ASSESSING WATER-RELATED RISK FACTORS FOR BURULI ULCER: A CASE-CONTROL STUDY IN GHANA

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Abstract. To assess water-related risk factors of Buruli ulcer, a case-control study of 102 patients (51 cases and 51 controls) was undertaken by matching age group, sex, and bacille Calmette-Guérin (BCG) vaccination history in Ghana. The factors used here for matching have previously been implicated as factors of Buruli ulcer, an emerging infectious disease. This is the first study to delineate a set of previously suspected, water-related risk factors, in a case-control study matching for age group, sex, and BCG vaccination status. The results of both bivariate and multivariate analyses presented a significantly high odds ratio (OR) only for swimming in rivers on a habitual basis (OR = 18.00, P < 0.01) among the major water-related risk factors. Use of water from rivers and ponds for drinking, cooking, bathing, and washing purposes were not significant risk factors. Our data suggest that swimming, or activities on riverbanks associated with it, is a risk factor.

INTRODUCTION

Buruli ulcer occurs in patchy foci in at least 27 countries in Africa, Asia, South America, and the western Pacific. The precise current distribution is not known and the incidence is probably underestimated. The majority of the reported cases are from west and central Africa: Benin, Côte d’Ivoire, Gabon, Ghana, Liberia, Nigeria, Togo, Uganda, and Zaire. Since Bayley reported the first case of Buruli ulcer identified in Ghana in 1971, awareness of the public health importance of the disease has been increasing.1,3,13–15

The disease was first described by Cook in Uganda in 1897 and the etiologic agent was characterized by MacCalum and others in Australia.17 Since a great number of cases of the disease were identified in the Buruli area of Uganda, it is referred to as Buruli ulcer. Buruli ulcer is caused by Mycobacterium ulcerans and often followed by extreme deformities and disabilities.18 Due to the ugly appearance of the deformities it leaves, Buruli ulcer is greatly feared and stigmatized in the endemic areas, and is often attributed to witchcraft and curses.19 Patients tend to be socially alienated. Since the treatment costs are extremely high, the appropriate medical care is not affordable for most local residents without financial support to either the health facilities or patients.20 At least, treatment of this disease puts a considerable strain on health budgets.

Children less than 15 years of age are predominantly affected compared with adults.3,18,21 It is also reported that the prevalence in adult females is higher than in adult males, while there are no sex differences in the distribution of cases among children.1,22 An earlier study in Uganda23 reported that bacille Calmette-Guérin (BCG) might confer protection against the disease or delay the onset of symptoms. However, a more recent case-control study24 found that BCG vaccination did not protect against onset of the disease, though it shortened its duration.

Trauma to the skin followed by the introduction of M. ulcerans from a source of contamination is probably how infection begins. However, the mode of transmission has not yet been clearly demonstrated. A study in Benin reported the possibility of non-genetic familial person-to-person infection.2 Recently, a case of the disease following a human bite was reported.24,25 However, person-to-person transmission is clearly not a major route of transmission.

Epidemiologic studies suggest a connection with water because use of (or residence near) a river or pond has consistently been identified as a risk factor.3,8,10,26 Portaels and others suggested bites by or contact with insects inhabiting plant roots in swamps as a possible mechanism of M. ulcerans transmission.27 Marston and others,3 in one of only two published case-control studies of the disease,21 found that the wearing of long pants was protective. Nevertheless, the mechanism of transmission of the disease through water-related human activities is not yet identified.1 This study attempts to assess water-related risk factors in Ghana by controlling several other major possible risk factors.

METHODS

Study area and population. The site of the study, the Amanse West District in the Ashanti Region of Ghana, has the greatest number of reported cases of Buruli ulcer of the 18 districts in the region28 and the second greatest number in the country.15 The district is a typical tropical rain forest area of 1,320 km² with an estimated total population of 130,000 in 135 communities. It is approximately 60 km southwest of Kumasi, the Ashanti regional capital. As one of the least developed districts in the country, infrastructure and socioeconomic conditions are poor.20

Within the district, the majority of Buruli ulcer-endemic communities are located in the south along the two rivers, the Offin and the Oda. Tontokrom is the most heavily affected community in the district with an estimated prevalence of 22%.2 In addition to St. Martin’s Catholic Hospital located in Agrovesum, which has been providing Buruli ulcer patients within and outside the district with curative services since 1993, there are four government health centers and one private clinic in the district.

