.

Unfortunately, very little is known about various aspects of the disease, notably the mode of transmission of its causative organism. This chapter attempts to bring together all relevant information on what is currently known about the disease, in order to suggest possible preventive and control measures.

Model for prevention and control

It is helpful under the circumstance to go back to the traditional model of looking at communicable diseases in terms of the agent, transmission route(s), environment and host factors and relate them to Buruli ulcer. Preventive and control efforts can be targeted at any of the above and often at more than one at a time.

The agent

The causative agent is the organism or factor responsible for the disease and, in the case of Buruli ulcer, it is *Mycobacterium ulcerans*. To cause disease the agent must find a way of being transmitted through the environment to a susceptible host. The agent must also be able to survive in the environment or host or both and multiply.

M. ulcerans multiplies asexually. The reservoir of infection is the patient or the environment, especially marshy soil and vegetation along slow-flowing streams and rivers. Its latency in the environment is not very clear.

Transmission

The mode of transmission of *M. ulcerans* is mostly speculative. What is certain is that the organism can be transmitted through any abrasion or breach of the skin. What is not certain is whether it can be transmitted directly through the intact skin or, say, after an insect bite. It is also not clear whether direct person-to-person transmission is possible.

It is known that once it enters the subcutaneous tissue of the host, *M. ulcerans* causes its pathology by producing a toxin.

Excising the preulcerative lesions before they ulcerate is known to be curative in most cases and, even when they recur, the crippling deformities characteristic of Buruli ulcer are usually averted.

The environment

Environmental factors often determine the success and severity of any infection, together with host factors. Burli ulcer is found in warm, humid environments especially in settings rich in vegetation and marshy soil or stagnant or slow-flowing water bodies. Damming of rivers and streams is known to create a favourable environment for the growth of the organism and thereby the disease it causes. Buruli ulcer shows some seasonality with increasing incidence during the rainy season, coinciding with the periods when farmers work in the fields.

Poverty is also an important environmental factor as it determines financial and geographical access to services, thereby influencing the morbidity due to the disease. Another important environmental factor is the level of education, which influences personal and environmental cleanliness, uptake of services and health educational messages.

Host factors

Even though people of all ages are susceptible to Buruli ulcer, children under 15 years are mostly affected. The sex distribution is about equal, although more males than females are affected among the young, while the converse is the case among adults. It is believed, however, that the above observation is likely to be due to differential exposure of the sexes to the organism in the environment rather than different susceptibility to infection.

Parts of the body exposed and prone to injury, such as the lower and upper extremities, are more susceptible to infection than more inaccessible parts like the trunk and armpits. Recurrences at the same or other sites are common, indicating that immunity to infection, if any, is minimal.

Preventive and control options

Any component of the disease chain, i.e. agent, host, transmission path or the environment, can be attacked to address the problem of Buruli ulcer, and preferably more than one at a time.

Immunity to infection can be boosted by vaccination if available. Unfortunately as of now there is no vaccine against *M. ulcerans*. The use of BCG might offer protection against Buruli ulcer (*61*) but the short duration of BCG protection would require repeated vaccination targeted at the population at risk group. Adequate nutrition is known to improve resistance to many infections but this has not yet been studied in Buruli ulcer.

Another primary preventive measure is avoidance of contact with the environment. This is obviously difficult to achieve, especially among farmers in endemic communities, as they will invariably come into contact with the organism in the environment as they go about their daily farming and other activities. The use of protective clothing to cover the exposed parts of the body may prove beneficial (*7*).

Health education on maintaining proper personal and environmental cleanliness, and the proper care of all abrasions and cuts, is also to be encouraged. Prompt treatment of injuries with antiseptic cream may also offer some protection, although this strategy has not been evaluated. Were an antitoxin available, it could be given once infection begins.

Health education at present should focus on early identification and reporting, so that pre-ulcerative lesions may be excised before they ulcerate. Close collaborative work with the school system and disease control programmes such as those on leprosy, Guinea worm, schistosomiasis, onchocerciasis and yaws could enhance the early detection of cases.

Addressing environmental factors is not easy. Efforts should be made to avoid creating artificial marshy environments through damming of rivers, especially in endemic or nearby communities. Provision of boreholes near where people live will reduce the frequency of contact with the organism in the environment. Buruli ulcer is not only a medical problem but a developmental one also. Consequently, much effort should be put into the general socioeconomic development of the area, including road construction and provision of educational and health facilities.

As most people in Buruli-endemic communities are poor, the need to provide free or heavily subsidized services for the management of Buruli ulcer cannot be overemphasized. Otherwise the whole purpose of educating people to report for early excision will be defeated, as they will not be able to access the services even when these are available.

Experience in Ghana has shown that a major way of preventing the horrible deformities associated with Buruli ulcer is the setting up of a system for identifying and excising pre-ulcerative Buruli lesions, preferably in all health facilities in endemic districts. A community outreach programme for this purpose (see below) is also an option. Such a system is usually combined with an information, education and communication package and a community-based surveillance system. Fortunately, medical assistants can be trained to perform the excision procedures, and thus the system should not depend solely on the availability of medical practitioners.

Summary of preventive and control measures for all endemic communities

1. Information, education and communication messages on:
- the disease;
- proper care of all injuries;
- proper personal and environmental cleanliness;
- wearing of protective clothing as applicable;
- avoidance of contact with swampy areas;
- early detection and reporting of **all skin lesions** to the nearest facility for screening.

2. Establishment of a community-based surveillance system.

3. Setting up of an accessible system for excision of all suspicious skin lesions before ulceration.

4. BCG vaccination of infants as part of an EPI programme.

5. Provision of boreholes in communities.

6. General socioeconomic development of the area.

7. Subsidized or free services for Buruli ulcer patients.

8. Rehabilitation of those already deformed by the disease.

Health education: example of ongoing work in Ghana

The outreach education and treatment programme for rural villages in Amansie West District, Ghana is a collaborative project between the Ghanaian Ministry of Health (Ashanti Region), the School of Medical Sciences at Kumasi and St George's Hospital Medical School, London, United Kingdom. The programme provides health education to promote the recognition of early lesions, and immediate excision of early nodules in the village itself.

Forty villages in the Amansie West District were selected and the following programme, carried out over two days in one village per week, was started in March 1998.

Day 1: health education

Colour posters are displayed in prominent positions around the village and in the evening, when the villagers have returned from their farms, a team consisting of a primary health care worker, a community nurse, an environmental health officer and a communicable disease control officer show a colour video explaining the clinical features and treatment. The simplicity of early treatment by nodule excision is contrasted with the extensive ulceration and limb deformities consequent on late presentation, and the importance of early referral is stressed by patients and health care workers. Music for the video has been specially written by a Ghanaian musician. After the video, a question and answer session is held.

Day 2: surgery

A small surgical team excises nodules in the village using sterile equipment and clothing supplied by St Martin's Hospital, Agroyesum, the local district hospital. Excised nodules are preserved for microbiological, histological and immunological studies.

Follow-up care

A nurse from the district hospital visits patients in their communities on the 3rd, 5th and 7th day postoperative to change dressings and remove stitches. Those with infected wounds are referred to the hospital for further management.

This programme is highly cost-effective. The average costs (direct and indirect) of treatment for Buruli ulcer care at St Martin's Hospital are US$ 783 per person (*9*). The total cost for the first 10 months of this project is estimated at US$ 16 365, consisting of US$ 6650 running costs and US$ 9715 initial one-off payments. Therefore, if only 20 patients were identified and treated, the project would still be cost-effective. In fact, 15 patients had their lesions excised in the first 10 visits and a further 11 were referred to hospital.

This is a practical and effective way of promoting early recognition of *M. ulcerans* infection, treating large numbers of lesions at low cost and extending health services to those in remote areas. It can easily be linked to the establishment of ongoing surveillance.

Strategies for Buruli ulcer surveillance

Public health surveillance is the systematic collection, analysis and timely dissemination of public health data for use in the planning, implementation, and evaluation of public health practice. Therefore, the purpose of Buruli ulcer surveillance is to identify where the disease is and who is affected (i.e. geographical distribution and disease burden) and to determine how prevention efforts should be directed. This information can then be used to identify health service and training needs, to establish epidemiological and laboratory research priorities, and to evaluate the success or failure of implemented control and prevention strategies.

Three factors mitigate against the effectiveness of a passive national notifiable disease surveillance system for Buruli ulcer: (a) the disease typically occurs in the most remote areas where villagers have little contact with the health care system; (b) the disease is often not considered by villagers to have an effective medical treatment; and (c) treatment is often sought from traditional healers. Before setting up a permanent, ongoing surveillance system, therefore it would be wise to start by conducting a survey to establish the extent and burden of the disease, in order to identify a more cost-effective strategy for active surveillance in selected endemic areas.

