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Risk Factors for Buruli Ulcer Disease (*Mycobacterium ulcerans* Infection): Results from a Case-Control Study in Ghana

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Background. Morbidity due to Buruli ulcer disease (BUD), a cutaneous infection caused by *Mycobacterium ulcerans*, has been increasingly recognized in rural West Africa. The source and mode of transmission remain unknown.

Methods. To identify BUD risk factors, we conducted a case-control study in 3 BUD-endemic districts in Ghana. We enrolled case patients with clinically diagnosed BUD and obtained skin biopsy specimens. *M. ulcerans* infection was confirmed by at least 1 of the following diagnostic methods: histopathologic analysis, culture, polymerase chain reaction, and Ziehl-Neelsen staining of a lesion smear. We compared characteristics of case patients with confirmed BUD with those of age- and community-matched control subjects using conditional logistic regression analysis.

Results. Among 121 case patients with confirmed BUD, leg lesions (49%) or arm lesions (36%) were common. Male case patients were significantly more likely than female case patients to have lesions on the trunk (25% vs. 6%; $P = .009$). Multivariable modeling among 116 matched case-control pairs identified wading in a river as a risk factor for BUD (odds ratio [OR], 2.69; 95% confidence interval [CI], 1.27–5.68; $P = .0096$). Wearing a shirt while farming (OR, 0.27; 95% CI, 0.11–0.70; $P = .0071$), sharing indoor living space with livestock (OR, 0.36; 95% CI, 0.15–0.86; $P = .022$), and bathing with toilet soap (OR, 0.41; 95% CI, 0.19–0.90; $P = .026$) appeared to be protective. BUD was not significantly associated with penetrating injuries ($P = .14$), insect bites near water bodies ($P = .84$), bacille Calmette-Guérin vaccination ($P = .33$), or human immunodeficiency virus infection ($P = .99$).

Conclusions. BUD is an environmentally acquired infection strongly associated with exposure to river areas. Exposed skin may facilitate transmission. Until transmission is better defined, control strategies in BUD-endemic areas could include covering exposed skin.

Buruli ulcer disease (BUD), an infection due to *Mycobacterium ulcerans*, has been an increasingly recognized cause of morbidity in rural Africa [1–4]. The development of effective strategies for control of BUD has been hampered by several factors [4, 5]. Although infection is postulated to be environmentally acquired, the natural reservoir and precise mode(s) of *M. ulcerans* transmission are unclear. Diagnostic methods to confirm *M. ulcerans* infection are expensive and ill-suited to low-resource areas. The effectiveness of antimycobacterial drug therapy has not been proven [6, 7]; consequently, surgery is the recommended treatment option.

Unique among mycobacteria, *M. ulcerans* produces a macrolide toxin, mycolactone, that promotes coagulation necrosis of subcutaneous adipose tissue surrounding sites of bacterial colonization [2, 8–10]. BUD involves 3 clinical stages: preulcerative, ulcerative, and

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inactive disease [4, 5]. Clinical presentations of active BUD range from painless subcutaneous nodules to extensively undermined ulcers. Preulcerative lesions—including nodules, papules, plaques, and edema—can develop into large ulcers within weeks to months. Ulcers can heal spontaneously, producing a depressed stellate scar characteristic of inactive BUD. Four diagnostic techniques are recognized for confirmation of *M. ulcerans* infection, but in areas of endemicity lacking laboratory resources, the diagnosis of BUD relies on clinical judgment.

Epidemiologic studies of BUD have implicated stagnant water bodies in *M. ulcerans* transmission [2]. In Australia, an outbreak of BUD occurred in a suburban community near a golf course irrigated with dammed water [11, 12]. No additional cases occurred after irrigation was halted [11]. In Africa, BUD primarily afflicts rural farmers in swampy environments and was extensively studied in a Ugandan refugee camp [4, 13]. BUD outbreaks in Nigeria and Australia have been associated with environmental changes, such as flooding or damming [11, 14, 15]. Despite suggestive epidemiologic evidence, *M. ulcerans* has never been cultured from environmental specimens [12, 16]. *M. ulcerans* DNA has been detected in vegetation and water associated with the Australian outbreak [17]. These observations have led to the hypothesis that *M. ulcerans* naturally exists in riverine environments and that people acquire infection through contact with contaminated water or vegetation. Disruption of cutaneous integument is thought to introduce the organism into subcutaneous tissue, but, because of delayed disease development, the injury is not remembered [18]. Alternative hypotheses include aerosol transmission from *M. ulcerans*–contaminated water sources [15] or insect vectors [19].