Study design. A case-control study was used to assess the water-related risk factors for Buruli ulcer. To estimate the
level of risks specifically related to water such as type of water source and water-related behavior in daily life, the other major reported risk factors were matched between cases and controls or controlled against possible confounding.

**Study population.** The cases and controls were drawn from the catchment population of St. Martin’s Catholic Hospital, including inpatients and outpatients seen in July and August 1999.

**Case definition and confirmation.** All the 51 patients with a diagnosis of Buruli ulcer were considered for inclusion as cases. These were confirmed by applying the Ziehl-Neelsen test and polymerase chain reaction (PCR) methods to the tissue samples taken from their lesions for routine diagnosis purposes. The World Health Organization (WHO) recommends that the results of at least two such tests should be positive to confirm a diagnosis of Buruli ulcer. In the event, all cases were confirmed.

**Laboratory methods.** For the Ziehl-Neelsen test, specimens were prepared by flooding a smear on a slide with Ziehl-Neelsen carbol-fuchsin, heating for 3–5 minutes, rinsing with gentle stream of running water, counterstaining with methyl blue chloride (1 gram/liter), and air-drying. For the PCR, specimens were prepared by homogenizing tissue samples in a microfuge tube in 500 μL of digestion buffer (0.01 M phosphate-buffered saline), centrifuging for five minutes, decanting the buffer, and resuspending the pellet in distilled water. *Mycobacterium ulcerans* DNA was isolated after heating the pellet at 95°C. DNA amplification was performed in an automated thermal cycler.

**Recurrent cases.** The WHO criteria for recurrent cases were used: i.e., a patient with previous surgical treatment of Buruli ulcer who presents with another current lesion(s) at the same or different site(s) within one year from the end of the last treatment.1

**Matching criteria.** Considering the known epidemiology of the disease trends, individual matching was used as to age group and sex. Three age groups were arranged by using two cut-off points of 15 and 50 years old.1,23 Because of the possible association with BCG vaccination, frequency matching by vaccination status was used rather than individual matching because of the difficulty of identifying controls individually matched for all three factors: sex, age group, and vaccination status (Table 1). Since person-to-person transmission is not a major route of transmission, matching by familial infection (both genetic and non-genetic) was not undertaken, but it was included as a risk factor.

**Sample size.** The sample size was calculated so as to detect an association when the odds ratio (OR) was ≥3.5 with \( \alpha = 0.05 \) and \( \beta = 0.80 \). Since the source of water among 30% of the rural residents in the country was either river, pond, or dugout,34 the proportion of controls exposed to water sources of those types was assumed to be 0.3. This implied a requirement of 42 cases along with the same number of controls.35,36 Therefore, anticipating a 20% refusal rate, all 51 current inpatients and outpatients diagnosed as having Buruli ulcer at St. Martin’s Catholic Hospital during the study period between July and August 1999 were selected as cases.

**Selection of controls.** Fifty-one controls were selected by systematic sampling based on treatment record from the inpatients and outpatients who received services at the hospital during the study period for health problems other than Buruli ulcer, such as malaria, respiratory infections, and diarrhea. The controls were selected from the same districts where cases resided. After selection of each case, subsequent treatment records were perused in chronological order until the first suitable control was found.

**Data collection.** To obtain data on types of water sources, hygienic behavior, swimming habit, and other household members’ infection, structured interviews with cases and controls were conducted during home visits by using questionnaires. Interviews with current inpatients were undertaken at the hospital. When individuals were less than five years of age, their parents were interviewed. Akan, the local language in Ashanti Region, was used for interviews. The questionnaire was filled out by the interviewer during the interview. Repeated home visits were made until all the target interviewees were covered by the study. The full list of risk factor data collected for each case and control was as follows: age, sex, BCG status, types of water sources used, whether their drinking water was boiled and hands were regularly washed, habitual swimming in ponds and in rivers, any other cases in the household, involvement in agricultural work by the patient and by their parents/partner.

