Am. J. Trop. Med. Hyg., 75(4), 2006, pp. 768–774

BCG VACCINE EFFECTIVENESS AGAINST BURULI ULCER: A CASE-CONTROL STUDY IN BENIN

FABIENNE NACKERS,* MICHÈLE DRAMAIX, ROCH CHRISTIAN JOHNSON, CLAUDE ZINSOU, ANNIE ROBERT, ELISA DE BIURRUN BAKEDANO, JUDITH R. GLYNN, FRANÇOISE PORTAELS, AND RENÉ TONGLET†

Unité d'Epidemiologie, Ecole de Santé Publique, Université Catholique de Louvain, Brussels, Belgium; Laboratoire de Statistiques Médicales, Université Libre de Bruxelles, Brussels, Belgium; Programme National de Lutte contre l'Ulcère de Buruli, Cotonou, Benin; Centre de Dépistage et de Traitement de l'Ulcère de Buruli, Lalo, Benin; Centre Sanitaire et Nutritionnel Gbemoten, Zagnanado, Benin; Infectious Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, London, United Kingdom; Department of Microbiology, Institute of Tropical Medicine, Antwerp, Belgium

Abstract. BCG remains the only possible prophylactic intervention against Buruli ulcer (BU). Estimating its public health impact on BU control is an important issue. We conducted a case-control study to investigate the vaccine effectiveness of routine BCG vaccine against BU in southern Benin. From August 2002 to August 2003, BCG vaccination status was obtained for 279 clinically diagnosed BU cases and 988 age- and sex-matched neighborhood controls. BCG coverage, which was estimated by the presence of a scar or a vaccination record, was 64.5% in cases and 67.2% in controls. There was no evidence of a protective effect of routine BCG vaccination against BU in southern Benin (vaccine effectiveness adjusted for socioeconomic status = 12%, 95% confidence interval = −24% to 37%).

INTRODUCTION

In 1998, *Mycobacterium ulcerans* disease was declared by the World Health Organization as an emerging skin disease of public health concern.[1] Usually called Buruli ulcer (BU), the disease has been reported in at least 30 (mostly tropical) countries.[2] The major burden is in west Africa[3–5] where the clinical lesion starts as a painless subcutaneous nodule, plaque, or edema that secondarily ulcerates with characteristic undermined edges.[6] Bones can also be involved.[7] Although there is an increasing evidence of the efficacy of antibiotic therapy, treatment still relies on surgical excision of infected and necrotic tissues. Since BU mostly affects rural populations with limited geographic, financial, and cultural access to health services, patients usually present late with severe clinical forms of the disease, including extensive skin destruction, multiple lesions, secondary infection and/or bone involvement. Even with adequate treatment, permanent disabilities can remain.

Control strategies against BU are limited because of a lack of knowledge of potential risk factors and the absence of a specific vaccine. The protective effect of BCG vaccination in preventing BU remains controversial. Two trials conducted in Uganda[8,9] reported short-lasting protection (47%), and a case series in Benin suggested that BCG could protect against BU osteomyelitis.[10,11] Conversely, case-control studies conducted in Ghana[12,13] did not provide any evidence of a protective effect of BCG against BU. In a recent large hospital-based case-control study conducted in Benin, Debacker and others[14] reported an increased risk of BU after BCG vaccination in adults and children more than five years of age. Since BCG is the only prophylactic intervention, estimating its public health impact on BU control is an important issue.

The frequency of BU varies widely from village to village, even within the same district,[15,16] which presumably reflects differences in environmental exposure to *M. ulcerans.* Studies of risk factors for disease must take into account this variation in exposure. We present a case-control study with neighborhood controls to assess the effectiveness of routine BCG vaccination in preventing BU in southern Benin.