Two previous case-control studies explored risk factors for BUD [20, 21]. In Côte d'Ivoire, farming near the river was a risk factor, and wearing long pants was protective against BUD [20]. An earlier study in Uganda failed to implicate any specific risk behaviors [21]. Both studies were limited by the use of clinically diagnosed cases of BUD that lacked laboratory evidence for *M. ulcerans* infection. Consequently, we conducted a case-control investigation with systematic laboratory confirmation to identify modifiable risk factors for BUD in Ghana.

METHODS

Study design and case definitions. We conducted a matched case-control study in 3 districts of Ghana (Amansie West, Asante Akim North, and Upper Denkyira) where BUD was highly prevalent [22]. These districts are characterized by abundant rivers, streams, swamps, and environmental changes due to logging and mining. An established BUD treatment center in a local hospital serves each district.

BUD lesions during the active stage of disease were categorized as preulcerative (nonulcerative edematous plaque or subcutaneous nodule) or ulcerative (painless cutaneous ulcer with induration and undermined borders) [4, 23]. Inactive BUD was defined as a depressed stellate scar from a previously diagnosed episode of active BUD. A probable case of BUD was defined as BUD in a patient aged $\geq$2 years who presented to 1 of the 3 study hospitals between 23 August and 30 November 2000 with active or inactive BUD lesions.

A confirmed case of BUD was defined as a probable case with evidence of *M. ulcerans* infection revealed by $\geq$1 diagnostic test: Ziehl-Neelsen (ZN) staining of smears of lesion exudates, histopathological analysis, mycobacterial culture, or PCR. An eligible control subject was defined as a person residing in the case patient's village who was matched by age category and had no signs or symptoms of BUD or tuberculosis. One age- and village-matched control subject was selected from houses nearest the case patient's residence by means of a defined method. For 6 case patients with BUD who resided in isolated homesteads, control subjects were selected from the nearest village.

Recruitment and consent. Case patients were identified from hospital wards and through active community recruitment. Study enrollment was voluntary; trained personnel explained procedures and obtained written informed consent or assent from case patients and control subjects and/or their parents or adult guardians in their native language. The study protocol was approved by ethics committees at the Centers for Disease Control and Prevention (Atlanta), Emory University (Atlanta), Groningen University (The Netherlands), and the Ghana Health Service. Unlinked anonymized HIV testing was performed in accordance with Ghana's National HIV Control Program guidelines.

Data collection. Study personnel administered a standardized questionnaire to participants concerning demographic, environmental, and behavioral risk factors. Participants were asked about activities performed during the year before BUD onset (case patients) or during the past year (control subjects). Tissue specimens and lesion swab specimens were obtained from case patients with probable BUD undergoing surgical excision, debridement, or wound cleaning. Serum specimens were collected from case patients and control subjects for BUD assay development.

Laboratory methods. BUD was confirmed by at least 1 of the following methods: ZN staining for detection of acid-fast bacilli in smears of lesion exudates [24], ZN staining for detection of acid-fast bacilli in histologic sections [25], mycobacterial culture [26], and PCR [27, 28]. Unlinked anonymized serum specimens were screened for HIV-1 and -2 antibody by an enzyme immunoassay (BioRad Genetic Systems), and results were confirmed using HIV-1 Western blot analysis (Calypte Biomedical).

Statistical methods. Questionnaire responses were double entered using EZC software (N.A. Hills Computing Services Ltd.); cleaning and analysis of data was performed with SAS,

Table 1. Distribution of diagnostic test result profiles among 144 case patients from Ghana with probable Buruli ulcer disease who underwent examination by at least 1 laboratory method.