**Statistical analysis.** The WHO allows four types of water sources (piped water, well/borehole, river, and pond) in its standardized report forms for Buruli ulcer cases.1 Also, only these four types are accessible and used among the study population. Thus, these four types were used as the categories for water sources in this study. A fifth type, rainwater collection, was found to be extremely rare when the survey form was pretested. Data obtained through the interviews were field checked, entered into a computer, and then analyzed using SPSS for Windows version 11.0 (SPSS, Inc., Chicago, IL).

**Statistical methods.** First, a descriptive univariate analysis on cases was undertaken. Second, cases and controls were compared using bivariate analysis to give, for each potential risk factor variable, the OR, its 95% confidence interval and the corresponding \( P \) value using the McNemar chi-square test. Third, logistic regression was undertaken for multivariate analysis, taking each patient’s disease status (case or control) as a dependent variable. As the independent variables, we used the risk factors that showed significant associations (\( P < 0.05 \)) in bivariate analyses. Logistic regression analysis was undertaken using forward stepwise variable entry. Variables were entered if they showed a significant association (\( P < 0.05 \)) in the bivariate analysis and removed if not significant (\( P > 0.10 \)) in the regression.

### Table 1
**Characteristics of Buruli ulcer cases used for matching**

<table>
<thead>
<tr>
<th>Age category (years)</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14</td>
<td>15</td>
<td>29.4</td>
</tr>
<tr>
<td>15–49</td>
<td>11</td>
<td>21.6</td>
</tr>
<tr>
<td>≥50</td>
<td>4</td>
<td>7.8</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–14</td>
<td>19</td>
<td>37.3</td>
</tr>
<tr>
<td>15–49</td>
<td>1</td>
<td>4.8</td>
</tr>
<tr>
<td>≥50</td>
<td>1</td>
<td>4.8</td>
</tr>
<tr>
<td>BCG vaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>18</td>
<td>35.3</td>
</tr>
<tr>
<td>No</td>
<td>33</td>
<td>64.7</td>
</tr>
</tbody>
</table>

*For individual or frequency matching, the same number of controls were selected for each category. BCG = bacille Calmette-Guérin.
Ethics. Informed consent to participate in the study was obtained verbally from both cases and controls after treatment and before the interview. In the case of children, consent was obtained from their parents. There were no refusals. Ethical approval was given by St. Martin’s Catholic Hospital, following procedures laid down by the Ghana Ministry of Health.

RESULTS

Univariate analysis: characteristics of cases. All 51 cases treated at St. Martin’s Catholic Hospital during the study period took part in the study (Table 1). Of these 51 cases, 34 (67%) were children less than 15 years of age, but there were no cases less than two years of age. Among children (0–14 years of age), slightly more than half the cases were female (19 of 34, 56%), while most cases in both adult age groups were male (15 of 17, 88%). This difference was statistically significant ($\chi^2 = 9.11, P = 0.003$). Of the 51 cases, 36 (76%) were recognized as recurrent cases. Forty-two were from the Amansie West District and all the others were from adjacent districts. There were 102 active lesions in the 51 cases (mean of number of lesions per patient case = 2.0). However, the unit of analysis in this study is the patient, not the lesion.

Bivariate analysis: ORs. The results of comparing cases and controls are shown in Table 2. Since multiple responses were allowed, the exposure groups in the table are not discrete. We therefore report the OR of Buruli ulcer for those reporting use of each type of source (or each type of behavior) compared with those not reporting it.

Seventeen cases (33%) and nine controls (18%) used more than one type of water source for drinking and cooking. Of all the cases and controls, only one case differentiated water sources according to purposes of water use. The household of this case used both well and river water for drinking and cooking, but only river water for bathing and washing. In Table 2, it is shown as using river water. All six cases using a piped supply were from the districts adjacent to the Amansie West District.

