Volume 73, Number 1
Printed in the U.S.A.
(ISSN 0148-916X)

INTERNATIONAL JOURNAL OF LEPROSY
and Other Mycobacterial Diseases

VOLUME 73, NUMBER 1 MARCH 2005

Ulcerative Cutaneous Mycobacteriosis Due to *Mycobacterium ulcerans:* Report of Two Mexican Cases[1]

Josefa Novales-Santa Coloma, Gisela Navarrete-Franco, Pedro Iribe, and Larissa Dorina López-Cepeda[2]

ABSTRACT

We report two patients from Central Mexico, with ulcerated cutaneous lesions containing acid-fast bacilli (AFB) and ultimately diagnosed as *Mycobacterium ulcerans* disease. The first patient had a long history (11 years) of disease involving multiple lesions of both upper and lower extremities. Histopathological changes included necrosis of the subcutaneous tissue with large numbers of extracellular AFB. Cultures at 32°C were "positive for mycobacteria," but were not further identified. The polymerase chain reaction for *M. ulcerans* performed on skin bopsies was positive. The lesions improved after treatment with rifampin and isoniazid (INH) for one month, followed by ethambutol and streptomycin.

The second case followed trauma to the right hand, which spread over 2 years to the right upper extremity, the back, and both legs, with a loss of digits and metacarpal bones of the right hand. The histopathological findings were similar to the first case, including presence of AFB. PCR for *M. ulcerans* on extracts of skin biopsies was positive. Rifampin, INH, pyrazinamide, and levofloxacin resulted in marked improvement of the ulcer; ethambutol and streptomycin were later used, also.

We report these cases because they are rare (approximately 6 previous cases were reported from Mexico), and both are unusually disseminated. They are significant in alerting the medical community to *M. ulcerans* infection, which is still active in Mexico, and the treatment used has not been reported previously.

RÉSUMÉ

Cet article décrit la maladie de deux patients habitant la région centrale du Mexique, qui souffraient de lésions cutanées ulcérées contenant des bacilles acido-alcoolo-résistants (AAR) et qui ont finalement été diagnostiqués comme souffrant de maladie causée par

[1] Received for publication on 11 December 2003. Accepted for publication on 29 November 2004.

[2] J. Novales-Santa Coloma, M.D., Dermatologist and Chief of Dermatopathology Department; G. Navarrete-Franco, M.D., Dermatologist and Dermatopathologist, Centro Dermatologico, Dr. Ladislao de la Pascua, México City, Mexico; P. Iribe, M.D., Dermatologist and Dermatopathologist; L. D. López-Cepeda, M.D., Dermatologist, Centro Dermatológico Pascua.

Reprint requests to: Josefa Novales, M.D., Vertiz 464 Col. Buenos Aires, c.p. 06780, Mexico D.F., E-mail: larisslo@yahoo.com.mx

Mycobacterium ulcerans. Le premier patient présentait des commémoratifs d'une maladie chronique de 11 années, caractérisée par de nombreuses lésions de l'extrémité des membres. Les lésions histopathologiques comprenaient une nécrose du tissu sous-cutané, associée à de très nombreux bacilles AAR extracellulaires. Des cultures réalisées à 32°C furent « positives pour les mycobactéries ». La réaction de polymérisation en chaîne (PCR) pour *M. ulcerans* à partir de biopsies cutanées confirma le diagnostic. Les lésions rétrocédèrent après traitement par la rifampicine et l'isoniazide (INH) pendant 1 mois, suivi par l'éthambutole et la streptomycine.

Le second cas était une maladie similaire observée après un traumatisme de la main droite, qui a progressé chroniquement sur 2 années de l'extrémité du bras droit vers le dos, les deux jambes et qui s'est compliquée de la perte des doigts et des os métacarpiens de la main droite. Les lésions histopathologiques étaient similaires au premier cas, avec notamment la présence de bacilles AAR. La PCR pour *M. ulcerans* à partir d'extraits de biopsies cutanées fut positive. Rifampicine, INH, pyrazinamide et lévofloxacine a permis d'atteindre une amélioration spectaculaire des lésions ulcérées ; l'éthambutole et la streptomycine furent ensuite utilisées.