No. of positive results	Test result profile				No. (%) of case patients (n = 144)
	Culture	Histopathological analysis	PCR	Ziehl-Neelsen staining	
Zero	−	−	−	−	23 (16)
One					45 (31)
	+	−	−	−	1 (<1)
	−	+	−	−	6 (4)
	−	−	+	−	38 (26)
	−	−	−	+	0 (0)
Two					30 (21)
	+	−	−	+	0 (0)
	−	+	−	+	0 (0)
	−	−	+	+	0 (0)
	+	+	−	−	7 (5)
	+	−	+	−	3 (2)
	−	+	+	−	20 (14)
Three					37 (26)
	+	+	−	+	1 (<1)
	−	+	+	+	3 (2)
	+	−	+	+	0 (0)
	+	+	+	−	33 (23)
Four	+	+	+	+	9 (6)

NOTE. +, Positive test result; −, not tested, no growth on culture, contaminated specimen, other histopathologic diagnosis, or negative test result.

versions 8.2 and 9.0 (SAS Institute). Continuous variables were dichotomized at the median value. Univariate conditional logistic regression analysis was performed using PROC PHREG. Analysis was limited to matched sets containing case patients with confirmed BUD and age- and village-matched control subjects. Variables that attained significance at a P value <.10 and selected variables with associations from previous studies were retained for multivariable analyses.

Multivariable conditional logistic regression models were constructed using PROC LOGISTIC. The BESTFIT procedure was used to identify a subset of variables for subsequent stepwise and forward selection. The variable "participated in farming" was used instead of detailed farming activities, and separate models were explored to identify risk factors among persons who participated in farming. Activities that remained statistically significant in submodels among farming participants were recategorized as 3-level variables ("did not farm," "farmed without behavior," and "farmed with behavior") and retained for forward multivariable selection. Confounding and effect modification were assessed in the final model.

RESULTS

We enrolled 158 case patients with probable BUD (including 5 with inactive disease) and 149 age- and village-matched control subjects in the study. Tissue specimens were obtained from 144 case patients with probable active disease, and 124 specimens were adequate for histopathologic examination. Of these 144 case patients, 121 (84%) had evidence of *M. ulcerans* infection revealed by at least 1 diagnostic method and were classified as having confirmed BUD.

BUD confirmation. The 4 diagnostic methods confirmed the presence of BUD at variable rates. PCR analysis confirmed the highest number of probable cases of BUD (106 [75%] of 142 case patients), followed by histopathologic analysis (79 [64%] of 124), culture (54 [38%] of 144), and ZN staining (13 [13%] of 102). Forty-five cases (31%) were confirmed by a single positive test result; of these, 38 were confirmed by PCR, 6 by histopathologic analysis, and 1 by culture (table 1). Of 126 case patients with probable BUD who had at least 2 diagnostic tests performed, disease in 76 (60%) was confirmed by at least 2 diagnostic methods (table 1). Disease stage did not influence the probability of confirmation. Confirmation rates among case patients classified as having preulcerative disease (32 [82%] of 39), preulcerative and ulcerative disease (31 [82%] of 38), or ulcerative disease (58 [76%] of 76) were not significantly different (P = .70, by the Wilcoxon rank-sum test).

Characteristics of case patients with confirmed BUD. Children comprised the majority of 121 case patients with con-

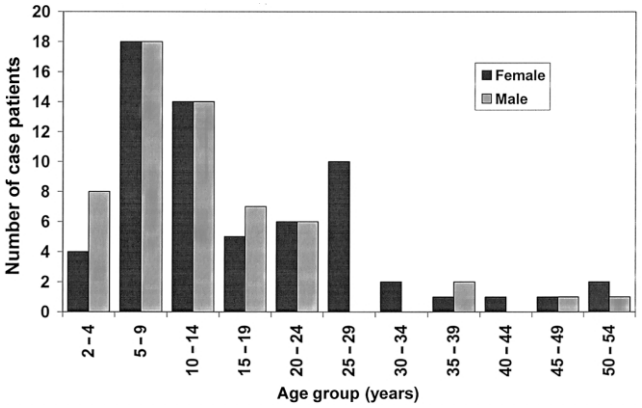

Figure 1. Age and sex distribution of 121 Ghanaian case patients who presented to the study hospitals with confirmed Buruli ulcer disease between 23 August and 30 November 2000.