No controls used a piped water supply. We therefore combined piped supplies and wells/boreholes into a single category, piped supply or well/borehole, assuming the risks of these two types of water source were similar. This pooling process of two categories of water source is supported by the findings of an earlier study that Buruli ulcer is more frequent among households using water from a river or pond than in those using a piped supply, a borehole, or a well.26

Use of water from a river was associated with a significantly greater risk of disease ($OR = 2.38, P < 0.05$). However, use of water from a piped supply or well/borehole was not significantly protective ($OR = 0.55, P = 0.332$). Use of water from a pond was not significantly associated with disease, but the numbers of involved were small.

Of the four types of water-related behaviors we recorded, only swimming in rivers showed a significant positive association ($OR = 18.00, P < 0.01$). Only two controls swam on a habitual basis. Both were males from households where water from a well and a river was used. No cases or controls reported boiling water for drinking or swimming in ponds. And all cases and controls claimed to wash their hands before meals. Therefore, ORs could not be calculated for these three variables. Living with other household member(s) infected with Buruli ulcer appeared to be associated with an increased risk of disease in this study ($OR = 2.20$), but the association was not significant. Only six (12%) more cases than controls were exposed to this risk factor, suggesting that only 12% of the cases could be attributed to intra-household transmission. Being engaged in agricultural work did not present a significant risk factor, either for patients themselves or for their parents/partners.

Multivariate analysis: logistic regression. The risk factor variables that were significantly associated with Buruli ulcer ($P < 0.05$) were applied as the independent variables to logistic regression. These were swimming in rivers and domestic use of river water. As a result, the final model of the regression was composed of only one risk factor variable, swimming in rivers ($OR = 18.00, P < 0.01$). The constant in the logistic regression model was 0.105. Naturally, the OR (18.00) is the same as that in the bivariate analysis.

We further analyzed the details on swimming in rivers. The number of cases who swam on a habitual basis was 19 and accounted for 37% of the total number of cases. Although no

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**Table 2**

Results of bivariate analysis of risk factors for Buruli ulcer*

<table>
<thead>
<tr>
<th>Risk factor variables</th>
<th>Cases</th>
<th>Controls</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water source†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piped supply</td>
<td>6</td>
<td>0</td>
<td>0.55</td>
<td>0.24–1.59</td>
<td>0.332</td>
</tr>
<tr>
<td>Well/borehole</td>
<td>33</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River</td>
<td>22</td>
<td>11</td>
<td>2.38</td>
<td>1.15–5.90</td>
<td>0.049‡</td>
</tr>
<tr>
<td>Pond</td>
<td>7</td>
<td>4</td>
<td>1.75</td>
<td>0.65–7.09</td>
<td>0.549</td>
</tr>
<tr>
<td>Water-related behavior</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boil water for drinking (always twice or three times a day)</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Wash hands before meal (always twice or three times a day)</td>
<td>51</td>
<td>51</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Swimming in rivers§</td>
<td>19</td>
<td>2</td>
<td>18.00</td>
<td>3.77–362.13</td>
<td>&lt;0.001¶</td>
</tr>
<tr>
<td>Swimming in ponds</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Familial cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other household member(s) infected</td>
<td>12</td>
<td>6</td>
<td>2.20</td>
<td>0.90–7.25</td>
<td>0.210</td>
</tr>
<tr>
<td>Agricultural occupation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>6</td>
<td>10</td>
<td>0.50</td>
<td>0.20–1.82</td>
<td>0.381</td>
</tr>
<tr>
<td>Patient’s parent/partner</td>
<td>38</td>
<td>27</td>
<td>2.38</td>
<td>1.15–5.90</td>
<td>0.265</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>51</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* CI = confidence interval; NA = not applicable.
† Multiple responses allowed (more than one type of water source reported for 16 cases and 9 controls).
‡ $P < 0.05$.
§ Swimming on a habitual basis (once every two days or more).
¶ $P < 0.01$. 