Nous rapportons ces deux cas parce qu'ils sont rares (approximativement 6 cas furent rapportés auparavant au Mexique) et les deux présentaient une forme disséminée inhabituelle. De plus, ils sont importants pour alerter la communauté médicale vis-à-vis du risque d'infection par *M. ulcerans*, qui est encore présent au Mexique, ainsi que pour les modalités de traitement qui n'ont pas encore été rapportées.

RESUMEN

En este artículo presentamos los casos de dos pacientes del Centro de la República Mexicana, con lesiones cutáneas ulceradas causadas por *Mycobacterium ulcerans.* El primer paciente tenía una historia clínica prolongada (11 años) de su enfermedad y mostraba múltiples lesiones en las extremidades superiores e inferiores. Los estudios histopatológicos revelaron necrosis del tejido subcutáneo, con grandes números de bacilos ácido-resistentes (BAAR). Los cultivos a 32°C fueron "positivos para micobacterias" pero no se hizo el intento de identificar a los microorganismos. La reacción en cadena de la polimerasa (PCR) para *M. ulcerans* en una biopsia de piel fue positiva. En este paciente las lesiones remitieron después del tratamiento con rifampina e isoniazida (INH) durante 1 mes, seguido por etambutol y estreptomicina.

El segundo caso se descubrió atendiendo un trauma en la mano derecha del paciente. La infección se diseminó en el transcurso de 2 años a la extremidad superior derecha, la espalda, y ambas piernas, y ocasionó la pérdida de los huesos digitales y metacarpales de la mano derecha. Los hallazgos histopatológicos fueron similares a los del primer caso, incluyendo la presencia de bacilos ácido-resistentes. La PCR para *M. ulcerans* en el extracto de una biopsia de piel también fue positiva. La rifampina, la INH, la pirazinamida y la levofloxacina condujeron a una notable resolución de la úlcera, después también se usaron etambutol y estreptomicina.

Reportamos estos casos porque son raros (en México sólo se han reportado 6 casos previos) y porque ambos fueron inusualmente diseminados. El reporte de estos casos es importante porque alertará a la comunidad médica sobre la infección por *M. ulcerans* como una enfermedad todavía existente en México. El tratamiento utilizado en estos casos no se ha reportado previamente.

CLINICAL CASES

Case 1. A 76-year-old woman, occasional farmer, born and lived in Esperanza Tarimoro, (Guanajuato, Central Mexico), presented in 1995 with an eleven-year history of an evolving dermatosis, affecting the left forearm and elbow, index and middle fingers and back of the right hand, the left thigh on its anterior and lower sides, the knee, and posterior mid-left leg. The lesions consisted of 10 nodules between 2 to 4 cm in diameter, red-violet in color, with a hard consistency, and non-pitting edema. Some also had ulcerations and were draining bloodstained serum at the center (gummae). Seven ulcers measured 2 to 10 cm in diameter had open, undermined borders that allowed the introduction of a clamp. The underside was necrotic and partially covered with purulent secretion (Fig. 1, Case 1). At the right knee were three circular scars, 1

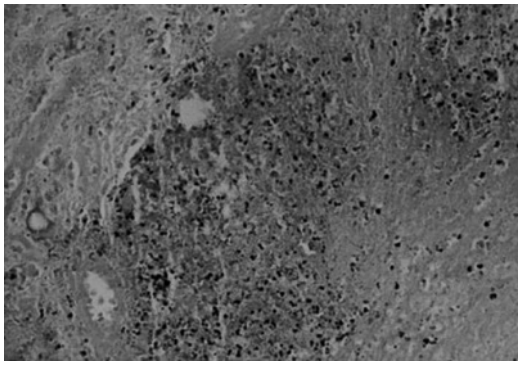

FIG. 1. Case 1. Ulcerative cutaneous mycobacteriosis (UCM). Note undermined borders and different sized ulcers with some purulent secretion.