Fortunately, there is an existing programme in West Africa (Benin, Côte d'Ivoire, Ghana and Togo) that could serve as an ideal model for Buruli ulcer surveillance. Like Buruli ulcer, dracunculiasis (Guinea worm) presents as a cutaneous disease. The Guinea Worm Eradication Programme derives its success through an ongoing, active surveillance system, using village health workers trained in the accurate recognition of a cutaneous dracunculiasis. Patients are identified by village-based health workers who report their cases to a district coordinator. These surveillance data are then forwarded to the regional and national levels, where the information is used to monitor the effectiveness of the programme. The Guinea Worm Eradication Programme owes its success to the partnerships established between the communities where surveillance is conducted, ministries of health, the World Health Organization, nongovernmental organizations and the Global 2000 Programme. Other reasons for its success were the incentives provided to patients to report to the health facility for treatment and also to volunteers for reporting cases. To be successful, a Buruli ulcer surveillance system arising from the Global Buruli Ulcer Initiative could rely on this partnership strategy. In fact, Buruli ulcer surveillance could be less costly if it were conducted in partnership with the Guinea Worm Eradication Programme, making use of the existing surveillance infrastructure.

However, fail-safe measures would have to be in place to ensure that this strategy would not dilute the performance of the Guinea Worm Eradication Programme.

To conduct Buruli ulcer surveillance, reliable case definition is required. Case definitions developed in this book will be used together with forms given in Annex 7. A summary of recommended standard for Buruli ulcer surveillance is presented in Annex 8.

To initiate Buruli ulcer surveillance, health care facilities must be strengthened in order to provide treatment for all patients identified. Villages eligible for surveillance could be identified using the following methodology. The Geographical Information System (GIS) used by the Global 2000 Programme would be used to identify all villages and health centres in the four countries located within a certain distance of bodies of fresh water. The number of villages randomly selected for surveillance would depend on the resources available to support health care services for Buruli ulcer patients and the training needs for health care providers. Existing health care facilities with expertise in treating Buruli ulcer that are not selected for surveillance using this randomization process could be added to the surveillance sites.

Training materials would be developed in both English and French. Surveillance kits would also be developed for use by the village-based workers. These kits might contain pictures of Buruli ulcer and simple questionnaires to be administered by the workers. Training for village-based workers would be organized in each country and would include, where possible, a trip to a highly endemic area so that the workers could see cases first-hand and practise administering the questionnaire. Village-based workers would learn how to educate their fellow villagers about the early recognition of nodules and ulcers, and refer patients with nodules and ulcers for appropriate excision or wound care. Data would be collected regularly and reported to the ministry of health and to the Global 2000 Programme, using the mechanisms already in place for dracunculiasis surveillance.

Once the surveillance system is operational, a case–control study could be performed at the village level to identify modifiable risk factors for infection. Identification of risk factors would be used to develop prevention strategies, and these strategies could be monitored for their effectiveness with ongoing surveillance. Environmental samples (vegetation, soil and water) could also be collected to check for sources of *M. ulcerans*, and water samples could be tested for nitrogen level, the presence of indicator bacteria, pH and other variables associated with pollution. Finally, serum samples and clinical isolates of *M. ulcerans* could be obtained from consenting patients for the development of a serological diagnostic test for infection (with or without active disease), and clinical isolates of *M. ulcerans* could be collected for the development of an antitoxin or vaccine. When and if an antitoxin or vaccine should ever become available, these well characterized, highly seroprevalent populations who are at risk of developing Buruli ulcer disease would be ideal sites for a field trial of vaccine efficacy.

National plans to control Buruli ulcer

Organization

Based on the recommendations of the Yamoussoukro Conference, national plans should be developed along the following guidelines as outlined in Table 6. Efforts should be made to strengthen community-based surveillance system to increase early case detection.

Table 6. Development of national control programmes

Organizational level	Essential elements	Support
• National • Regional • District • Subdistrict • Community village	• Political commitment • Advocacy • Health education • Appropriate training • Adequate treatment facilities • Financial support • Early detection and treatment	• Financial • Educational materials • Transport • Equipment, including computers

Recording and reporting of Buruli ulcer cases

In order to ensure a uniform documentation of cases and to monitor and evaluate the performance of control programmes, a systematic way of recording and reporting of cases is needed. The initial proposed forms are shown in Annex 7. These forms may be used in whole or adapted to the needs of the country.

- **BU 01** is a clinical form and is intended to allow a detailed collection of information on each patient. It is recommended that this form is used at the district, regional and tertiary hospitals.
- **BU 02** is for the registration patients. This could serve as a district, regional and national register. It could also be adapted to serve the needs of lower levels e.g. health centre, community and school.
- **BU 03 to BU 05** are for reporting aggregated data on cases from the district through regional level to the national level and then to WHO.
- **BU 06 to BU 08** are for reporting aggregated data of treatment outcomes of cases from the district through regional level to the national level and then WHO.

Indicators

In order to adequately monitor and evaluate the performance of control programmes, some simple and measurable output indicators are needed. The following list of indicators may be used to monitor programme performance at the district, regional and national levels.

- Number of cases recorded over a period of time
- Proportion of various forms of the disease
- Proportion of cases confirmed by laboratory methods
- Ratio of nodules to ulcers
- Proportion of patients presenting with disabilities
- Proportion of patients with sequelae after treatment
- Recurrence rate
- Mortality rate

Chapter 10

Economic and Social Impact

Dr Kingsley Asiedu & Dr Samuel Etuaful

Economic cost

The economic costs of diseases are important for policy-makers in making decisions on the allocation of scarce health resources. They are also important for the patient in making decisions. Several factors affect decision-making on diseases. These include mortality rate, number of people affected, morbidity and economic losses associated with the disease. Diseases with a high mortality rate tend to attract more attention. Conversely, diseases that tend to affect the poor and have a low mortality rate are accorded less importance.

The economic cost of Buruli ulcer has not been adequately studied. One recent study on the economic and social cost of Buruli ulcer in the Amansie West district of Ghana shows that the impact is high (*10*). The main findings of the study are presented below.

Treatment costs

Direct costs referred to the cost of services provided during the period of hospitalization. These included the cost for hospitalization, surgery, laboratory tests, daily wound dressing, drugs and miscellaneous costs. They excluded the cost of labour.

Indirect costs were calculated based on the productivity losses incurred by the patient and the attending relative, the cost of feeding the patient and the attending relative, and miscellaneous expenses estimated at 25% of the sum of productivity losses and cost of feeding.

Based on the available data on 102 cases of Buruli ulcer treated at a district hospital in Ghana between 1994 and 1996, the average hospitalization was 130 days and the average total treatment cost per person was estimated at US$ 783 (direct cost US$ 234 and indirect cost US$ 549). The distribution of these costs is shown in Fig. 5.

The cost of drugs, dressing materials and hospitalization constituted 90% of the total direct costs. All of these costs depend on the period of hospitalization, which at present is prolonged.

The cost of feeding the patient and the attending relative constituted 49% of the total indirect costs, followed by productivity losses and miscellaneous. Productivity losses were low because the majority of the patients were children and were not considered to be in the labour force. As the disease begins to affect more adults, the productivity losses and economic costs would be higher. Feeding costs are an important consideration for hospitalized patients, especially when the period of hospitalization is prolonged.

Figure 5a. Distribution of direct costs (78)