firmed disease. The median age was 12 years (range, 2–53 years), and 72 (62%) were <15 years old (figure 1). There were statistically significant differences in age between male case patients and female case patients ($P = .037$, by the Wilcoxon score test); both sexes were equally represented among children and young adults aged <20 years (47% were female), but female sex predominated among adults aged ⩾20 years (70% of whom were female; figure 1). Although most patients presented with a median (and mode) of 1 lesion (range, 1–7), a total of 39 (32%) of 121 had >1 lesion. Disabling sequelae were observed in 15 case patients (12%); 11 (9%) had contracture deformities, 2 (2%) had amputated limbs, and 2 (2%) had osteomyelitis.

BUD lesions occurred most commonly on the leg (59 case patients [49%]) and arm (44 [36%]), with slightly greater frequency on the left side (62 [52%]), compared with the right side (49 [41%]) or both sides (9 [8%]). More case patients (74 [61%]) had lesions on distal extremities (i.e., from the elbow to the hand or from the knee to the foot), compared with 34 (28%) who had lesions on the proximal extremities. Male case patients were significantly more likely than female case patients to have developed BUD lesions on the trunk ($P = .009$) but not on the arm or leg (table 2).

Univariate analysis. A total of 116 age- and village-matched control subjects were enrolled; no eligible control subjects were located for 5 case patients with confirmed BUD. Table 3 presents demographic characteristics of the 116 case patients and 116 matched control subjects. Median age and distribution by sex, ethnicity, and district of residence were similar among enrolled case patients and control subjects.

Conditional logistic regression analysis was performed to identify factors associated with BUD (table 4). Previous bacille Calmette-Guérin (BCG) vaccination, verified by the presence of a scar on the upper right deltoid, was not associated with BUD ($P = .33$). No association was observed between BUD and HIV infection ($P = .99$), although 6 case patients tested positive for HIV-1, and 1 control subject tested positive for HIV-2.

Case patients and control subjects exhibited no apparent differences with respect to primary drinking water sources (table 4). Case patients were significantly more likely than control subjects to report wading in a river or stream ($P = .032$) and walking >5 min to their primary water source ($P = .030$). Case patients were no more likely than control subjects to recall penetrating injuries (e.g., cuts, scratches, thorn pricks, or splinter wounds) or insect bites that occurred near bodies of water ($P = .14$ and $P = .84$, respectively).

More control subjects than case patients reported farming as an occupation ($P = .079$) and active participation in farming ($P = .054$). Control subjects more commonly reported wearing trousers ($P = .044$) and top clothing (i.e., shirts; $P = .007$) while farming. Case patients were not more likely than control subjects to report a family member with a history of BUD ($P = .15$).

Control subjects were significantly more likely than case patients to sometimes or always bathe with costly toilet soap (i.e., wrapped bar soap; $P = .023$), whereas no difference was observed with respect to the use of less expensive, mass-produced Key soap or locally produced amonkye soap ($P = .22$ and $P = 1.0$, respectively). Control subjects more frequently reported sharing indoor living space with livestock or pets ($P = .014$), whereas similar numbers of case patients and control subjects reported owning and handling livestock or pets ($P = 1.0$ and $P = .76$, respectively). Living indoors with chickens was reported significantly more often by control subjects than by case patients ($P = .014$).

Multivariable analysis. In the final multivariable model for BUD (table 5), wading in a river or stream was a risk factor, whereas sharing indoor living space with livestock and bathing with toilet soap were protective factors. Participating in farming was not independently associated with BUD, but it confounded the other variables. Compared with persons who did not farm,

Table 2. Comparison of lesion locations in female and male case patients from Ghana with confirmed Buruli ulcer disease.