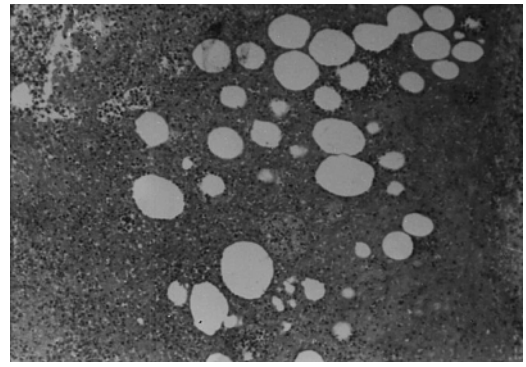

FIG. 3. Case 1. UCM. Histologic aspect: large necrotic zones in the subcutis (H&E Original magnification 20×).

cm in diameter, with erythema and marked swelling.

At the onset there were small, erythematous nodules on the legs. Some of these had healed spontaneously one year earlier, and new lesions had appeared on her arms, which ulcerated after 9 mos. Previous treatments included home remedies. The initial clinical diagnosis was deep nodular tuberculosis (Hutchinson type). General medical examination revealed a patient in good general condition. Laboratory analysis showed aleukocytosis with 14,900 white blood cells (83% segmented cells), an incremented globular sedimentation rate of 25 mm/hr, and normal hepatic function and chest x-rays. The skin test with 5TU purified protein derivate (PPD) was negative.

Two skin biopsies were taken, one from the border of the ulcer on the left forearm and the other from the nodule on the left thigh. Histologically, we observed large necrosis zones affecting the middle and deep dermis and hypodermis in both biopsies (Figs. 2 and 3). Fite-Faraco stains revealed large numbers of acid-fast bacilli (AFB) in necrotic areas, some in clusters and forming "globi," (Figs. 4 to 7), similar to those described by MacCallum ([15]) and Connor([28]).

Further analysis of an acid-fast smear of the purulent secretion from borders and bottom ulcers, using a Ziehl Neelsen stain, revealed clusters of AFB. Cultures on Lowenstein-Jensen media at 32°C were positive for mycobacteria. The cultures were damaged before further identification could be obtained from a referral center. The final diagnosis was Ulcerative Cutaneous Mycobacteriosis (UCM), species unknown.

Treatment with rifampin 600 mg/day and isoniazid 300 mg/day, and daily soaks of ul-

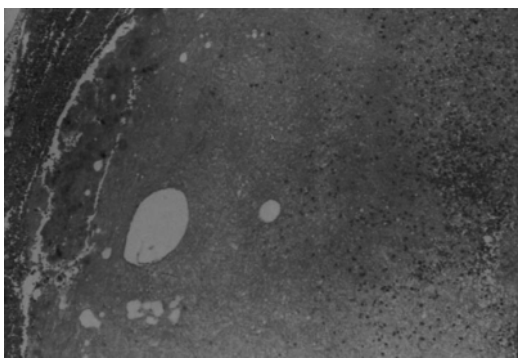

FIG. 2. Case 1. UCM. Histologic aspect: large necrotic areas in the deep dermis and subcutis (H&E Original magnification 10×).

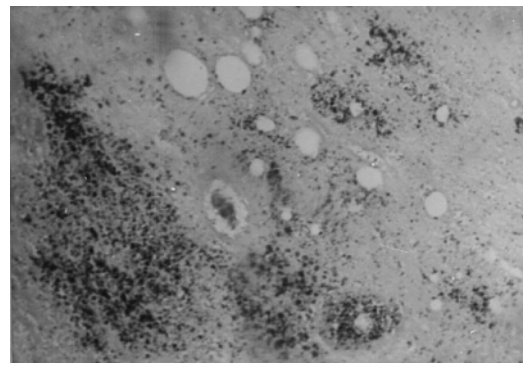

FIG. 4. Case 1. UCM. Numerous acid-fast bacilli isolated or in groups in necrotic zones (H&E Original magnification 10×).