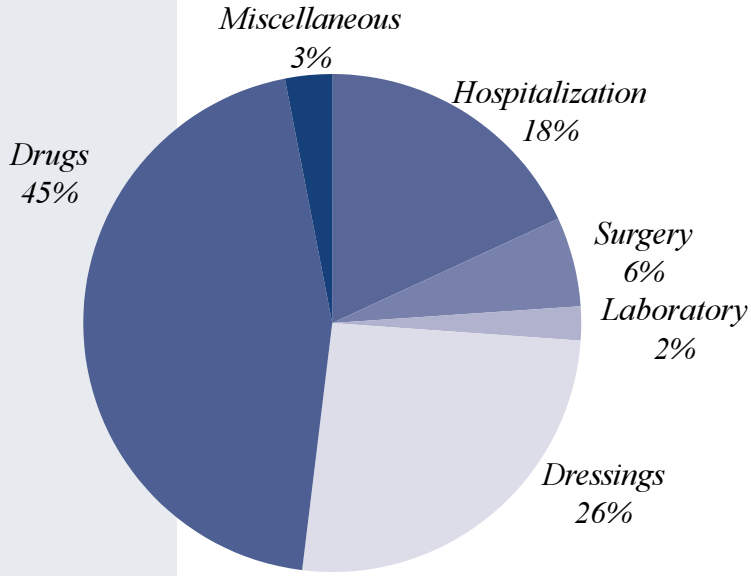


Figure 5b. Distribution of indirect costs

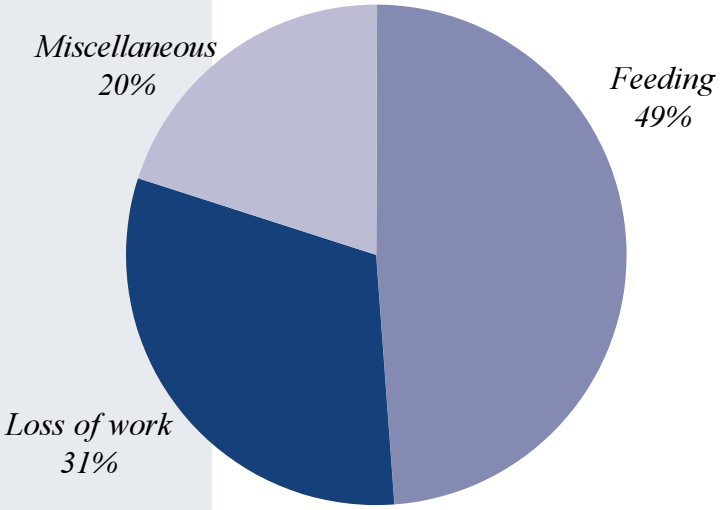
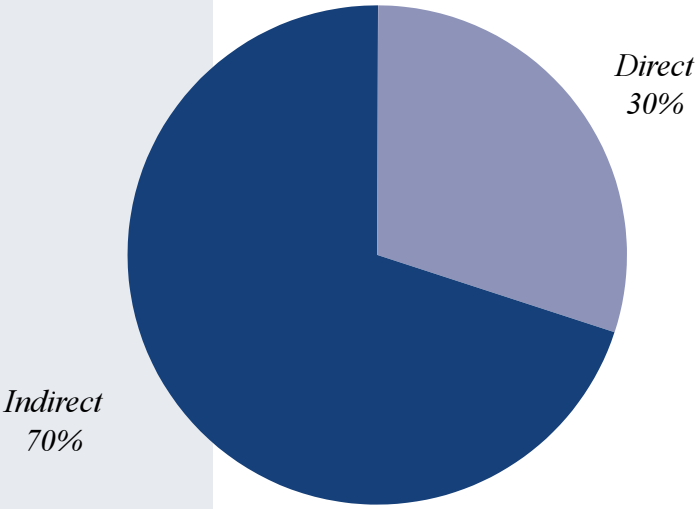


Figure 5c. Distribution of total costs



In conclusion, this preliminary study shows that Buruli ulcer is a very expensive disease. The average treatment cost per patient is more than what most people can afford, let alone the poor. The indirect costs constituted about 70% of total treatment costs despite conservative estimates of earnings at US$ 1 per day and the cost of feeding a patient of about US$ 1 per day. It is important that policy efforts to reduce the economic burden of Buruli ulcer focus on reducing the prolonged periods of hospitalization through early detection and treatment (see below).

Cost savings of an early detection and treatment programme

Preliminary analysis of the benefits of a programme to detect and treat cases shows that substantial amounts of money could be saved, in addition to alleviating the human suffering that is currently associated with the disease (*77*). Based on 102 cases treated at St. Martin's Catholic Hospital, Agroyesum, Ghana between 1994 and 1996, it was estimated that about 1% of patients present to the health services in the early (nodule) stage, while 79% seek treatment in the late stage when there is extensive skin ulceration requiring hospitalization of more than 100 days. Chronic functional disability (amputations and contracture deformities) affected 22% of the patients. In the current situation whereby the majority of patients seek care late, total costs of treating 100 cases of Buruli ulcer at this hospital were estimated at US$ 66 337 (direct cost = US$ 20 512 and indirect cost = US$ 44 825). Under an effective programme aimed at early detection and treatment, the total treatment costs could be reduced to US$ 15 383 (direct cost = US$ 5208 and indirect cost = US$ 10 175). The total potential saving of US$ 50 000 could buffer any reasonable programme). The complication rate could be reduced to almost zero. Given the growing number of cases in recent years and the potential savings that could achieved, as well as the reduction in human suffering, endemic countries should intensify efforts to detect and treat cases in the early stages.

Social impact

Impact on families

Buruli ulcer affects poor people in remote rural areas, where access to health services is difficult. Average hospitalization is about 3 months and, in most hospitals in developing countries, hospitalization of a patient requires the concomitant "hospitalization" of a healthy relative to provide indirect care such as cooking, washing of the patient's clothing, fetching of water, etc. Furthermore, in most hospitals patients must provide their own food. Clearly, the issue of hospitalization is a huge burden on the patients and their families. In countries without social programmes to take care of the disabled, families constitute the social safety net and, in the event of illness and disability, the burden falls on the poor family. The long-term care of people disabled by disease by the family could lead to considerable loss of productivity and greater poverty.

Impact on children

The indirect impact of chronic diseases on children is well known in the case of tuberculosis. The indirect impact of tuberculosis on children includes taking them out of school, either because they have the disease or because a parent suffering from the disease has become unproductive and therefore poor. The indirect consequences of sickness in adults are that children have to drop out of school or are forced to work to support the family. This could also happen with adults suffering from Buruli ulcer. Because hospitalization of a patient requires the need to have an attendant relative, usually a woman, another indirect effect on the children is the lack of parental care of those left behind at home.

About 70% of those affected by Buruli ulcer are children under 15 years. The direct consequences for children is two-fold. First, prolonged morbidity often lead to serious disruption of school or even discontinuation of schooling. Second, complications such as amputations and contracture deformities are frequent, and children disabled by the disease would not be able to work on the field. These children would grow into adulthood and become a burden to society.

Impact on women

The social impact on women of diseases such as tuberculosis and leprosy is well known. The full social impact of Buruli ulcer on women has yet to be ascertained. First, studies on the social consequences of tuberculosis in Pakistan indicate that this disease may lead to stigmatization, social isolation, diminished marriage prospects and divorce, particularly in women (*79*). Buruli ulcer, with its physical and cosmetic problems, could have similar repercussions. Second, the role of women in rural communities is enormous. They are involved in farming and other income-generating activities. These could be reduced considerably if they are left with a permanent disability as a result of the disease. Such disabilities could potentially limit their ability to carry out activities such as trading, farming, food preparation, obtaining water and breastfeeding. The inability to perform these tasks could negatively reduce their income-generating potential and the health and welfare of their children.

Chapter 11

Research Coordination

Dr Kingsley Asiedu & Dr John Hayman

Major research areas were identified by the WHO Task Force on Buruli ulcer in 1998. Of immediate importance are:

- Epidemiology of the disease (mode of transmission, risk factors, and the burden of the disease);
- Environmental factors that favour the emergence of the disease;
- Rapid methods of diagnosis;
- Drug treatment;
- Trial of serial BCG vaccination and vaccine development;
- Operational research.

Research coordination

International Mycobacterium ulcerans Study Team (IMuST)

To ensure international coordination of research into Buruli ulcer, WHO in 1998 established the working group called IMuST. It consists of scientists and clinicians interested in *Mycobacterium ulcerans* – its ecology, microbiology, toxicology and molecular biology, as well as the pathology, epidemiology, diagnosis, treatment and prevention of the disease it produces. Dr John Hayman, Box Hill Hospital, Box Hill, Australia is the coordinator of IMuST.

The main objectives of the WHO/IMuST are:

- Increase the awareness of *M. ulcerans* disease in the international medical community, monitor the incidence of the disease in all countries, and in particular ensure the rapid identification of new foci;
- Encourage and co-ordinate research activities on *M. ulcerans* infection, ensuring that there is maximum international collaboration;
- Propose consensus documents in specialized areas;
- Produce an accepted standard for grading and staging the disease, so that treatment modalities may be accurately compared.

Institutional collaboration

WHO intends to establish Collaborating Centres to support research and training into Buruli ulcer. Some of the objectives are:

- To provide a microbiological, molecular biological and histological diagnostic service on samples from patients or confirm the diagnosis from samples received from other laboratories.
- To provide data for epidemiological studies by analysis of environmental samples and by typing isolates from patients in different areas and foci of infection.

- To facilitate the adoption of standardized case definitions and standardized classification and staging of the disease, with a view to comparing the effectiveness of various treatment regimens.
- To provide a central registry of cases, with a view to rapid identification of new foci, documentation of variations in incidence, particularly in relation to intervention strategies and variation in seasonal conditions such as rainfall.
- To provide a repository of histological material, reference cultures and serological samples which will be available for scientific study through the International *Mycobacterium ulcerans* Study Team (IMuST).
- To support research into epidemiology, pathogenesis of the disease, prevention, treatment and support for patients with the disease.
- To facilitate exchange of information among researchers.
- To establish training programmes on Buruli ulcer for health professionals and researchers, particularly those from endemic countries as part of capacity building.

Institutions involved in Buruli ulcer research

The following are some of the institutions currently involved in research into Buruli ulcer. Most of these institutions have representation on the WHO Task Force on Buruli ulcer.