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cases less than six years of age had a habit of swimming in rivers, those 6–14 years old who swam in rivers (10 cases) accounted for 67% of all cases among children. Of the 19 cases, 13 (68.4%) were males and the others were females. Of these six female cases with the habit of swimming in rivers, five were between 6 and 11 years of age (Figure 1), and the other was 33 years old. Conversely, the male cases swimming habitually in rivers ranged in age from 10 to 59 years.

**DISCUSSION**

The predominance of children (2–14 years of age) among the cases is consistent with the finding of previous studies that children are the most affected by Buruli ulcer.\(^1\)\(^,\)\(^3\) Swimming in rivers on a habitual basis was the only significant risk factor among the eight variables studied through multivariate analysis. The strength of the association with swimming in rivers on a habitual basis (OR = 18.00, \(P < 0.01\)) was the greatest of the risk factor variables examined in this study and in previous studies. For instance, Marston and others reported ORs associated with residence in an encampment (OR = 3.45, \(P < 0.01\)) and rice farming (OR = 2.57, \(P < 0.05\)).\(^3\) An earlier case-control study matching sex and age in the same district did not find significant risk factors related to water and thus did not discuss swimming in rivers.\(^2\)\(^1\) A study in Côte d’Ivoire assessed the various risk factors, but reported that swimming in rivers was not associated with a significant risk of Buruli ulcer.\(^3\) That study selected unmatched cases and controls, al-

**FIGURE 1.** Cumulative number of cases who swam in rivers, by sex.
though it included a multivariate analysis. Our matching for the well-known risk factors may have enabled our study to assess swimming in rivers as the critical water-related risk of Buruli ulcer with greater precision.

No case with the habit of swimming was found among children less than six years of age (Figure 1). Such younger children are probably too young to walk to the river by themselves. This supports the results of a study in Uganda.78 Swimming in rivers in rural areas is carried out generally for multiple purposes: 1) play or exercise, 2) fishing, 3) washing clothes, 4) fetching water, and 5) bathing. It is likely that the major purpose of children’s swimming in rivers is play and exercise. Conversely, while only one (17%) of the six female cases with a habit of swimming in rivers belonged to the economically active and reproductive age group (15–49 years of age), six (46%) of the 13 male cases with that habit belonged to this age group. Those male adolescents and adults may swim in rivers not only for play and exercise but may also take the opportunity to fish, fetch water, and bathe. Swimming in rivers probably implies a variety of activities and their combinations.

Our sample of cases had the converse sex distribution from other studies. Other studies, including a nationwide survey of Ghana, have demonstrated a predominance of males among children (<20 years of age) with Buruli ulcer, but more females among adult cases (≥20 years of age).15 This difference could be due to sampling error because our sample of cases is rather small.

Portaels and others hypothesized that bites of insects or contact with insect feces could be a mode of transmission.27 This could occur by contact with vegetation growing in stagnant water or poorly drained soil, as hypothesized for Uganda by Revill and Barker.37 Marston and others reported that wearing shoes or sandals could reduce the odds of disease by 67% (OR = 0.33), although it was not statistically significant.3 Unlike other activities in and near rivers (fishing, washing clothes, fetching water, and bathing), people who swim are unlikely to wear long pants or shoes. Taking these into account, the results of our study are consistent with the possibility that resting barefoot and scantily clad on riverbanks with abundant vegetation (where insects live) might bear the greatest part of the risk during the entire process of swimming activity. Unfortunately, our data cannot distinguish these activities from swimming itself.

We cannot exclude the possibility of transmission in the river itself. Rather, our results underline the need for further research. There are limitations in generalizing the findings of this study with small sample size at one hospital. However, we recruited all the current cases at St. Martin Catholic Hospital, where a larger number of Buruli ulcer patients are reported than at any other local health facility, which should have helped to reduce selection bias. Another limitation of our study is that we did not ascertain exactly how people swim in rivers and rest on riverbanks, what happens, and the detailed breakdown of time spent in the entire process of swimming. These factors need to be studied further.

The socioeconomic burden and loss through Buruli ulcer are contributing to a vicious cycle of poverty in the disease-endemic areas. To break this vicious cycle, elucidation of the transmission route remains an urgent task.

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