METHODS

Setting. The study was conducted in southern Benin. Buruli ulcer is endemic in this area with more than 5,700 patients reported since 1989.[2] In 2002, the area included two reference centers for the treatment of BU: the Center Sanitaire et Nutritionnel Gbemoten (CSNG) at Zagnanado, Zou region, and the Center de Dépistage et de Traitement de l'Ulcère de Buruli (CDT/UB) at Lalo, Mono-Couffo region. Patients come directly to these centers, often motivated by previously treated patients, and are also referred through village outreach activities.[4] In 2003, the prevalence of human immunodeficiency virus among Beninese adults 15–49 years of age was estimated to be between 1.1% and 3.3%.[17]

Since the implementation of the Beninese Expanded Program of Immunization (EPI) in 1982, the vaccination policy is to administer BCG at birth. BCG had always been administered intradermally on the upper third of the dorsal surface of the left forearm. It was not possible to obtain information on the strains and doses used in Benin but it is likely that they varied over time.

Cases. From August 2002 to May 2003 at the CSNG and from August 2002 to August 2003 at the CDT/UB, all patients clinically diagnosed with BU by medical officers specialized in BU treatment were eligible for enrollment. Patients living outside Benin were excluded, as were those living in the cities of Cotonou and Porto-Novo, because it is unlikely that they contracted BU in those urban areas.

Controls. The study protocol aimed to recruit four controls per case. Neighborhood controls were individually matched to cases by sex and age (categories: < 1, 1–5, 6–12, 13–19, 20–29, 30–39, and ≥ 40 years). A door-to-door systematic procedure was used for control selection. Field investigators started at the case's house, chose a random direction, and visited the nearest house. They listed all members of the

* Address correspondence to Fabienne Nackers, Ecole de Santé Publique, Université Catholique de Louvain, Clos Chapelle-aux-Champs 30/EPID3058, 1200 Woluwé-Saint-Lambert, Belgium. E-mail: Fabienne.Nackers@epid.ucl.ac.be
† Deceased.

household and identified potential controls fitting the matching criteria. If several subjects were identified, they chose the closest in age to the case. The procedure was repeated in each consecutive house until four appropriate controls were found. If identified potential controls were absent, one or two additional visits were planned. Controls were examined to rule out active or healed BU.

Data collection. Clinical information was abstracted from medical records. Lesions were classified according to clinical form: nodule, plaque, edema, ulcer, osteomyelitis, mixed, and other lesions. Specimens of tissue and of exudates from some patients were analyzed by Ziehl-Neelsen staining, culture, IS*2404* polymerase chain reaction, and histopathologic examination[18] to confirm the clinical diagnosis. Participants were examined for a BCG scar. When available, vaccination records were checked. All subjects were asked about prior BCG vaccination. For children less than 15 years of age, parents or guardians were used as informants. Evidence of BCG vaccination was defined by a BCG scar or a BCG vaccination record. Individuals with a doubtful scar were excluded from the analysis. In a separate analysis, we also excluded subjects without a scar or vaccination record but who provided a history of BCG vaccination. Scar size was not recorded.

Participants or guardians answered a questionnaire. Questions on socioeconomic characteristics included education level of the participants, education level and occupation of the head of the participant's household, type of house, and possession of selected items by the household (radio, bicycle, motorbike, car, and dugout canoe). The household was defined as all subjects sharing the same roof and the same cooking pot. Factors recorded to estimate likely environmental exposure to *M. ulcerans* included participation in farming, water-contact frequency with distinction between stagnant and flowing water, and main drinking water source and drawing water at this source. Information on whether young children accompanied adults during farming or obtaining water was recorded.

Interviews were conducted in French, Fon, or Adja by nurses or biomedical technicians trained in the study protocol, BCG scar reading and recognizing BU lesions. Hospital and field teams included different investigators not blind to the disease status of the participants.

Statistical analysis. Estimating from a preliminary pilot study that 25% of the controls and 35% of the cases had no BCG scar, the proportion of discordant pairs was expected to be 43%. To have 80% power to detect whether the odds ratio (OR) is significantly different from 1 at the 5% level, with a VE of 40% considered to be clinically relevant, the sample should include 208 cases and 832 matched-controls in a 1:4 matched ratio.[19] In practice, we aimed to recruit 300 cases to allow subgroup analysis.