- Victorian Infectious Diseases Reference Laboratory, Melbourne, Australia
- Institute of Tropical Medicine, Antwerp, Belgium
- Armed Forces Institute of Pathology, Washington DC, USA
- Centers for Disease Control and Prevention, Atlanta, GA, USA
- Emory University, Atlanta, GA, USA
- Rocky Mountains Laboratories, Hamilton, MT, USA
- St. George's Hospital Medical School, London, England
- Bactériologie et Hygiène, Faculté de Médecine Pitié-Salpétrière, Paris, France

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Annex 1.

Recommendations and proposed plan of work of the first meeting of the WHO Task Force on Buruli Ulcer, Geneva, Switzerland, 16–18 February 1998

In February 1998, the first meeting of the WHO Task Force on Buruli Ulcer was held at WHO headquarters in Geneva. The Task Force recommended that WHO should:

1. establish a Global Buruli Ulcer Initiative and advocate for support to endemic countries;
2. establish a Global Buruli Ulcer Working Group composed of world experts on the disease, to be named the International *Mycobacterium ulcerans* Study Team (IMuST), which should seek to develop control and research activities and help coordinate the world's efforts against Buruli ulcer;
3. identify 2–3 endemic countries and perform situational analyses in order to have a clear understanding of current efforts and the additional resources that will be needed to fight the disease;
4. support the selected countries to establish centres where adequate management of patients can be provided;
5. once the centres have been established and proper training of staff has been accomplished, undertake surveys to determine the burden of the disease;
6. assist in the implementation of adequate control measures aimed at early detection, standard case definitions for surveillance and clinical management of patients, and develop standard guidelines for treatment and referral of patients;
7. support research in the following priority areas:
   i. assessing the operational steps in the implementation of adequate control measures;
   ii. understanding environmental changes that favour emergence of the disease;
   iii. defining the chemical structure of the toxin;
   iv. refining DNA fingerprint techniques to study the mode of transmission and the behaviour of the organism;
   v. determining the activities of known antimicrobial drugs on *M. ulcerans*, starting with animal models;
   vi. establishing international diagnostic and reference laboratories to support research by facilitating exchange of materials;
8. encourage and coordinate ongoing research and facilitate exchange of information among researchers.

Proposed plan of work

The Task Force proposed a plan of work addressing in general the following areas.

1. Support to endemic countries to strengthen the capacity of their health systems to effectively deal with Buruli ulcer (establish adequate surgical and laboratory facilities in strategic locations throughout the countries).
2. Appropriate training of health care personnel in critical areas such as surgery, pathology, microbiology, epidemiology and physiotherapy.
3. Early detection of cases by strengthening the surveillance and health education systems in collaboration with other programmes especially at the community level.
4. Clinical management of patients using standard treatment guidelines, i.e. common case definitions and categorizations.

5. Clinical and basic science research, particularly in the areas of drug treatment (starting on mouse models and later on humans), fingerprinting (with the aim of allowing the needed epidemiological data to clarify the mode of transmission), environment (with the aim of understanding the recent emergence of cases), toxin and immunotherapy (with the aim of purifying the toxin and its chemical structure in order to begin immunotherapy studies).
6. Establish three international diagnostic and reference centres to support research efforts, and also to serve as strain and histopathology specimen banks to be available to all researchers.

Annex 2

Report on the First International Conference on Buruli Ulcer Control and Research, Yamoussoukro, Côte d'Ivoire, 6–8 July 1998

Objectives of the Conference

In view of the growing public health importance of Buruli ulcer and the little attention paid to the disease in the past, the Conference was organized to bring together world experts and decision-makers who could contribute to the fight against the disease.
The primary objectives of the Conference were:

1. to raise awareness of the significance of the disease globally;
2. to increase recognition of the disease among donor agencies, WHO partners and non governmental organizations, and to seek support for endemiccountries to deal with the disease;
3. to review the current scientific knowledge on Buruli ulcer;
4. to gain consensus on WHO's proposed plan of work for control and research; and
5. to adopt a declaration on Buruli ulcer.

Co-sponsors

The Conference was co-sponsored by the World Health Organization, the Government of Côte d'Ivoire; the Sasakawa Memorial Health Foundation, Japan, the Association Française Raoul Follereau, the Damien Foundation, Belgium and the Humanitarian Aid Relief Team, Provo, Utah, USA.

Participants

The Conference was well attended, attracting over 200 participants from more than 20 countries. Among the participants were Presidents Henri Konan Bedié of Côte d'Ivoire, Jerry Rawlings of Ghana and Mathieu Kerékou of Benin; the then Director-General of WHO, Dr Hiroshi Nakajima; the WHO Regional Director for Africa, Dr Ebrahim Samba; WHO Representatives from Benin, Côte d'Ivoire, Ghana, and Togo; WHO Secretariat; Mr Michel Récipon, President of AFRF; ministers of health; members of the Diplomatic Corps; representatives of other United Nations bodies in Côte d'Ivoire; representatives of donor agencies, nongovernmental organizations and the private sector; clinicians and scientists, and the general public.

Highlights of the Conference

The signing of *The Yamoussoukro Declaration* on Buruli Ulcer by the Presidents of Benin, Côte d'Ivoire and Ghana, and by the Director-General of WHO, served to stimulate all the participants and to spawn hope that such proclamations will be followed by meaningful actions. The Declaration also served to back WHO's efforts to address the disease. The speeches of the three Presidents emphasized the important link between health and overall development. The Director-General's speech stressed the need to simultaneously address traditional diseases such as malaria and tuberculosis, as well as emerging diseases like Buruli ulcer, if international efforts to control communicable diseases in general are to have any significant impact in the 21st century. The final resolution of the Conference is presented in Annex 4.

Activities of nongovernmental organizations

1. The President of AFRF, Mr Michel Récipon, pledged the commitment of AFRF to collaborate with the WHO Global Buruli Ulcer Initiative in the control of Buruli ulcer in Benin and Côte d'Ivoire.
2. MSF Luxembourg announced that it has already started a Buruli ulcer project in Benin.
3. HART presented its activities in Ghana.

Presentations from endemic countries

Presentations by health workers from Benin, Côte d'Ivoire, French Guiana, Ghana and Togo served to focus attention on both their common and specific problems. These presentations also served to introduce these workers to the participants – a most important outcome. Following this introduction, scientists, clinicians and other health professionals were more readily able to discuss their ideas with personnel from the field.

Annex 3

The Yamoussoukro Declaration on Buruli ulcer

1. We, the participants at the Conference on Buruli Ulcer Control and Research, recognize that Buruli ulcer (also called Bairnsdale ulcer in Australia), the third most common mycobacterial disease after tuberculosis and leprosy, is a foremost cause of human suffering. In West Africa, in particular, the disease is rapidly emerging as a serious public health problem.

2. Buruli ulcer affects, primarily, poor communities, and especially children and women with limited access to health care. We are concerned that with the increasing number of cases and the associated health complications, Buruli ulcer could hinder efforts to improve the economic and social development of the communities most affected.

3. We are deeply concerned that very little is known about this disease. We believe that multidisciplinary research, including vaccine and drug development, could result in minimal non-invasive treatment of the disease. We recognize that early detection and early surgical treatment before ulceration are curative and avoid the complications currently associated with the disease.

4. We further recognize that, because the people most affected have limited access to health care and usually seek help at an advanced stage of the disease, when complications are devastating, hospitalization is often prolonged and hence costly to the health services, the patients and their families.

5. We, the participants at the Conference on Buruli Ulcer Control and Research, and more especially, Heads of State and government representatives from the most affected countries, are committed to mobilize the resources needed to establish, as soon as possible, effective action to control Buruli ulcer as an integral part of primary health care.

6. We pledge to make all efforts:

   a) to provide simple surgical facilities at the peripheral level for the treatment of nodular and early ulcerative disease;
   b) to improve and sustain health education on Buruli ulcer at all levels;
   c) to establish as soon as possible surveillance systems and to conduct surveys to determine the burden of the disease, provided that treatment can be offered to all patients identified;
   d) to collaborate in research on transmission and prevention of the disease;
   e) to monitor the implementation of the above measures.

7. We stress that these efforts be supported by the international community as a whole. We therefore call on governments, organizations of the United Nations system, bilateral agencies, development banks, nongovernmental organizations, foundations, and research institutions:

   a) to cooperate directly with endemic countries to undertake the activities needed;
   b) to develop partnerships and cooperation with organizations and programmes involved in health system development, so that effective treatment can reach all those affected.

8. We, therefore, affirm our support for the WHO Global Buruli Ulcer Initiative, which will address problems associated with Buruli ulcer and strengthen general health care delivery, thus contributing to building up capacity in endemic countries.