Lesion location	No. (%) of females ($n = 64$)	No. (%) of males ($n = 57$)	P[a]
Arm	26 (41)	18 (32)	.3466
Leg	34 (53)	25 (44)	.3638
Trunk	4 (6)	14 (25)	.009
Head	2 (3)	1 (2)	1.000

NOTE. Three females and 2 males had multiple or contiguous lesions on 2 body areas.

[a] By 2-sided Fisher's exact test.

Table 3. Demographic characteristics of 116 case patients with confirmed Buruli ulcer disease and 116 age- and village-matched control subjects—Ghana, 2000.

Characteristic	Case patients	Control subjects	*P*
Female sex	62/116 (53)	57/116 (49)	.25
Median age, years (range)	12 (2–53)	11.5 (2–50)	1.00
District of residence			
Amansie West	26/116 (22)	25/116 (22)	.32
Ashanti Akim North	11/116 (9)	11/116 (9)	NC[a]
Upper Denkyira	56/116 (48)	58/116 (50)	.16
Other	23/116 (20)	22/116 (19)	.32
Ethnicity			
Ashanti	56/116 (48)	61/116 (53)	.25
Denkyira	27/116 (23)	27/116 (23)	NC[a]
Other	33/116 (28)	28/116 (24)	.30
Highest educational level			
None	21/116 (18)	18/116 (16)	.47
Primary school	64/116 (55)	67/116 (58)	.53
Middle school	9/116 (8)	7/116 (6)	.57
Secondary school	22/116 (19)	24/116 (21)	.60
Median no. of people in household (range)	7 (2–30)	8 (2–23)	.055
Owns land[b]			
Adults ⩾18 years of age	16/36 (44)	21/36 (58)	.35
Children <18 years of age, father owns land	3/80 (4)	1/80 (1)	.62
Farming occupation[c]			
Adults ⩾18 years of age	25/36 (69)	29/36 (81)	.41
Children <18 years of age, father's occupation	70/80 (89)	75/80 (94)	.28

NOTE. Data shown are no. of participants with the characteristic/no. of participants surveyed (%), unless otherwise indicated.

[a] Not calculable (NC), because this characteristic did not differ between case patients and control subjects.

[b] Adults aged ⩾18 years ($n = 72$) were asked whether they owned land. Participants aged <18 years ($n = 160$) were asked whether their father owned land.

[c] Adults aged ⩾18 years ($n = 72$) were asked for their occupation. Participants aged <18 years ($n = 160$) were asked for their father's occupation.

persons who wore a shirt while farming had a lower risk of BUD. No interaction was observed between any of the risk factors presented in table 5 and sex or age, although children were less likely to participate in farming than were adults (median age of farmers and nonfarmers, 17 and 8 years, respectively).

DISCUSSION

Confirmed BUD case series. This study presents 121 BUD cases with laboratory evidence of *M. ulcerans* infection and corroborates results from previous case series that relied on clinical diagnosis [13, 29]. The preponderance of BUD lesions on the extremities and, in males, on the trunk (table 2) implies that transmission requires exposed skin. The overrepresentation of adult women and children (figure 1) suggests that some undiscovered common behavior increases their risk for BUD. Fetching water has been hypothesized to be a common BUD risk factor for women and children [16], yet we found no significant association between BUD and domestic water exposures (e.g., fetching water, washing dishes, and washing clothes) or primary water sources (table 4).

The high rate of laboratory confirmation in our study, regardless of disease stage, indicates that clinical diagnosis is adequate for determining treatment in BUD-endemic areas. The development of a low-cost, simple rapid diagnostic test for BUD would nevertheless greatly aid clinical judgment and BUD surveillance in these areas.

Risk and protective factors for BUD. Our data strengthen the hypothesis that BUD is an environmentally acquired infection associated with exposure to rivers and streams in tropical climates. Previous investigations have documented an elevated risk for BUD among Ugandan tsetse control workers who frequented swampy areas [30] and among persons who farmed near the Lobo River in Côte d'Ivoire [20]. Our study in BUD-endemic districts of Ghana identified wading in a river or stream as an independent risk factor. The heightened risk

Table 4. Univariate analysis of selected risk factors for Buruli ulcer disease among 116 matched case-control pairs from Ghana.