The ORs and 95% confidence intervals (CIs) were calculated using conditional logistic regression. Associations were tested using the likelihood ratio test (LRT) and, for ordered categorical variables, the LRT for trend. Vaccine effectiveness (VE%) was calculated as $(1 - OR) \times 100$.[20] Estimation of VE was adjusted for socioeconomic status (SES) and for factors estimating likely environmental exposure to *M. ulcerans*; age, sex, and neighborhood were accounted for by the matched design and analysis. The SES was measured by house type, education level of the head of the household, and a score of ownership based on household possession of a radio, bicycle, motorbike, dugout canoe, or car. Items were weighted according to the inverse frequency of households owning this item in rural Benin in 2001[21] and then summed. Higher scores therefore reflected both greater ownership and owning less common (and by inference more valuable) items. Vaccine effectiveness was assessed separately for age groups older and younger than 20 years (born after the implementation of the EPI and therefore likely to have been vaccinated at birth), by sex, for laboratory confirmed BU, and for patients with BU osteomyelitis. Differences between subgroups were tested for interaction using LRT. Reliability of scar reading was estimated using a kappa coefficient for multiple observers. Data were entered in Epi-Info version 6.04 (Centers for Disease Control and Prevention, Atlanta, GA) and analyzed with STATA version 9.0 (Stata Corporation, College Station, TX).

Ethics. Data were collected after informed consent of the participant or participant's guardian. Potential controls with BU lesions were offered appropriate treatment. The study was reviewed and approved by the Beninese Public Health Minister.

RESULTS

Study population. Among the 446 eligible patients, 2 refused participation in the study, 3 were excluded because of doubtful scars, 1 was excluded because of missing BCG data, 42 were excluded because a BU lesion on the left forearm made identification of a BCG scar impossible, and 78 (17.5%) were not recruited because of work overload in the health centers. Information on BCG status was thus available for 320 patients. Mainly because of wrongly listed addresses and impassable roads preventing field teams from reaching villages, suitable controls could be matched for only 229 (71.6%) of these patients. Fifty patients hospitalized for BU at the CSNG between January and August 2002 and living in villages vis-

TABLE 1
Description of the cases (n = 279) of Buruli ulcer in Benin

Variable	No.	%
Clinical form†		
Edema	8	2.9
Ulcer	127	45.7
Nodule	7	2.5
Plaque	61	21.9
Osteomyelitis	7	2.5
Others	3	1.1
Mixed	65	23.4
Clinical stage†		
Not ulcerative	92	33.1
Ulcerative	137	49.3
Healing	34	12.2
Several stages	12	4.3
Others	3	1.1
Number of positive laboratory test results‡		
0	45	16.1
1	34	12.2
≥ 2	100	35.8
Untested	100	35.8
Hospitalized at the CDT/UB, Lalo*	92	33.0
Hospitalized at the CSNG, Zagnanado*	187	67.0

* CDT/UB = Centre de Dépistage et de Traitement de l'Ulcère de Buruli; CSNG = Centre Sanitaire et Nutritionnel Gbemoten.
† One missing value.
‡ Ziehl-Neelsen staining, histology, culture and/or polymerase chain reaction.

TABLE 2
Demographic characteristics of the participants

	Cases No. (%) n = 279	Controls No. (%) n = 988	*P*
Female	131 (47.0)	467 (47.3)	*
Age (years)			*
< 6	36 (12.9)	114 (11.5)	
6 to < 13	99 (35.5)	360 (36.4)	
13 to < 20	36 (12.9)	136 (13.8)	
20 to < 30	39 (14.0)	133 (13.5)	
30 to < 40	18 (6.5)	72 (7.3)	
≥ 40	51 (18.3)	173 (17.5)	
Region of residence			*
Atlantique	14 (5.0)	43 (4.4)	
Ouémé-Plateau	122 (43.7)	429 (43.4)	
Zou	52 (18.6)	179 (18.1)	
Mono-Couffo	91 (32.6)	337 (34.1)	
Ethnicity†			0.43‡
Fon	222 (80.1)	792 (80.7)	
Adja	48 (17.3)	169 (17.2)	
Others	7 (2.5)	21 (2.1)	