9. We affirm our determination to intensify action against Buruli ulcer. Recognizing the link between Buruli ulcer and overall development, we adopt this Declaration and commit ourselves to its implementation.

Signed by:
Dr Hiroshi Nakajima, *Director-General, World Health Organization*
His Excellency Henry Konan Bédié, *President of Côte d'Ivoire*
His Excellency Mathieu Kérékou, *President of Benin*
His Excellency Jerry John Rawlings, *President of Ghana*

Date: 6 July 1998, Yamoussoukro, Côte d'Ivoire

Annex 4

The Final Resolution of the International Conference on Buruli Ulcer Control and Research, Yamoussoukro, Côte d'Ivoire, 6–8 July 1998

In accordance with the *Yamoussoukro Declaration*, signed on 6 July 1998 by the Heads of State of Benin, Côte d'Ivoire, and Ghana, and which underlined the personal and socioeconomic consequences of Buruli ulcer and which stressed the involvement of these Governments to mobilise resources to establish a control programme integrated into Primary Health Care, the participants unanimously recommend that patients afflicted with Buruli ulcer are treated free of charge, as is done for tuberculosis and leprosy. This recommendation will require national programmes for the training of regional health staff, and treatment facilities which are adapted to all levels of health care delivery and which will allow the implementation of an epidemiological surveillance system. The development of these national programmes will be built upon basic and applied research, which will be coordinated at an international level with support of WHO and all other parties concerned.

Declared at Yamoussoukro, 8 July 1998

The Participants Assembled

Annex 5

Country assessment reports

Prof. Jacques Grosset, Prof. Jean-Marie Kanga, Prof. Françoise Portaels, Dr Augustin Guedenon, Dr Napo Tignokpa, Dr Robert Scherpbier & Dr Kingsley Asiedu

Background

At the first meeting of the WHO Task Force on Buruli Ulcer in February 1998, at WHO headquarters in Geneva, one of the recommendations was that WHO should conduct a rapid assessment of the Buruli ulcer situation in selected countries in order to have a better understanding of the problem. As a result, a team of WHO consultants visited Benin, Côte d'Ivoire, Ghana and Togo between March and July 1998 with the following objectives:

1. to determine the scale of the Buruli ulcer problem in Benin, Côte d'Ivoire, Ghana and Togo;
2. to assess the resources currently available and the additional resources required to develop an effective control programme for the disease; and
3. to discuss with the government authorities the importance of the disease as a public health problem in those countries.

The summary and recommendations for each country are presented below.

Côte d'Ivoire

Summary

The first case of Buruli ulcer was reported in 1978. Between 1988 and 1997, over 10,000 cases have been recorded with more than half between 1995 and 1997 (see Fig. A5.1). In addition, there has also been increasing geographical spread of the disease. Five Centres currently treating Buruli ulcer patients were visited during the mission in order to assess the situation. These were the Dermatology Centre of Treichville University Hospital, Abidjan; the Kongouanou Centre in Yamoussoukro region; St Michael's Health Centre at Zoukougbeu in Daloa region; the Raoul Follereau Institutes at Manikro, Bouaké region and Adzopé, Abidjan region. The Kongouanou and Zoukougbeu Centres have no surgical facilities, hence the only treatment available was wound dressing. The average period of hospitalization in all these Centres was in excess of 6 months, and the average cost of treatment per patient was between US$800 and US$1500. Inadequate resources to control the disease has hampered the effective implementation of the programme's activities.

Recommendations

- The government of Côte d'Ivoire must commit itself to an effective programme to control the disease.
- Buruli ulcer should be considered a social disease like leprosy and tuberculosis, and those afflicted by the disease should be treated free of charge.
- The Raoul Follereau Institutes in Adzopé and Manikro should be supported and used for the treatment for Buruli ulcer.
- The Dermatology Centre at Treichville University Hospital, Abidjan should be brought up to standard to allow for better management of patients.
- The national programme should establish a better surveillance system for the disease.

Figure A5.1a. Buruli ulcer situation in Côte d'Ivoire

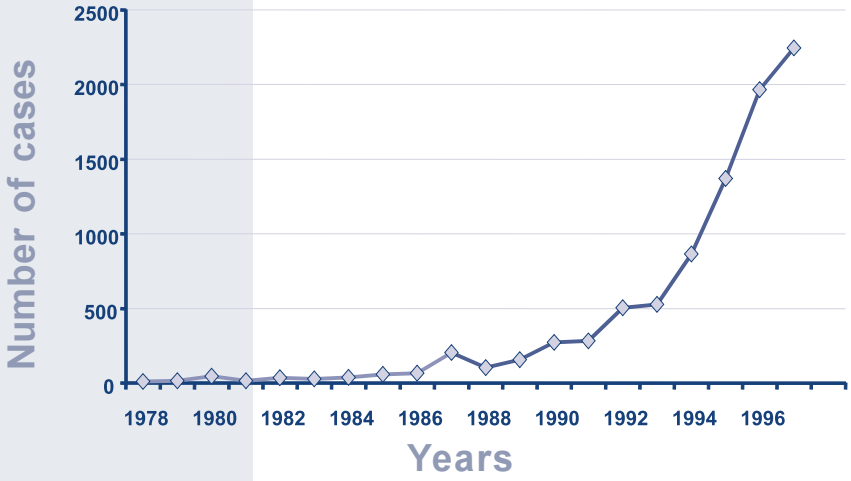


Figure A5.1b. Map of Côte d'Ivoire showing the geographical distribution of Buruli ulcer in 1989.

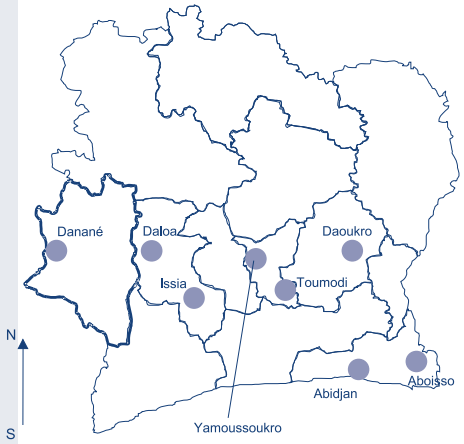
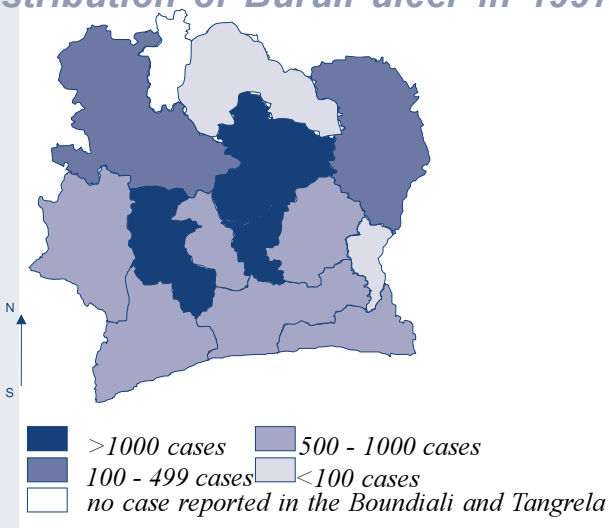


Figure A5.1c. Map of Côte d'Ivoire showing the geographical distribution of Buruli ulcer in 1997.



Benin

Summary

Since 1988, approximately 2300 cases have been reported, most of them as a result of passive detection. Four of the six regions (Atlantic, Mono, Ouémé and Zou) have been affected and the trends in the disease are shown in Fig. A5.2. In response to the growing problem, the Government of Benin has drawn up a national strategic plan to deal with the disease. This plan is yet to be approved by the Ministry of Health and implemented. The Health Centre at Lalo, the Leprosy Centre at Davougon and Zangnanado Nutritional Centre were visited. In all there were many patients with Buruli ulcer. Zangnanado Nutritional Centre is currently the centre treating the majority of patients in Benin. The median length of hospitalization at this centre is 2 months. Some endemic villages were visited, where large numbers of people with active disease spontaneously presented. It was concluded that Buruli ulcer was indeed a public health problem in Benin. However, given the resources currently available, effective control may be difficult unless additional ones are mobilized.

Recommendations

- The Government of Benin should quickly finalize the national programme plan and implement it as soon as possible, starting with the resources currently available.
- The national programme should include surgeons to train staff, treat patients and ensure standardization of care.
- Based on the similarities between the problems encountered with leprosy and Buruli ulcer, the control of Buruli ulcer should be linked to the leprosy programme.
- Because of the lengthy hospitalization, the Government should contribute towards the cost of feeding patients, as is done for leprosy patients.
- The Zangnanado Centre should be improved and provided with more support, particularly in terms of medical staff.
- The draft agreement between MSF Luxembourg and the Republic of Benin should be finalized as quickly as possible to allow the Lalo Centre to manage Buruli ulcer cases.
- Peripheral centres at Tchi in Mono and Bonou in Ouémé should be equipped to manage simple cases (simple excisions) and wound dressing.