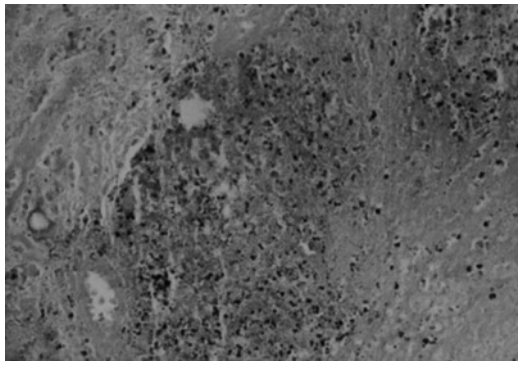

FIG. 5. Case 1. UCM. Acid-fast bacilli isolated or in clusters in necrotic zones (H&E Original magnification 20×).

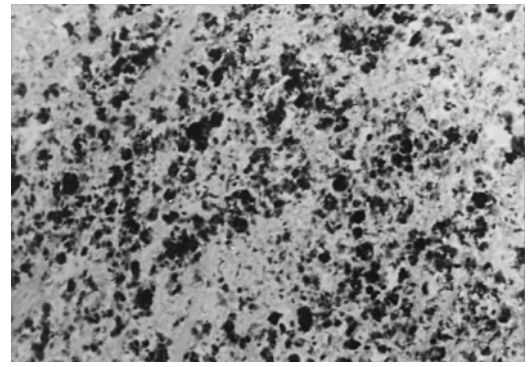

FIG. 6. Case 1. UCM. Acid-fast bacilli isolated or in clusters in necrotic zones (Fite Faraco. Original magnification 40×).

cers in sulfate solution (1:1000) for one month resulted in improvement. Rifampin was discontinued after the patient developed clinical hepatitis attributable to this drug. Two weeks later, after hepatic function tests returned to normal, treatment was initiated with ethambutol 600 mg/day and streptomycin 1 gm/day for 15 days, then reduced to 1 gm every three days, for a total of 30 gr. With this regimen, the ulcers healed. At this stage the patient returned to her hometown and was lost to follow-up.

Case 2. A 23-year-old male horse-meat merchant who lived in Chimalhuacán, Mexico (Central Mexico), presented with a 2-year evolving dermatosis, affecting the lower right arm and elbow, forearm, and dorsum of the right hand, anterior aspect of both legs, and posterior aspect of the left leg. There were four nodules of 2 cm in diameter and 10 ulcers between 1 and 5 cm in diameter with the characteristics described for the first case. Some ulcers were communicating and alternated with small areas of apparently normal skin. The largest ulcerative lesion was on the forearm, measuring 15 × 25 cm in diameter, with viscous crust, surrounded by scar tissue (Fig. 8), in the area previously grafted. Scars were present, 3 cm in diameter. We also observed absence of all of the fingers of the right hand, with exception of the thumb and the metacarpal bones.

The disease presented after trauma to the right hand from a prick with a horse bone chip; 15 days later, there was reddening and swelling of the area that quickly extended to the forearm. At the trauma site, the patient observed necrosis, which was excised, but despite this the disease continued. The patient was then sent to the Instituto Nacional de Ortopedia in Mexico City in August 1997, where the forearm lesion was excised, four fingers were amputated, and the metacarpal zone was covered with a skin graft. Results were poor and the disease continued. Two years later, the patient was sent to the Centro Dermatologico Pascua (CDP), where the initial clinical diagnosis was cutaneous tuberculosis. The skin test with 5TU PPD showed 5 mm of induration, and the physical examination was otherwise normal. With the history of trauma precedents, long evolution, and poor response to the prescribed treatment, we considered the possibility of a diagnosis of UCM.

Four biopsy specimens were taken from the necrotic area on the ulcers of the fore-

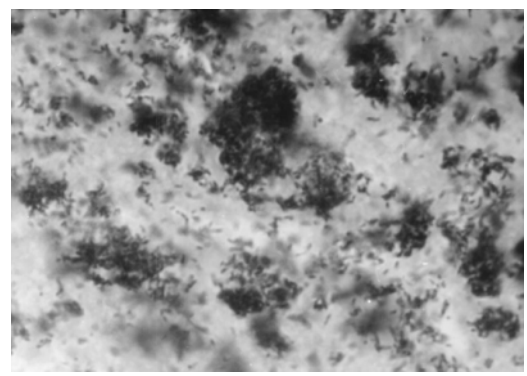

FIG. 7. Case 2. UCM. Acid-fast bacilli isolated or in clusters in necrotic zones (Fite Faraco. Oil immersion).