Characteristic	No. (%) of case patients (n = 116)	No. (%) of control subjects (n = 116)	Univariable OR (95% CI)[a]	P[b]
Demographic/household				
Farming occupation	95 (82)	104 (90)	0.47 (0.20–1.09)	**.079**
No. of people in household >7	49 (42)	63 (54)	0.58 (0.33–1.01)	**.055**
Duration of village residence >9 years	49 (43)	63 (55)	0.43 (0.22–0.84)	**.014**
Family member had Buruli ulcer	14 (12)	22 (19)	0.58 (0.28–1.22)	.15
Lived in a mud house	90 (78)	96 (83)	0.63 (0.28–1.38)	.24
Exposure to water				
Primary source of drinking water				
River or stream	47 (41)	45 (39)	4.0 (0.45–35.8)	.22
Open borehole	19 (16)	19 (16)	…	NC[c]
Borehole with pump	41 (35)	48 (41)	0.56 (0.25–1.27)	.17
Waded in a river or stream	55 (47)	40 (34)	1.94 (1.06–3.54)	**.032**
Swam, waded, or bathed in a river or stream	77 (66)	65 (56)	1.86 (0.97–3.56)	**.062**
Bathed in water from an open borehole	27 (23)	19 (16)	2.60 (0.92–7.29)	**.069**
Washed clothes	61 (53)	64 (55)	…	.55
Washed dishes	49 (42)	55 (47)	…	.31
Fetched water	74 (64)	83 (72)	0.59 (0.30–1.17)	.13
Walking time to primary drinking water source >5 min	61 (53)	47 (41)	2.08 (1.07–4.02)	**.030**
Walking time to laundry water source >7 min	35 (30)	22 (19)	2.30 (1.09–4.83)	**.028**
Swam, washed, or played in mining pits with standing water	7 (6)	10 (9)	…	.37
Crossed a body of water	67 (58)	62 (53)	…	.44
Received cuts, scratches, thorn pricks, or splinter wounds near water	41 (35)	51 (44)	…	.14
Received insect bite near water	24 (21)	25 (22)	…	.84
Exposure to infectious agents				
Previous bacille Calmette-Guérin vaccination	63 (54)	56 (48)	…	.33
HIV-1 or -2 positive	6 (5)	1 (0.9)	…	.99
Soap use while bathing				
Sometimes or always used amonkye soap	97 (84)	103 (90)	0.50 (0.20–1.24)	.22
Sometimes or always used Key soap	111 (96)	110 (96)	…	1.0
Sometimes or always used toilet soap	68 (59)	81 (70)	0.44 (0.22–0.89)	**.023**
Animal exposure				
Owned livestock or pets	40 (34)	40 (34)	…	1.0
Handled livestock or pets	51 (44)	49 (42)	…	.76
Shared indoor living space with livestock or pets	12 (10)	26 (22)	0.36 (0.16–0.82)	**.014**
Shared indoor living space with chickens	10 (9)	22 (19)	0.33 (0.13–0.84)	**.020**
Bitten or scratched by animals	9 (8)	16 (14)	0.42 (0.15–1.18)	.10
Farming activity				
Participated in farming	54 (47)	65 (56)	0.48 (0.22–1.0)	**.054**
Digging	29 (25)	45 (39)	0.27 (0.11–0.67)	**.005**
Weeding	46 (40)	60 (52)	0.36 (0.16–0.82)	**.014**
Harvesting	45 (39)	58 (50)	0.35 (0.15–0.83)	**.017**
Sowing	45 (39)	57 (49)	0.40 (0.18–0.91)	**.029**
Plowing	16 (14)	23 (20)	0.30 (0.08–1.09)	**.067**
Raised cassava	53 (46)	64 (55)	0.45 (0.20–0.99)	**.047**
Raised corn	51 (44)	62 (53)	0.50 (0.24–1.03)	**.060**
Raised yam	42 (36)	52 (45)	0.52 (0.25–1.09)	**.082**
Other farming activities	9 (8)	1 (1)	9.0 (1.14–71.0)	**.037**