Figure A5.2a. Buruli ulcer situation in Benin. Distribution of new cases between 1989 and 1998 in Zangnanado

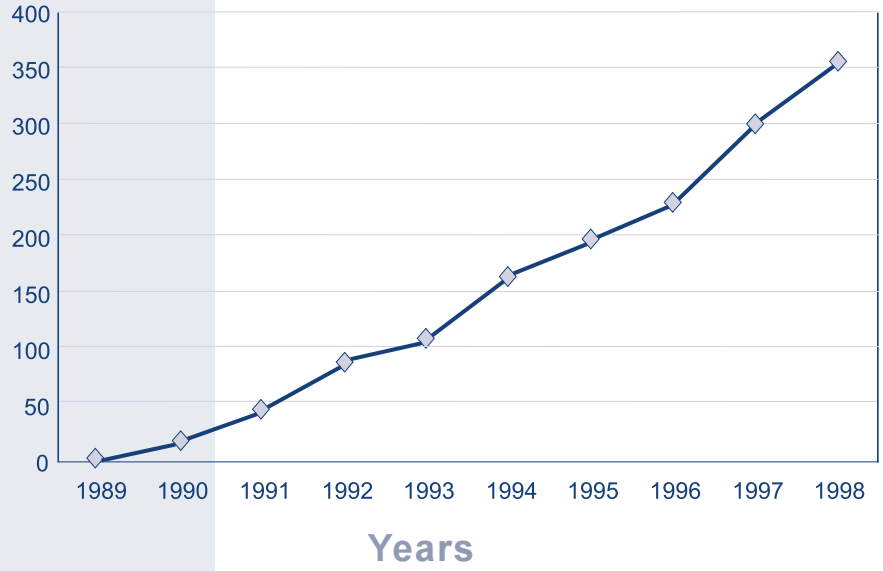
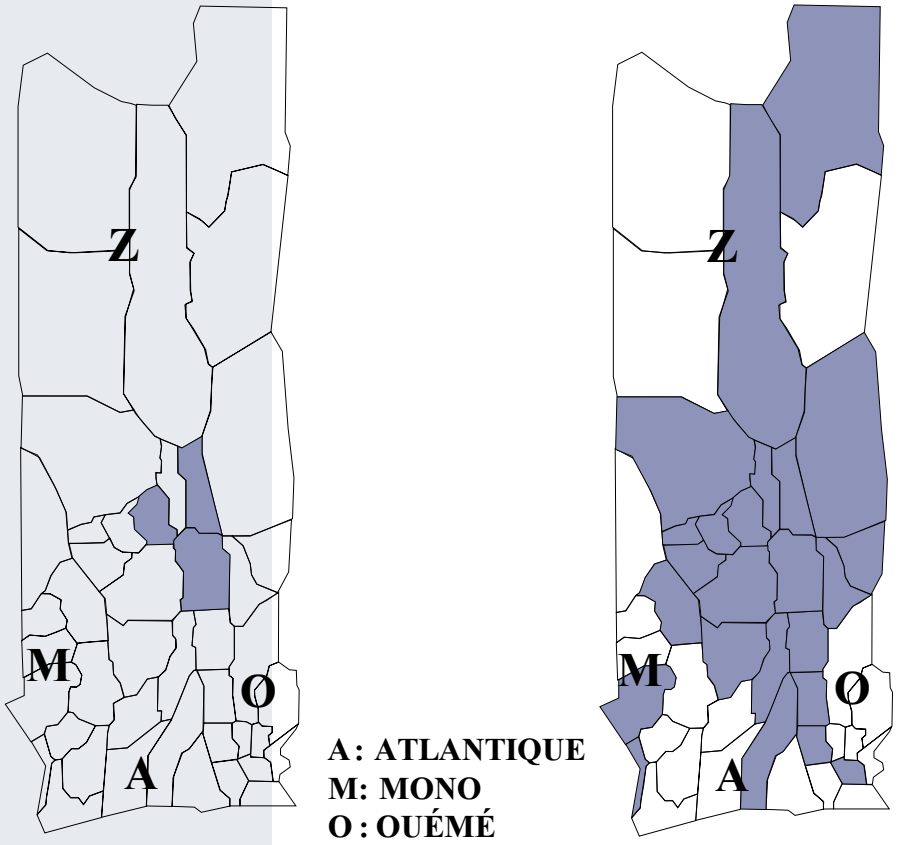


Figure A5.2b. Map of Benin showing the geographical distribution of Buruli ulcer in 1989.

Figure A5.2c. Map of Benin showing the geographical distribution of Buruli ulcer in 1997.



Togo

Summary

The first two cases were described by Meyers et al. in 1996, since when about 40 cases have been surgically treated at the St Jean Dieu Hospital, Afagnan. This hospital is well equipped and staffed to provide complete treatment for Buruli ulcer patients. The extent of Buruli ulcer as a public health problem in Togo is not yet clear. As such, no efforts are currently in place to address the disease. The disease exists in the southern part of the country (Tabligbo, Vogan and Aneho). The estimated average treatment cost per patient at St Jean Dieu Hospital was between 0.5 and 1 million CFA francs (US$ 800–1600). This cost is covered by a charity called LILIANA.

Recommendations

- Buruli ulcer is not yet a public health problem in Togo and a national programme for this disease was not considered necessary.
- Prevalence studies should be carried out as soon as possible to determine the extent of the problem.
- The excellent infrastructure set up for leprosy could be used for these studies.
- Togo is situated between two countries (Benin and Ghana) where the disease is highly endemic. Since it seems that Buruli ulcer is not yet a major health problem in Togo, the government of this country should rapidly set up a surveillance system allowing early detection and treatment of cases.

Ghana

Summary

The first case of Buruli ulcer was reported in 1971. Between 1993 and 1997, nearly 2000 cases have been reported. Six of the 10 regions and 35 of the 110 districts of the country are affected. The exact magnitude of the problem is not known. The Ashanti region is the worst affected region in the country, accounting for about 60% of all reported cases. In response to the growing problem of Buruli ulcer, the Ministry of Health has set up a Buruli Ulcer Task Force to advise the Government on the control of and research on the disease. The assessment was conducted in the Ga district of the Greater Accra region, in the Asante Akim North and Amansie West districts of the Ashanti region, and in the Upper Denkyira district of the Central region. Except for the Ga district, all the other districts have equipped surgical facilities and basic laboratories for the management of patients. All the institutions visited emphasized the strain put on their limited resources by the increase in Buruli ulcer patients. Structural intervention for prevention of disabilities and for rehabilitation (e.g. physiotherapy) do not exist.

Recommendations

- The Government of Ghana should start to put into operation an effective programme to address the disease.
- Surveys should be conducted to assess the extent and distribution of the disease and set up ongoing surveillance.
- Resource gaps and needs (training, equipment and facilities for diagnosis, treatment, prevention and rehabilitation) should be identified. Community health workers should be trained and involved in early diagnosis.
- The treatment of the disease should be free of charge in accordance with the final resolution of the Yamoussoukro Conference, and patients and health care providers should be made aware of this.
- Treatment should be decentralized as much as possible, by providing to various levels of the health care system dressing materials and other logistics to ensure effective treatment of patients.
- A rehabilitation programme should be an integral part of a national control programme.
- A simple surgical facility should be provided at the Amasaman Health Centre to allow treatment of early lesions, and the laboratory should be equipped to allow the detection of acid-fast bacilli from ulcers.
- Agogo hospital should be used to teach the disease in the nursing school and to train doctors in the surgical management of the disease.
- The Noguchi Memorial Institute for Medical Research should be contacted for the possibility of collaborating with other centres in Buruli ulcer research activities.