* Matching variable.
† Two matched sets lost due to missing data in cases.
‡ By conditional logistic regression.

ited by the field investigators were retrospectively recruited with suitable controls. They were included in the analysis, resulting in a total of 279 matched sets. Cases included in the matched analysis were similar to the eligible cases not included regarding sex and age, but they were more likely to have come from the Ouémé region and to be vaccinated with BCG (64.5% versus 50.5%; $P = 0.018$).

In 7 of more than 200 villages visited, local authorities refused to allow persons to participate in the study. Eight controls were reported to have refused to answer the questionnaire but individual refusals were not systematically recorded. Three controls with suspected BU lesions, 3 with missing BCG data, and 25 with doubtful scars were excluded. A total of 988 controls were matched to the 279 cases (average matching ratio = 1:3.5).

Clinical description of cases. The median age was 13.4 years (range = 11 months to 80 years) and 47% were female. Ulcer was the most frequent form (46%) followed by the mixed (23%) and the plaque forms (22%) (Table 1). A total of 24 (8.7%) patients had bone involvement (7 without active skin lesions and 17 with a mixed form of BU). Recurrence accounted for 21 (7.5%) of the cases. Forty-eight percent of the cases were confirmed by at least one of the four laboratory tests (Table 1).

Sociodemographic characteristics and SES. Matching resulted in sex, age, and region of residence being similar in cases and controls (Table 2). The predominant ethnic group was Fon. Cases were more likely than controls to live in cemented or brick houses and were of higher SES as measured by the score of ownership and education level of the head of the household (Table 3). However, there was no evidence that education level of cases and controls more than 20 years of age differed (Table 3). Among those 6–19 years of age, 83 (61.9%) of the cases were currently attending school compared with 306 (66.0%) of the controls ($P = 0.38$).

BCG vaccination coverage. BCG coverage estimated by the presence of a scar or a vaccination record was 64.5% among 279 cases and 67.2% among 988 controls. As shown in Table 4, 11 participants with a scar or a BCG vaccination record claimed not to have been vaccinated. Thirty (30.3%) of 99 cases and 117 (36.1%) of 323 controls without a scar or vaccination record claimed to have been vaccinated. Overall,

TABLE 3
Socioeconomic characteristics of the participants

	Cases No. (%)	Controls No. (%)	OR (95%CI)*	*P*†
House type				< 0.001
Mud, wood, straw	75 (27.3)	369 (38.5)	1	
Mud, sheet metal roof	139 (50.6)	538 (56.2)	1.49 (1.03, 2.14)	
Cement, brick	61 (22.2)	51 (5.3)	10.04 (5.64, 17.84)	
Head of the household's education level				0.006‡
None	149 (56.9)	581 (64.3)	1	
Primary uncompleted	59 (22.5)	190 (21.0)	1.30 (0.81, 1.83)	
Completed primary or higher	54 (20.6)	132 (14.6)	1.77 (1.19, 2.59)	
Participant's education level (> 20 years)				0.43
None	72 (67.9)	273 (73.8)	1	
Primary uncompleted	20 (18.9)	55 (14.9)	1.51 (0.79, 2.91)	
Completed primary or higher	14 (13.2)	42 (11.4)	1.44 (0.62, 3.34)	
Head of the household's occupation				0.028
Farmer, fisher	167 (61.9)	661 (70.8)	1	
Manual, trade, driver	63 (23.3)	189 (20.2)	1.35 (0.93, 1.96)	
Professional	24 (8.9)	53 (5.7)	2.02 (1.13, 3.64)	
Village/religious authority, traditional healer	6 (2.2)	17 (1.8)	1.53 (0.56, 4.13)	
Retired, none	10 (3.7)	14 (1.5)	3.15 (1.16, 8.54)	
Score of ownership§				< 0.001‡
1	28 (10.4)	159 (17.1)	1	
2	91 (33.7)	400 (42.9)	1.40 (0.85, 2.30)	
3	101 (37.4)	287 (30.8)	2.20 (1.33, 3.64)	
4	50 (18.5)	86 (9.2)	4.36 (2.40, 7.95)	