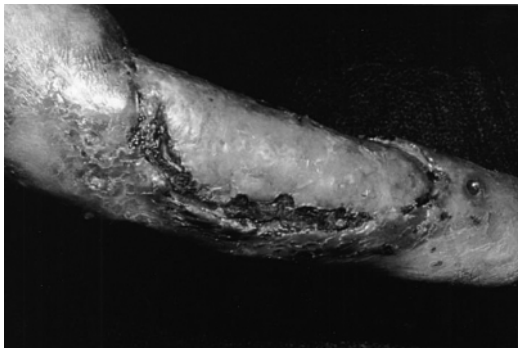

FIG. 8. Case 2. UCM. Largest ulcer with infiltrated borders.

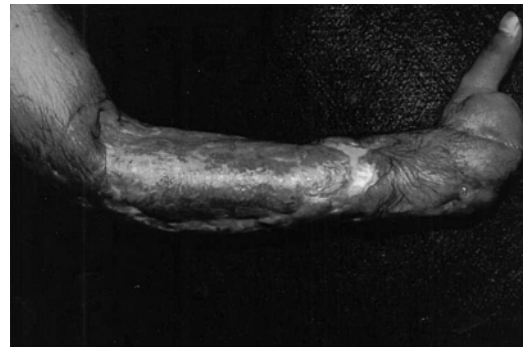

FIG. 9. Case 2. UCM. Improvement of ulcers of the forearm after four months of treatment.

arm and leg nodules. Microscopic findings were similar in all specimens and identical to those previously described in Case 1, confirming the clinical diagnosis.

Bacilloscopies revealed AFB and an enzyme-linked immunosorbant assay (ELISA) was positive for *Mycobacterium* sp., but cultures for mycobacteria were negative. Routine laboratory determinations were within the normal range.

Surgical cleaning and soaks with sulfate solutions (1:1000) were carried out twice a day. Rifampin 600 mg/day, isoniazid 300 mg/day, pyrazinamide 300 mg/day and levofloxacin 400 mg/day, were administrated for two months, with great improvement of the ulcers, marked by a decrease of purulent drainage and necrosis. The nodules, however, persisted. Surgical excision of nodules was performed and microscopic analysis showed necrotic zones and numerous acid-fast bacilli. Streptomycin 1 gr intramuscularly (IM) every three days (30 gr total dose) and ethambutol 1200 mg/day were then administered. Additionally, surgical cleaning was performed once a week and topical soaks with sulfate solutions was performed daily. After streptomycin was discontinued, rifampin 600 mg/day was resumed with ethambutol 1200 mg/day again for 10 months, and the cutaneous lesions healed. (Fig. 9). At present, the patient is under periodic monitoring and shows no lesions, and a prosthesis has been fitted to the patient's right hand.

Paraffin blocks of biopsies from both patients were sent to Dr. Francois Portaels at the Institute for Tropical Medicine, Antwerp, Belgium, for analysis by polymerase chain reaction. Specimens from both patients were found to contain DNA sequence IS2404 from *M. ulcerans*.

DISCUSSION

The skin ulceration caused by *Mycobacterium ulcerans* was described for the first time by MacCAllum, *et al*. in Australia in 1948 ([16]). In 1950 in the Belgian Congo (now the Democratic Republic of Congo) the first African case was reported ([29]), and in the same year, Fenner ([7]) identified the bacillus and named it *Mycobacterium ulcerans*. Since 1959, several authors have described numerous patients with this disease in tropical and subtropical regions of Central and West Africa([13]). Buruli ulcer is recognized as a public health problem, for example, in Uganda, Nigeria, Gabon, Ghana, Cameroon, Liberia, the Ivory Coast ([1, 6, 17, 28, 30]), Malaysia ([22]), New Guinea ([14]), Togo ([15]), French Guyana ([5, 25]), and the Republic of Benin ([20]). In the Americas, it is an exceptionally rare disease and only a few cases have been reported. In 1953, Lavalle, *et al*., reported the first UCM case in Mexico ([11, 13]) and until 1990, only five additional cases from Guanajuato State in Central Mexico were reported ([13, 14, 15]).