(continued)

Table 4. ***(Continued.)***

Characteristic	No. (%) of case patients (n = 116)	No. (%) of control subjects (n = 116)	Univariable OR (95% CI)[a]	P[b]
Walking time from farm to primary water source that was greater than the median time	16 (14)	27 (23)	0.48 (0.22–1.01)	**.054**
Walking time from home to farm that was greater than the median time	23 (20)	34 (29)	0.54 (0.28–1.06)	**.075**
Clothing worn while farming				
Trousers	37 (32)	49 (42)	0.48 (0.23–0.98)	**.044**
Top (shirt)	37 (32)	54 (47)	0.37 (0.18–0.77)	**.007**
Closed shoes	12 (10)	15 (13)	…	.53
Dress	28 (24)	25 (22)	…	.57
Wrap	10 (9)	8 (7)	…	.57
Clothing worn, nonfarming activity				
Closed shoes, wading	9 (8)	8 (7)	…	.80
Trousers, wading	39 (34)	32 (28)	1.47 (0.76–2.83)	.25
Closed shoes, walking in the bush	13 (11)	18 (16)	…	.30
Trousers, walking in the bush	53 (46)	64 (55)	0.61 (0.33–1.11)	.11
Insect protection				
Sometimes or always used mosquito coils	62 (53)	48 (41)	1.78 (1.00–3.17)	**.051**
Sometimes or always used bednets	28 (24)	31 (27)	…	.514
Behavior and beliefs				
Believed that poor hygiene causes Buruli ulcer	3 (3)	14 (12)	0.21 (0.06–0.75)	**0.016**
Treated cuts with roots	6 (5)	14 (12)	0.38 (0.14–1.08)	**.069**
Other activities				
Participated in mining	10 (9)	15 (13)	0.38 (0.10–1.4)	.15
Fished	13 (11)	11 (9)	…	.62
Hunted, trapped, or caught wild game	9 (8)	13 (11)	…	.35
Walked through the bush	86 (74)	88 (76)	…	.74
Walked through the forest	12 (10)	14 (12)	…	.51

[a] ORs were omitted when $P > .25$.
[b] Boldface type indicates differences that were significant at $P \leq .1$ using conditional logistic regression.
[c] Not calculable (NC), because this characteristic did not differ between case patients and control subjects.

for BUD may have resulted from direct contact with contaminated water bodies and/or indirect exposure to riverine environments that harbor *M. ulcerans*.

Our analysis corroborates the observation that wearing protective clothing is associated with decreased BUD risk [20] and furnishes important clues about BUD transmission. In the Côte d'Ivoire study, wearing long trousers was protective against BUD. We found that wearing clothing that covered the upper body while farming was protective. Wearing trousers was also univariately significant in our study but was not retained in the multivariable model, because of collinearity with wearing top clothing. Taken together with the predominance of trunk lesions in males, these results suggest that exposed skin facilitates infection. This finding is consistent with both prevailing hypotheses about insect vectors and penetrating injuries as potential modes of BUD transmission [2]. The insect-vector theory has been reinforced by the recent discovery of a biofilm containing *M. ulcerans* that covers the roots of aquatic plants [31]. Animals feeding on this biofilm may serve as intermediate hosts and as prey for carnivorous water insects (Naucoridae) that can accumulate *M. ulcerans* in their salivary glands [32]. In the laboratory, mice developed characteristic lesions after bites from *Naucoris cimicoides* experimentally infected with *M. ulcerans,* but the relevance of this mode of transmission to human disease is unclear [32]. We were unable to detect independent associations between BUD and insect bites, cuts, scratches, and other wounds received near bodies of water (table 4). These negative results may be explained by lengthy delay between exposure and active disease and subsequent failure to recall the original trauma. Although insect vectors may play a role in endemic disease, the Australian BUD outbreak is difficult to explain by this mechanism [11]. Therefore, *M. ulcerans* may be transmitted by >1 route.