* OR = odds ratio; CI = confidence interval.
† By conditional logistic regression.
‡ By conditional logistic regression. Test for trend.
§ Household possession of a radio, bicycle, motorbike, dugout or car; items were weighted according to the inverse frequency of households owning this item in rural Benin in 2001[21] and then summed and categorized.

TABLE 4
BCG vaccination status of cases and controls according to the source of information

Clinical examination for a BCG scan and BCG vaccination record	History of BCG vaccination according to the subject or subject's guardian*			
	Yes	No	Do not know	Total
Cases				
Presence of a BCG scar	148	4	12	164
BCG vaccination record (± BCG scar)	15	1	0	16
Not BCG vaccinated (no scar, no record)	30	59	10	99
Total	193	64	22	279
Controls				
Presence of a BCG scar	567	6	44	617
BCG vaccination record (± BCG scar)	43	0	3	46
Not BCG vaccinated (no scar, no record)	117	158	48	323
Total	727	164	95	986

* Missing value in one control with a BCG scar and one control without BCG scar or BCG vaccination record.

84.0% of the 920 participants claiming to have been vaccinated with BCG had a recognizable scar or a BCG vaccination record.

Sixty-two subjects (16 cases and 46 controls) had a BCG vaccination record. They were less than 12 years of age and were vaccinated before five months of age. Scar examination in two of them was not possible because the BU lesion was on the vaccination site; 13.3% (8 of 60) had no scar.

A subsample of subjects were examined independently for BCG scar by four investigators of CSNG (n = 53) and three investigators of CDT/UB (n = 27). Kappa coefficients were 0.78 for CSNG and 0.90 for CDT/UB, which indicated good intra-observer agreement on the presence of a scar.

BCG vaccine effectiveness. The BCG VE was 15% (95% CI = –15% to 37%, $P = 0.29$) (Table 5). When adjusted for SES, as measured by education level of the head of the household, score of ownership and type of house, VE changed very little (Table 5). Adjusting for head of the household's occupation or for the participant's education level did not change the estimates. The estimates were also unchanged after additionally adjusting for factors measuring likely environmental exposure to *M. ulcerans* (contacts with flowing water, contacts with stagnant water, participation in farming, and drawing surface water for drinking).

Few participants had more than one BCG scar and the trend for an increased VE with increasing number of doses was not statistically significant (Table 5). Excluding subjects without scar or vaccination record but reporting a history of BCG vaccination increased the VE estimations (Table 5) but still provided weak evidence for a protective effect of BCG against BU.

Table 6 shows VE estimations for patient subgroups. The VE was higher in subjects more than 20 years of age but CIs were wide, and the test for interaction did not provide evidence for a true difference compared with VE in younger people. Also, the VE was higher in females than in males, but the interaction was not statistically significant. The VE was similar in laboratory-confirmed cases and unconfirmed cases. The VE tended to be higher against BU osteomyelitis compared with other forms of BU, but there was no statistical evidence for a difference, as reflected by the overlapping 95% CIs.