This mycobacteriosis has been given several names according to the place where it occurs or where it has been observed. For example, it was called Bairnsdale ulcer in Australia ([17]), Buruli ulcer in Uganda ([20]), and Tora ulcer and Mexican ulcer in México ([13]). Nevertheless, Lavalle proposed the name of Ulcerative Cutaneous Mycobacteriosis, caused by *Mycobacterium ulcerans* ([15]).

Mycobacterium ulcerans is a slowly grow-

ing, acid-fast organism generally considered to be an environmental saprophyte. It is usually observed in aquatic ecosystems in marshy terrain, a soil rich in silica, and in stagnant bodies of water or near rivers, at temperatures ranging between 32°C and 33°C, with pH between 5.5 and 6.9 ([8, 20, 21]). The bacillus grows best in Lowenstein-Jensen culture medium at 32°C.

The disease affects both sexes and all ages, but is more frequent in children between 5 and 14 yrs old ([28]). In the countries where it is endemic, it is frequent in farmers and may be considered an occupational disease. After tuberculosis and leprosy, *M. ulcerans* infection is considered the third most common mycobacterial disease affecting non-immunocompromised humans ([20]).

The exact manner of transmission of *M. ulcerans* is not known. It is assumed that a not-yet identified environmental factor exists that is related to slowly flowing or stagnant water and near rivers. There are some reports suggesting possible transmission by mosquito or insect bites ([3, 4, 24]). However, inoculation appears to occur via trauma to skin, on uncovered, unprotected regions of the body. Its topography in adults includes limbs, especially near joints, predominatly on legs (knees) and forearms (elbows) ([5, 8, 19]), but in children it can be found anywhere.

M. ulcerans affects humans by producing a heat-stable exotoxin that causes extensive, chronic, necrotizing damage to the papillary skin, subcutaneous fat and muscle (fascia and bone are also sometimes affected), resulting in deformity and disability ([12]).

The lesion begins as a small subcutaneous swelling, more palpable than visible, that grows slowly until it develops into a nodule that is adherent to the skin but not to deep tissues. These nodules are soft, undergo liquefaction, and finally ulcerate, with an oily, purulent discharge. Ulcers are often well defined and the borders are undermined. The base of the pristine ulcers contains a whitish, cotton wool-like slough and sometimes eschars. Skin surrounding the lesion becomes hyper-pigmented ([15]). Ulcers can be small or extensive, involving even an entire extremity or large portions of the trunk. Microscopic alterations are usually diagnostic, including extensive necrosis of the dermis and large numbers of extracellular AFB, in clumps or clusters ([2, 6, 16]).

Disease evolution can vary in severity. In some areas, ulcers heal slowly with fibrosis and retraction, even while the disease may progress in other areas. Secondary bacterial infection may develop, but the patient's general condition is not affected. There is no regional clinical lymphadenopathy nor fever.

The two cases described in this report are particularly interesting because of the unusual dissemination of the disease and the large number of nodules and ulcers. The possible mode of transmission was not apparent in the first case, whereas in the second case, the initial lesion followed trauma with a horse bone chip, similar to one of the Lavalle cases ([15]). This information allows us to suspect that a direct inoculation was made in this case.

The last report of Buruli ulcer the disease in Mexico was made several years ago, and no reports of other cases have been made since. This may be attributable to possible rarity of the disease in Mexico. As Lavalle suggests, the paucity of reports may be a result of a lack of awareness of the disease, or to the status of the public health services in endemic areas. Innate or acquired immunity of the populations may also contribute to low endemicity.

We conclude that the following features must be considered for the diagnosis of *M. ulcerans* infection: (i) a chronic dermatosis in a patient with good general health; (ii) the histopathological findings of extensive necrosis in the dermis and subcutaneous tissue and the presence of numerous extracellular acid-fast organisms ([2, 5, 6]).