Annex 6

Members of the WHO Advisory Group on Buruli ulcer

1. Dr George Amofah, Public Health Division, Ministry of Health, Accra, Ghana.

2. Prof. Jacques Grosset, Bactériologie et Hygiène, Faculté de Médecine, Pitié-Salpétrière, Paris, France.

3. Dr Augustin Guedenon, Dermato-Vénéréologue, Cotonou, Benin.

4. Dr John Hayman, Department of Pathology, Box Hill Hospital, Victoria, Australia.

5. Dr Paul Johnson, Department of Infectious Diseases, Monash Medical Center, Clayton, Melbourne, Australia.

6. Sr (Dr) R.H Taylor Joseph, Wewak Hospital, Wewak, East Sepin, Papua New Guinea.

7. Prof. Jean-Marie Kanga, Dermatology Centre, Treichville University Hospital, Abidjan, Côte d'Ivoire.

8. Dr Wayne Meyers, Armed Forces Institute of Pathology, Washington, DC, USA.

9. Prof. Françoise Portaels, Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium.

10. Dr Roger Pradinaud, Service de Dermatologie, Centre hospitalier général de Cayenne, Cayene Cedex, French Guiana.

11. Dr G.B. Priuli, Hôpital St Jean de Dieu, Lomé, Togo.

12. Dr Pamela Small, Cell Biology, Rocky Mountain Laboratories, Hamilton, MT, USA.

13. Dr Jordan Tappero, Centers for Disease Control and Prevention, Atlanta, GA, USA.

14. Dr Napo Tignokpa, Dermatology Centre, Leprosy Programme, Lomé, Togo.

15. Dr Mark Wansbrough-Jones, Division of Infectious Diseases, St George's Hospital Medical School, London, England.

16. Dr Yo Yuasa, Sasakawa Memorial Health Foundation, Minato-ku, Tokyo, Japan.

Annex 7

Recording and reporting forms for Buruli ulcer

Clinical form for Buruli ulcer — BU 01

A. Institutional information

1. *Name of institution, address:*_____
2. *Subdistrict:*_____*District:*_____*Region:*_____*Country:*_____
3. *Name of officer completing this form (last/first):*_____
4. *Title:*_____*Specialization:*_____

B. Patient information

5. *Health facility ID #:*_____*Date of admission(dd/mm/yy):*___/___/___
6. *Name(last/first)*_____
7. *Age:* |__|__|years/months **8.** *Sex:* ☐ M ☐ F
9. *Residential address:*_____
10. *Subdistrict*_____*District:*_____*Region:*_____*Country:*_____
11. *Occupation of patient:*_____
12. *Source of drinking water:* ☐ Pipe-borne ☐ Borehole/well ☐ River/stream ☐ Pond/stagnant
13. *Patient classification:* ☐ *New case*
Recurrence: ☐ *same site* ☐ *different site*
End of last treatment(dd/mm/dd) __/__/__
14. *Duration of illness before seeking care:* |__|__| months /weeks/days
15. *Use of traditional treatment* ☐ N ☐ Y
16. *History of cases in family/among relatives:* ☐ N ☐ Y
17. *History of trauma (penetrating or blunt) at site of lesion:* ☐ N ☐ Y
18. *BCG vaccination record or scar:* ☐ N ☐ Y

C. Location of lesion(s)

19. *Upper limbs:* ☐ Right ☐ Left *Lower limbs:* ☐ Right ☐ Left
☐ *Abdomen* ☐ *Back* ☐ *Buttocks, perineum*
☐ *Thorax* ☐ *Head & neck*

D. Clinical forms

20. *Active*: ☐ Nodule ☐ Papule ☐ Plaque ☐ Oedema ☐ Ulcer ☐ Osteomyelitis
Inactive: ☐ Scar due to Buruli ulcer ☐ Amputation due to Buruli ulcer ☐ Others,specify_____
21. *Disability present:* ☐ N ☐ Y
22. *Date of clinical diagnosis(dd/mm/yy):*___/___/___

E. Laboratory confirmation of clinical diagnosis

23. *Yes*, which one(s): ☐ ZN Staining ☐ Culture ☐ Histopathology ☐ PCR
Date(dd/mm/yy)___/___/____
☐ *No*, why not_____

F. Principal treatment(s)

24. ☐ Wound dressing only ☐ Excision only ☐ Excision + primary closure
☐ Excision +skin graft ☐ Amputation ☐ Heat
☐ Antimycobacterial agents, specify:_____
☐ Antibiotics and other drugs:_____
☐ Others, specify_____

G. Treatment outcomes

25. ☐ Healed without sequelae
☐ Healed with sequelae, specify:_____
☐ Referral for treatment of active lesions, where: _____Date:___/___/___
☐ Absconded/discharged against medical advice
☐ Died, Buruli ulcer related, specify:_____
☐ Died, not related to Buruli ulcer, specify:_____

H. Referral of sequelae for treatment/rehabilitation

26. ☐ *No*, why not:_____
☐ *Yes*, where:_____when (dd/mm/yy):___/___/___
27. Date of discharge (dd/mm/yy):___/___/___

This form BU 01 should be kept in the patient's records at the health facility where treatment is provided.

Registration of Buruli ulcer cases

BU 02

Month of _____

Name of institution _____

Subdistrict _____ *District* _____ *Region* _____ *Country* _____

N°	Name (last/first) (6)	Age (7)	Sex (8)	Residential address (9)	[a] Classification (13)	BCG Vaccination (18)	[b] Location of lesion (19)	[c] Clinical form(s) (20)	Date of clinical diagnosis (21)	Disability present (22)	[d] Laboratory confirmation of diagnosis (23)	Remarks
					New/rec	Yes/no				Yes/no	Yes(which)/no	

[a] Classification
New
Recurrent (Rec)

[b] Location of lesions
Upper limbs (UL)
Lower limbs (LL)
Abdomen (AB)
Back (BK)
Buttocks, perineum (BP)
Thorax (TH)
Head and neck (HN)

[c] Clinical forms
Nodule (N)
Papule (P)
Plaque (Q)
Oedema (E)
Ulcer (U)
Osteomyelitis (O)

[d] Laboratory confirmation of diagnosis
AFB Smear (AFB)
Culture (CUL)
Histopathology (HIS)
Polymerase chain reaction (PCR)

Quarterly Report on New and Recurrent Cases BU 03

Buruli ulcer **Patients registered during ___ Qtr of _____**

Name of country:_____
Number of regions reporting Buruli ulcer cases in the past year:_____
Number of districts reporting Buruli ulcer cases in the past year:_____
Name of officer completing this form:_____
Title:_____
Date of completion of this form (dd/mm/yy): ___/___/___
Address:_____
_____
Tel:_____Fax:_____
E-mail:_____

1 New and recurrent cases

	Laboratory confirmation						No laboratory confirmation						Subtotal				Total			
	<15 yrs		15-49 yrs		>49 yrs		<15 yrs		15-49 yrs		>49 yrs		Laboratory confirmation		No laboratory confirmation					
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
New																				
Recurrent																				

2 Clinical forms of new and recurrent cases

	Nodule	Papule	Plaque	Oedema	Ulcer	Osteo-myelitis	Mixed	Total
New								
Recurrent								

3 Disabilities in new and recurrent cases

	Disability present		Total
	Yes	No	
New			
Recurrent			

Buruli ulcer **Patients registered during ___ Qtr of _____**

Name of Country_____
Number of regions in the country_____
Number of regions reporting Buruli ulcer cases in the past year:_____
Name of officer completing this form:_____
Title: _____
Date of completion of this form (dd/mm/yy): ___/___/___
Address:_____
_____
Tel:_____Fax:_____
E-mail:_____

Treatment outcomes

	Healed without sequelae	Healed with sequelae	Absconded/ discharged against medical advice	Referred/ transferred	Died, Buruli ulcer related	Died, not Buruli ulcer related	Total
New							
Recurrence							
Total							

Annex 8

Summary of surveillance standards for Buruli ulcer

ICD A31.1 Buruli ulcer
(*Mycobacterium ulcerans* infection)

RATIONALE FOR SURVEILLANCE

Buruli ulcer is disease that has terrible consequences if not promptly diagnosed and treated. It is endemic in at least 25 countries worldwide, mostly in the tropical regions of the world. Awareness of this disease is low and may lead to significant under-recognition and under-reporting. There is little knowledge on the extent of the disease at national and international levels. A good surveillance system is needed to provide better data on the disease and to allow monitoring of control efforts.

RECOMMENDED CASE DEFINITIONS

Clinical case definition

Buruli ulcer is an infectious disease involving the skin, caused by *Mycobacterium ulcerans*, characterized by a painless nodule, papule, plaque or oedema, evolving into a painless ulcer with undermined edges, often leading to invalidating sequelae.

Case classification

Probable case

A case that meets the clinical definitions below. There are two types of the disease: **active** and **inactive**.

Active: Infections of different clinical forms.

- *Papule*: painless and raised skin lesions less than 1 cm in diameter.
- *Nodule*: painless, palpable firm lesion, 1–2 cm in diameter, situated in the subcutaneous tissue and usually attached to the skin.
- *Plaque*: usually painless, well-demarcated, elevated indurated lesion more than 2 cm in diameter.
- *Oedema*: diffuse, extensive, non-pitting, ill-defined margin, firm, may be painful with or without colour change over the affected skin.
- *Ulcer:* painless skin lesion characterized by a necrotic centre, undermined edges and oedematous skin. An early ulcerative lesion has a diameter of **less than 2 cm** and a late ulcerative lesion has a diameter of **more than 2 cm.**

Inactive: Healed lesions with characteristic depressed stellate (star-like) scar with or without sequelae. A *sequela* of Buruli ulcer is defined as a complication resulting either directly from the disease (contracture deformities, loss of organs such as breast, eye, and genitalia) or as a result of treatment (amputation of limbs).