TABLE 5
BCG vaccine effectiveness (VE) against Buruli ulcer

	All subjects				Subjects without missing data for socioeconomic status				
	Cases No. (%)	Controls No. (%)	VE (%) from matched analysis (95% CI)*	P†	Cases No. (%)	Controls No. (%)	VE (%) from matched analysis (95%CI)*	Adjusted‡ VE (%) (95% CI)	P†
All subjects included				0.29					0.48
Vaccinated (scar and/or vaccination record)	180 (64.5)	664 (67.2)	15 (–15, 37)		165 (66.0)	561 (67.8)	13 (–20, 37)	12 (–24, 37)	
Unvaccinated (no scar no vaccination record)	99 (35.5)	324 (32.8)			85 (34.0)	267 (32.2)			
Number of scars				0.25§					0.46§
0	99 (35.5)	324 (32.8)			85 (34.0)	267 (32.2)			
1	178 (63.8)	652 (66.0)	15 (–16, 37)		163 (65.2)	552 (66.7)	13 (–20, 37)	11 (–25, 37)	
> 1	2 (0.7)	12 (1.2)	47 (–159, 89)		2 (0.8)	9 (1.1)	28 (–276, 86)	26 (–307, 87)	
Subjects without scar or vaccination record but reporting a history of BCG vaccination excluded				0.07					0.06
Vaccinated (scar and/or vaccination record)	176 (71.8)	592 (76.3)	30 (–2, 51)		161 (72.8)	507 (77.5)	32 (–1, 54)	33 (–1, 56)	
Unvaccinated (no scar, no vaccination record and no reported history of BCG vaccination)	69 (28.2)	184 (23.7)			60 (27.2)	147 (22.5)			

* CI = confidence interval.
† By conditional logistic regression.
‡ Additionally adjusted for education level of the head of the household, score of ownership, and type of house.
§ LRT By conditional logistic regression for trend.

TABLE 6
BCG vaccine effectiveness (VE) against Buruli ulcer (BU) by age group and sex for confirmed BU and BU osteomyelitis

	Cases No. (% vaccinated)†	Controls No. (% vaccinated)†	VE (%) from matched analysis (95% CI)*	Adjusted‡ VE (%) (95% CI)*
Age, years[a]				
< 20	171 (71.4)	610 (72.1)	7 (–37, 38)	–7 (–67, 32)
≥ 20	108 (53.7)	378 (59.3)	24 (–21, 53)	33 (–15, 60)
Sex[b]				
Female	131 (58.8)	467 (66.4)	33 (–3, 57)	29 (–18, 57)
Male	148 (69.6)	521 (68.0)	–6 (–62, 30)	–6 (–67, 34)
Confirmed BU§	134 (67.9)	465 (71.6)	21 (–25, 50)	14 (–45, 49)
Not confirmed BU	145 (61.4)	523 (63.3)	10 (–33, 40)	10 (–42, 42)
BU osteomyelitis	24 (50.0)	78 (64.1)	48 (–48, 81)	53 (–34, 83)
Other BU	253 (66.0)	902 (67.7)	12 (–21, 36)	6 (–36, 35)

* CI = confidence interval.
[a] P values (conditional logistic regression) for modification of VE according to age groups = 0.52 (matched analysis) and 0.19 (adjusted for socioeconomic status [SES]).
[b] P values (conditional logistic regression) for modification of VE according to sex = 0.13 (matched analysis) and 0.26 (adjusted for SES).
† Vaccinated = scar and/or vaccination record; unvaccinated = no scar and no vaccination record.
‡ Adjusted for SES: head of the household's education level, score of ownership, and type of house.
§ Confirmed by at least one laboratory test (Ziehl-Neelsen staining, culture and IS*2404* polymerase chain reaction and histopathologic examination).

DISCUSSION

Our study does not provide evidence that BCG administered under routine conditions in Benin reduces the risk of BU among those vaccinated. Overall VE estimation adjusted for SES was 12% (95% CI = –24% to 37%). Although the CI is wide, strong protection is unlikely.

Our results are consistent with those of two case-control studies conducted in Ghana.[12,13] Those studies were designed to investigate several potential risk factors for BU, including presence of a BCG scar. Although the investigators did not report the OR (or VE), they reported no statistically significant difference in BCG scar prevalence between cases and matched neighborhood controls of similar age, as in our study.