Although it is difficult to culture this organism, it is now possible to identify the agent by PCR analysis carried out on the skin specimen ([10, 20]), although in our opinion these studies are not necessary for the diagnosis. In both cases presented in this report, PCR analysis for *M. ulcerans* DNA was performed on paraffin blocks of the skin biopsies, and these studies were done some years after the diagnoses were made. The treatments were initiated on the basis of clinical and histopathological findings, and the patients healed. This indicates that the molecular studies are not indispensable if there is an adequate clinical and histological study, but PCR contributes to support the diagnosis by identifying the mycobacterial DNA sequence IS2404.

Despite the fact that the first cases were adequately described more than 50 years ago, there remains no standard effective treatment. Surgical excision of skin lesions and, if necessary, skin graft application in the initial stages, are considered the best treatment, in addition to anti-mycobacterial agents. Hyperbaric oxygenation has been used experimentally ([26]). This schedule of antimicrobial treatment was applied in Case 2 as described in this report, obtaining a complete recovery after 12 months. The regimens used for treatment in both patients have not been reported previously, and they give affected patients the possibility of healing without important sequelae such as amputation. Additional preventive efforts, such as BCG vaccination ([23]) and wearing long pants in endemic regions to protect lower extremities, may help to reduce the incidence of the disease ([27]).

Acknowledgment. The authors would like to thank Dra. Verónica Morán, for her suggestions for this report; QFB Misael González, from Juárez Hospital from the Secretaría de Salud in Mexico City, for the bacteriologic and immunologic analysis; and Prof. Françoise Portaels from the Mycobacteriology Department, Institute for Tropical Medicine, Belgium, for the molecular biology (PCR) studies.

REFERENCES

1. Clancey, J. K., Dodge, O. G., Lunn, H. F., and Oduori, M. L. Mycobacterial skin ulcer in Uganda. Lancet **2** (1961) 951–954.
2. Connor, D., and Lunn, H. F. Buruli ulceration. A clinicopathologic study of 38 Ugandans with *Mycobacterium ulcerans* ulceration. Arch. Path. **81** (1966) 183–199.
3. Debacker, M., Zinsou, C., Aguiar, J., Meyers, W., and Portaels, F. First case of *Mycobacterium ulcerans* disease (Buruli ulcer) following a human bite. CID **36** (2003) 67–68.
4. Debacker, M., Zinsou, C., Aguiar, J., Meyers, W., and Portaels, F. *Mycobacterium ulcerans* disease (Buruli ulcer) following human bite. Lancet **360** (2002) 1830.
5. De Gentile, L., Mahaza, C., Rolland, F., Carbonnelle, B., Verte, J. L., and Chabasse, D. L'Ulcere cutane a *Mycobacterium ulcerans*. A Propos D'Une Observation en Provenance de Guyane Francaise. Bull. Soc. Path. Ex. **85** (1992) 212–214.
6. Dodge, O. G. Mycobacterial skin ulcers in Uganda and Experimental aspects. J. Path. Bact. **88** (1964) 167–174.
7. Fenner, F. The significance of the incubation period in infectious diseases. Med. J. Austral. **2** (1950) 813–818.
8. Grange, J. M. Infection and disease due to the environmental mycobacteria. Trans. R. Soc. Trop. Med. **81** (1987) 179–182.
9. Grange, J. M. Mycobacteria and the skin. Int. J. Trop. Dermatol. **21** (1982) 497–503.
10. Guimaraes-Peres, A., Portaels, F., de Rijk, P., Fissette, K., Patín, S. R., van Vooren, J., and Fonteyne, P. Comparison of two PCRs for detection of *Mycobacterium ulcerans*. J. Clin. Microbiol. **37** (1999) 206–208.
11. Hautmann, G., and Loti, T. Atypical mycobacterial infections of the skin. Dermatologic. Clinics **12** (1994) 657–667.
12. Krieg, R., Hockmeyer, W., and Connor, D. Toxin of *Mycobacterium ulcerans*: production and effects in guinea pig skin. Arch. Dermatol. **110** (1974) 783–788.
13. Lavalle, P. Infección humana por *Mycobacterium ulcerans*. Confirmación del primer caso observado en México. Dermat. Rev. Mex. **1** (1956) 5–15.
14. Lavalle, P., de Ovando, F., Novales, J., and Ayala, J. L. Micobacteriosis cutanea ulcerosa. Sobretiro. Dermat. Rev. Mex. **25** (1981) 325–347.
15. Lavalle, P., and Novales, J. Ulcerative cutaneous mycobacteriosis by *Mycobacterium ulcerans*. The Mexican experience. Clinical Mycobacteriology. Prous. Science, S.A.M. Casal Editor (1998) 367–376.
16. MacCallum, P., Tolhurst, J. C., Buckle, G., and Sissons, H. A. A new mycobacterial infection in MA. J. Path. Bact. **60** (1948) 93–122.
17. Marston, B. J., Diallo, M. O., Horsburgh, C.R., Jr., Diomande, I., Saki, M. Z., Kanga, J. M., Patrice, G., Lipman, H. B., Ostroff. S. M., and Good, R. C. Emergence of Buruli Disease in the Daloa Region of Cote D'Ivoire. Am. J. Trop. Med. Hyg. **52(3)** (1995) 219–224.
18. Meyers, W. M., Connor, D. H., McCullough, B., Bourland, J., Moris, R., and Proos, L. Distribution of *Mycobacterium ulcerans* infections in Zaire, including the report of new foci. Ann. Soc. Belge. Med. Trop. **54(3)** (1974) 147–157.
19. Meyers, W. M., Shelln, W. M., Connor, D. H., and Meyers, E. Human *Mycobacterium ulcerans* infections developing at sites of truama to skin. J. Trop. Med. Hyg. **23** (1974) 919–924.
20. Meyers, W. M., Tignokpa, N., Priuli, G. B., and Portaels, F. *Mycobacterium ulcerans* (Buruli ulcer): first reported patients in Togo. Br. J. Dermatol. **134** (1996) 1116–1121.
21. Muelder, K., and Nourou, A. Buruli ulcer in Benin. Lancet **336** (1990) 1109–1111.
22. Pettit, J. H. S., Marchette, N. J., and Rees, R. J. W. *Mycobacterium ulcerans* infection. Clinical and bacteriological study of the first cases recognized in South East Asia. Br. J. Dermatol. **78** (1996) 187–197.