Bacterial contamination of skin surfaces may facilitate *M. ulcerans* infection. The regular use of toilet soap (i.e., wrapped bar soap) while bathing may remove bacteria deposited on the skin, which is a plausible explanation for its protective multivariable effect. However, no protective association was ob-

Table 5. Multivariable model for risk factors for Buruli ulcer disease among persons in Ghana, 2000.

Risk factor	Multivariable OR (95% CI)	*P*
Waded in a river or stream	2.69 (1.27–5.68)	.0096
Shared indoor living space with livestock or pets	0.36 (0.15–0.86)	.022
Sometimes or always bathed with toilet soap	0.41 (0.19–0.90)	.026
Farmed while wearing top clothing		
Did not participate in farming	Reference	
Participated in farming and did not wear top clothing	0.60 (0.15–2.4)	.47
Participated in farming and wore top clothing	0.27 (0.11–0.70)	.0071

served among those who used less costly mass-produced Key soap or homemade amonkye soap, which are apparently similar markers for hygiene (table 4). Alternatively, use of costlier toilet soap may be a surrogate for higher socioeconomic status. Persons with higher socioeconomic status (SES) in rural African areas may have more limited environmental exposure to *M. ulcerans* because they live closer to their water sources or farms. SES may also be related to land ownership, household size, and duration of residence, which showed protective trends (tables 3 and 4). Sharing indoor living space with livestock, compared with handling or owning livestock, appeared to protect against BUD. Most persons who lived with livestock reported sharing indoor living space with chickens, which could be related to SES. Additional investigation into SES may require specially designed survey instruments to capture differences relevant to a resource-poor area.

Several negative findings from this study deserve comment. Although BCG vaccination appears to be effective against leprosy [33] and may protect against BUD within 1 year after vaccination [21, 34, 35], our study does not support the hypothesis that BCG vaccination provides lasting protection against BUD. However, our study was not designed to detect subtle degrees of BCG-mediated protection against disseminated BUD [36]. Although isolated BUD cases have been reported with concurrent HIV infection [20, 37–39], this study agrees with others in reporting no epidemiologic association between BUD and HIV infection. In further accordance with previous work [13], this study does not provide evidence for person-to-person transmission of BUD within the household, because case patients with BUD were no more likely to report having family members with a history of BUD than were control subjects.

Study limitations. This study was subject to standard limitations of case-control studies. It was not practical to blind interviewers to participants' disease status. The 4 diagnostic tests were weighted equally for case confirmation, and 45 case patients with BUD were included on the basis of a single positive test result. When the multivariable analysis excluded 7 case patients for whom disease was confirmed by PCR alone yet who had other histopathologically confirmed diagnoses, the OR estimates did not change substantially (data not shown). Matching case patients with control subjects by village of residence may have decreased the variability of relevant exposures, but such study inefficiency reduces the power to detect true associations.

Summary. We report a large case-control investigation of BUD with laboratory confirmation of cases. This analysis suggests that clinical diagnosis by skilled practitioners in resource-poor settings does not extensively misclassify BUD cases. Our findings support the hypothesis that BUD is an environmentally acquired infection linked with exposure to riverine areas. Exposed and/or contaminated skin may facilitate transmission, as suggested by the predominance of trunk lesions in males and the protective effects of clothing and soap. Although we attempted to identify modifiable risk factors to inform BUD prevention strategies, as in previous case-control investigations, our results do not suggest simple avenues for behavior change [20, 21]. Wearing protective clothing while farming could be encouraged but would not reduce BUD risk among children who infrequently participate in farming. Toilet soap use could also be promoted, but its protective effect may be related to SES rather than to hygiene. Wading, the strongest risk factor, may be an indirect marker of environmental exposure and was reported by fewer than one-half of the study participants. Interventions based on low-prevalence exposures are unlikely to eliminate BUD. Future research should investigate *M. ulcerans* ecology (vectors and reservoirs) and the contact patients with BUD have with flora and fauna in river environments. Case-control investigations of BUD outbreaks could also help elucidate modes of transmission. Until transmission is more clearly defined, early detection and prompt treatment may be the best public health strategies for reducing BUD morbidity.

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