In two Ugandan trials,[8,9] VE against BU was estimated to be 72% in the first 6 months[8] and 63% in the first year[9] after vaccination; it then waned to 0%. BCG was administered to subjects (from less than one to more than 25 years of age) with weak or negative tuberculin reactions. Besides important differences in geographic location and design, our results are difficult to compare with those trials because most of our participants received neonatal BCG and were included in the study more than one year after vaccination. Also, those studies estimated vaccine efficacy under ideal storage, handling, and administration conditions of a clinical trial, while our study estimated vaccine effectiveness, i.e., efficacy under field conditions.[22]

Because of the small area variation in the distribution of *M. ulcerans*[15,16] and the likely geographic variation of BCG vaccine coverage, we used neighborhood controls. We were able to adjust for factors linked to likely environmental exposure to *M. ulcerans*, and this did not change our estimates. Neighborhood controls have the disadvantage of possibly being less frequent users of health services. At the time of our study, the CSNG and CDT/UB were the main referral centers for treating BU in southern Benin. Both centers had important village outreach activities and some patients were referred to the centers through active case detection. Consequently, referral patterns of BU patients differed from those of patients treated for other diseases in the same centers. However, ascertainment of BU cases in the region is unlikely to have been complete because people are often reluctant to seek medical treatment of BU,[23] mainly because of fear of surgery and the cost of treatment, especially the indirect costs during the long duration of hospitalization.[24] We adjusted VE for SES to minimize this bias in likely health service usage patterns between cases and controls and VE changed very little. However, SES is difficult to measure and we cannot exclude residual confounding.

Because of work overload in the health centers, not all eligible patients were recruited, but this was regardless of their BCG status. For those recruited, matching was incomplete mainly because of wrongly listed addresses and impassable roads that prevented field teams from reaching villages. Included cases had a similar age and sex distribution compared with those not included, but included cases came from different geographic areas than case not included. In particular, included cases were more likely to come from the Ouémé-Plateau region where the highest BCG coverage has been reported.[21] Also, BCG coverage is likely to be lower in difficult to reach areas. Consequently, 64.5% of the cases included in the matched analysis were vaccinated compared with 50.5% of those not included. Since controls were matched in neighborhoods, this would not have biased our VE estimation.

Only half of the cases were laboratory confirmed and misclassified cases can decrease VE estimation.[25] However, there was no statistical evidence for a difference in BCG VE between laboratory confirmed cases and other cases.

Uncertainty about BCG vaccination status could have led to underestimation of BCG VE in our study. Vaccination records were seldom available and our definition of BCG vaccination status relied mainly on the presence of a BCG scar. Presence of a scar is recognized as a highly sensitive and reliable indicator of BCG vaccination status[26] except when administered within one month of birth.[27] Neonatal vaccination is a long-established policy in Benin, and we could estimate in our study that approximately 87% of the BCG vaccinations resulted in a scar. Since scar formation is probably independent of immune response,[27] this could have led to non-differential misclassification and VE underestimation. Therefore, we excluded subjects without BCG scars or vaccination records but who claimed to have received BCG and re-estimated BCG VE. Vaccine effectiveness estimates in-

creased to 33% (SES-adjusted) but the CI was wide and still provided weak statistical evidence for an effect of BCG in preventing BU in Benin.

Cases and controls were examined by different investigators. They were not blinded to the disease status of the participant or to the research hypothesis. Therefore, we cannot formally exclude differential observer bias. However, in both centers, teams were trained by the same member of the research team, and we have no reasons to believe that cases were more carefully examined for scars than controls.

The benefit of BCG revaccination had been reported against leprosy, in a setting where the initial dose appeared protective,[28] but not against tuberculosis.[28,29] In Uganda, there was no evidence for an enhanced protection of BCG revaccination against BU.[9] We observed a trend for an increased VE in subjects with more than one scar attributable to BCG vaccination. However, few subjects had more than one scar and the trend was not statistically significant. We also found a higher protective effect of BCG against BU osteomyelitis, which is consistent with the observations of Portaels and others,[10,11] but the numbers of patients were small.