23. Portaels, F., Aguiar, J., Debacker, M., Guèdènon, A., Steunou, C., Zinsou, C. and Meyers, W. *Mycobacterium bovis* BCG vaccination as prophylaxis against *Mycobacterium ulcerans* osteomyelitis in Buruli ulcer disease. Infect. Immun. **72** (2004) 62–65.
24. Portaels, F., Elsen, P., Guimaraes-Peres, A., Fonteyne, P. A., and Meyers, W. Insects in the transmission of *Mycobacterium ulcerans* infection. Lancet **353** (1999) 986.
25. Pradinaud, R. E. R., and Grosshans, E. Le Probléme des Mycobactérioses cutanées en Guyane Francaise. Bull. Soc. Fr. Derm. Syph. **79** (1972) 684–686.
26. Pszolla, N., Robindra, M., Strecker, W., Kern, P., Kinzl, L., Meyers, W., and Portaels, F. Buruli ulcer: a systemic disease. CID **37** (2003) 78–82.
27. Scholessberg, D. Other non-tuberculous mycobacteria and *Mycobacterium bovis*. In: *Tuberculosis and Nontuberculous Mycobacterial Infections*. New York: WB Saunders Company, 1999. pp. 401–402.
28. Uganda Buruli Group: Clinical features and treatment of pre-ulcerative Buruli lesions (*Mycobacterium ulcerans* infections). Br. Med. J. **2** (1970) 390–393.
29. Van Oye, E., and Ballion, M. Faudra-T-Il Tenir Compte de 'Une Nouvelle Affection. Á Baciles Acido-Résistants en Afrique (Note Prélimi naire). [Article in French]. Int. J. Lepr. Reprintes Articles **19(3)** (1951) 327–329.
30. Ziefer, A., Connor, D., and Gybson, D. W. *Mycobacterium ulcerans* infection of two patients in Liberia. Int. J. Dermatol. **6** (1981) 362–367.