Confirmed case

A probable case that is laboratory-confirmed by one or more of the following.

- Presence of alcohol-acid-fast bacilli (AFB) in a smear from necrotic base of ulcers.
- Positive culture of *M. ulcerans*;
- Characteristic histopathology on biopsy specimen;
- Positive polymerase-chain-reaction (PCR)-based test for *M. ulcerans*.

Categories of patients

New: A patient with no previous history or treatment for Buruli ulcer

Recurrent: A patient with a previous surgical treatment for Buruli ulcer who is now presenting with another lesion at the same or different site within one year of the end of the last treatment.

RECOMMENDED TYPES OF SURVEILLANCE

Individual patient records at the peripheral level for case management and follow-up.

Routine monthly or quarterly reporting of aggregated data of all cases from the peripheral level (district) to the intermediate level (regional) to central level (national) using WHO standard surveillance forms.

International: Annual reporting of aggregated data from central level to WHO

RECOMMENDED MINIMUM DATA ELEMENTS

Individual patient records (using WHO BU 01)

Unique identifier, name, age, sex, geographical information, location and clinical forms of the disease, presence or absence of disability, laboratory confirmation if any, principal treatment(s) received, treatment outcomes (healed with or without sequelae, absconded, referred or transferred, died) and referral of those with sequelae for rehabilitation.

Aggregated data for reporting – essential indicators for endemic countries (WHO BU 02, 03, 06)

- Number of cases (new and recurrence) registered in a given time
- Number of laboratory-confirmed cases
- Age and sex distribution of the cases
- Number of various clinical forms of cases
- Number of patients presenting initially with disabilities
- Number of patients with resulting sequelae after treatment
- Number of deaths related to Buruli ulcer.

RECOMMENDED DATA ANALYSES, PRESENTATIONS, REPORTS

Graphs: Number of cases or rates over time

Maps: Number of cases by geographical area.

Table: Number of cases, age and sex distribution, and number of patients with disability, recurrence rate and treatment outcomes.

PRINCIPAL USES OF DATA FOR DECISION-MAKING

- Estimate the magnitude of the problem
- Determine the geographical distribution of the disease
- Understand the natural history of the disease
- Plan for treatment and supplies
- Monitor disease trends and pattern
- Evaluate performance of control programmes

SPECIAL ASPECTS

- Previous residence or travel to a known endemic area should raise suspicion of Buruli ulcer.
- About 70% of the patients are children below 15 years of age.
- Males and females are equally affected.
- Most lesions are on the extremities: lower extremities lesions are twice as common as those on upper extremities.
- In areas where the disease is unknown, first cases should be confirmed by laboratory diagnosis. In known endemic areas, sample cases could be confirmed for quality assurance.

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Annex 9

Surgical trays and equipment for minor operations and skin grafting

This annex lists some of the instruments, equipment, and materials needed for minor operations (including excision of nodules) and skin grafting. Additional list for other surgical procedures at the district level is provided in the list of further readings at the end of the book.

Minor operations

Sponge forceps, 4 pairs
Tissue forceps
Scalpel handle and blade, 1
Small dissecting scissors, 1 pair
Stitch scissors, 1 pair
Sutures, 2/0, 3/0, and 4/0 chronic catgut, ties and with atraumatic needles
Sutures, 2/0 and 3/0 thread, ties and with cutting needles
Small, curved artery forceps, 3 pairs
Small, straight artery forceps, 3 pairs
Large, curved artery forceps, 2 pairs
Needle holder, 1
Rake self-retaining retractor, 1
Dissecting forceps, toothed, 1 pair
Dissecting forceps, non-toothed, 1 pair
Syringe, 5 ml with needle, 1
Syringe, 10 ml with needle, 1
Lidocaine 1%
Gallipot, 1
Kidney dish, 1
Skin hooks, 2
Towel clips, 4
Corrugated drain
Petrolatum gauze
Gauze swabs
Antiseptic solution
Adhesive tape
Sterile drapes
Sterile gloves, 2 pairs

Skin grafting

Skin-grafting knife, and blade, 1
Meshers and mesh plates
Scalpel handle with No. 10 blade, 1
Razor blade, 1
Sponge forceps, 2 pairs
Towel clips, 4
Small, straight artery forceps, 6 pairs
Small, curved artery forceps, 6 pairs
Dissecting forceps, non-toothed, 2 pairs
Dissecting forceps, toothed, 2 pairs
Dissecting scissors, straight, 1 pair
Dissecting scissors, curved, 1 pair

Dissecting scissors (Metzenbaum), 1 pair
Hook retractors, small, 2 pairs
Stitch scissors, 1 pair
Tissue forceps (Allis), 2 pairs
Skin hooks, 4
Gallipots, 2
Ruler, 1
Petrolatum gauze
Wooden boards with bevelled edges, 4
Antiseptic solution
Gauze swabs
Gauze packs (abdominal packs)
Cotton wool
Sterile drapes
Sterile gloves, 2 pairs

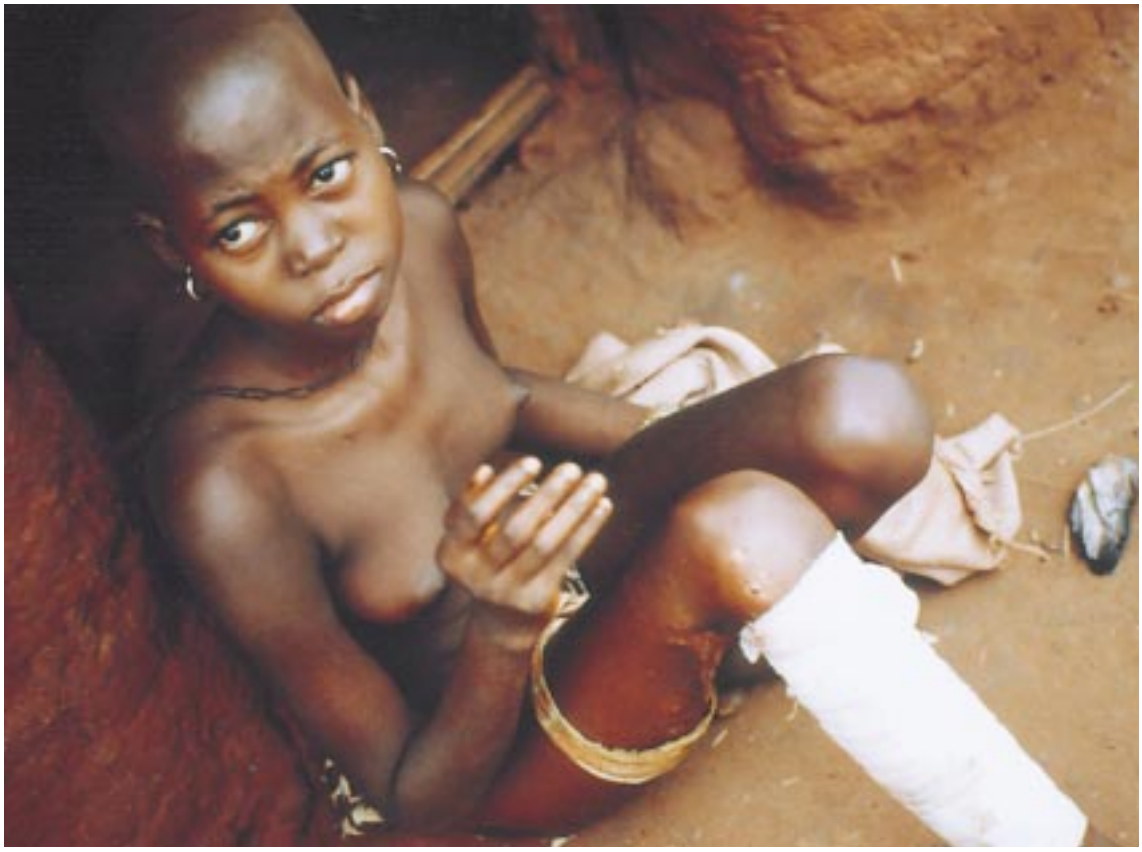
SELECTED WHO PUBLICATIONS OF RELATED INTEREST

1. Cook, J. et al. **General surgery at the district hospital**
   1988

2. Cook, J. et al. **Surgery at the district hospital: obstetrics, gyneacology, orthopaedic and traumatology**
   1991

3. Dobson, M.B.. **Anaesthesia at the district hospital**
   1988 (Second edition in preparation)

4. Levy-Lambert, E. **Manual of basic techniques for a health laboratory**
   1980 (Second edition in preparation)



A young girl crippled by Buruli ulcer in Benin. (Photo: Augustin Guedenon)

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Recognizing Buruli ulcer as an emerging public health threat, the World Health Organization established the Global Buruli Ulcer Initiative in 1998, to coordinate control and research efforts worldwide.

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World Health Organization

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