We found higher VE for subjects more than 20 years of age compared with younger persons. This is surprising because VE is expected to wane with time since vaccination,[30] and VE estimation in subjects more than 20 years of age measured the long-lasting protection conferred by BCG administered at least 20 years previously. Underestimation of VE could be serious if less than 90% of the vaccinations left a scar and more than 70% of the population has been vaccinated,[31] which is the case for subjects younger than 20 years of age included in our study. This underestimation could therefore have been more important in this group than in the older age group, in whom vaccination coverage was less than 60% and vaccines may have been given beyond the neonatal period. However, there was no statistical evidence for a true difference of BCG VE between age groups.

Although our BCG VE estimations could have been slightly underestimated, our data do not provide evidence of a strong protective effect of routinely administered BCG against BU in southern Benin. From a public health perspective, routine neonatal BCG appears of little value in controlling BU in this population. Thus, effective prevention of BU by immunoprophylaxis needs further vaccine development.

Received March 31, 2006. Accepted for publication June 1, 2006.

Acknowledgments: This article is dedicated to the memory of Professor René Tonglet, who passed away June 5, 2005. We sincerely thank the field investigators, the staff of the Programme National de Lutte contre de l'Ulcère de Buruli, CDT/UB, and CSNG for their help and hospitality, especially Soeur Julia Aguiar. We are grateful to all patients and villagers for participating in this study.

Financial support: This work was supported by the Damien Foundation (Brussels, Belgium), the Belgian Fonds de la Recherche Scientifique Médicale (grant no. 3.4501.03), and the Université Catholique de Louvain Fonds Spéciaux de Recherche (grant No 2002, 20c5). Fabienne Nackers analyzed the data while obtaining an MSc degree in Epidemiology at the London School of Hygiene and Tropical Medicine. She was supported by a British Medical Research Council Advanced Course Masters studentship and a grant from the Belgian Medical Foundation Mathilde Horlait-Dapsens. Roch Christian Johnson, Claude Zinsou, and Elisa de Biurrun Bakedano were supported by a grant from the Directorate-General for Development Cooperation (Brussels, Belgium; Project: Buruli Ulcer in Benin). Judith Glynn is supported by the United Kingdom Department of Health (Public Health Career Scientist Award).

Authors' addresses: Fabienne Nackers, Annie Robert, and René Tonglet, Ecole de Santé Publique, Université Catholique de Louvain, Clos Chapelle-aux-Champs 30/EPID3058, 1200 Woluwé-Saint-Lambert, Belgium, Telephone: 32-2-764-3320, Fax: 32-2-764-3328, E-mails: Fabienne.Nackers@epid.ucl.ac.be and Annie.Robert@epid.ucl.ac.be. Michèle Dramaix, Ecole de Santé Publique, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium, Telephone: 32-2-555-4051, Fax: 32-2-555-4049, E-mail: miwilmet@ulb.ac.be. Roch Christian Johnson, Programme National de Lutte contre l'Ulcère de Buruli au Bénin, Ministère de la Santé Publique, 06 BP 2572, Cotonou, Benin, Telephone: 229-331-827, Fax: 229-337-057, E-mail: rochjohnson@yahoo.fr. Claude Zinsou, Centre Sanitaire et Nutritionnel de Gbemoten, Zagnanado, Benin, Fax: 229-520-293. Elisa de Biurrun Bakedano, Centro Nacional de Medicina Tropical, Instituto de Salud Carlos III, C/Sinesio Delgado 4, Pabellón 13, 28029 Madrid, Spain, Telephone: 91-8-222-940, Fax: 91-3-877-756, E-mail: ebiurrun@isciii.es. Judith R. Glynn, Infectious Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom, Telephone: 44-20-792-72423, Fax: 44-20-7636-8739, E-mail: Judith.Glynn@lshtm.ac.uk. Françoise Portaels, Department of Microbiology, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium, Telephone: 32-3-247-6317, Fax: 32-3-247-6333, E-mail: portaels@itg.be.

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