Effect of well drilling on Buruli ulcer incidence in Benin: a case-control, quantitative survey

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Summary

Background Buruli ulcer is the third most common mycobacterial disease worldwide. The public health burden of this neglected tropical disease is large, particularly in poor areas of west and central Africa. The development of appropriate preventive strategies is hampered by an incomplete understanding of the epidemiology and transmission of the disease. We investigated the effect of the drilling of wells on Buruli ulcer incidence.

Methods In this case-control, quantitative survey, we obtained field data for Buruli ulcer incidence over a 10-year period from a specialised centre that collected data for the Ouémé and Plateau departments in Benin, and data for well drilling from the Ministry of Energy, Water and Mines in Benin. The coordinates of the wells drilled were obtained during site visits. A case-control study was then done to investigate the role of well water use in protecting against Buruli ulcer.

Findings We found a strong inverse correlation between the incidence of Buruli ulcer and the number of new wells drilled in the Bonou municipality ($r^2=0\cdot8818$). A case-control study (106 cases and 212 controls) showed that regular use of the water from the wells for washing, bathing, drinking, or cooking was protective against Buruli ulcer (adjusted odds ratio 0·1, 95% CI 0·04–0·44; p=0·0012).

Interpretation This study opens up new possibilities for developing an effective yet affordable policy to fight the disease on a substantial geographical scale. Our study shows that providing access to protected water is an efficient and feasable way to reduce the incidence of Buruli ulcer.

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Introduction

Buruli ulcer is caused by *Mycobacterium ulcerans*. The infection is characterised by painless cutaneous lesions leading to scarring, contracture deformities, amputations, and disabilities if left untreated.[1] Despite its discovery more than a century ago and its spread since the 1980s, Buruli ulcer remains one of the most neglected tropical infectious diseases. It is the third most common mycobacterial disease in the world after tuberculosis and leprosy. Most cases are reported in west Africa, and Benin is one of the countries in which endemicity is highest.

A key epidemiological feature of this disease is the distribution of cases in very well delimited foci. It typically occurs in poor rural communities with little economic or political influence. All epidemiological studies have shown that environmental changes (eg, deforestation, wetland creation) favour the spread of the disease.[2–4] The main reservoir of *M ulcerans* seems to be environmental, but the precise mode of transmission remains unknown, explaining descriptions of the condition as emerging from obscurity or as a mysterious disease.[5] Since the outbreak of the disease in the Buruli district of Uganda in the 1970s, the consensus view has been that human-to-human transmission does not occur. Field studies have shown a direct association between *M ulcerans* infection and swampy areas, and have identified aquatic environments as the main reservoir of the bacillus, as for most environmental mycobacteria. In this context, the main risk factor identified to date is close contact with unprotected water (eg, rivers, lakes, swampy areas),[6–11] with the inoculation of the bacillus in cutaneous tissue required for disease development.[12] It has also been suggested that urbanisation might result in a reduced likelihood of becoming infected with *M ulcerans*,[13,14] by reducing access to unprotected water or providing easier access to protected water sources.

Since 2010, the number of new cases of Buruli ulcer has decreased in several countries in which this disease is endemic, including Benin (appendix pp 1–2). Surprisingly, no epidemiological study has been done to identify the factors underlying this decrease in Buruli ulcer incidence, but several hypotheses have been put forward,[15–17] including: (1) the exhaustive diagnosis of chronic and active cases combined with active screening and treatment campaigns since 2007; (2) changes to the environmental reservoir of *M ulcerans*; (3) the introduction of medical treatments (antibiotics), suggesting

See Online for appendix

Research in context

Evidence before this study

We searched PubMed, without language restrictions, for articles published from database inception up until Dec 30, 2018, using the search terms "Buruli ulcer" OR "*Mycobacterium ulcerans*" AND "Epidemiology". 415 studies were identified and around 20 reported an association with contact with unprotected water sources (rivers) as the main risk factor for Buruli ulcer. Surprisingly, effects of well drilling on Buruli ulcer epidemiology have not been investigated in areas in which this disease is endemic, and the factors accounting for variations of Buruli ulcer incidence have not been identified, despite the decrease in the number of new cases observed in most African countries (Côte d'Ivoire, Ghana, Benin) in which Buruli ulcer is endemic.

Added value of this study

This study provides the first evidence, to the best of our knowledge, that the installation of wells can protect against Buruli ulcer in areas of endemic disease. We analysed a large cohort of laboratory-confirmed cases of Buruli ulcer (Pobè, Benin) over a 10-year period, including 1365 PCR-confirmed cases. We found that well drilling was strongly associated with a decrease in Buruli ulcer incidence in an area of endemic disease in Benin between 2006 and 2012. We also identified well drilling as a protective factor for the population in a case-control study. Our study provides quantitative demonstration of the impact of a clearly identified factor, well drilling, on the incidence of Buruli ulcer. As such, this identification provides operational means for the control of the disease by acting on the identified environmental factor. More generally speaking, this study is one of the rare reports showing the positive impact of well drilling installation in the field of neglected tropical diseases, with possible implications for other fields.

Implications of all the available evidence

The present study provides rationale for firm yet affordable public policies to be implemented at large scales in the affected areas, encouraging the drilling of wells to reduce the incidence of the disease. Accumulation of feedback data for the intervention should provide increasingly more accurate information on the optimal location for the well drilling, and integrating parameters relative to various underlying social aspects. Such voluntary policies should, in return, also provide valuable information concerning the fundamental aspects of the epidemiology of the disease.

a role for humans as a reservoir of the bacterium; and (4) a decrease in high-risk activities involving contact with *M ulcerans,* due to the use of protected water sources.

The identification of plausible protective factors against Buruli ulcer is crucial for the prevention and control of this disease. Protective factors might differ between regions, and could be environmental, socioeconomic, or behavioural. The main goal of this study was to investigate the effect of the drilling of new wells on the incidence of Buruli ulcer in a defined area, to determine whether well drilling, which provides the population with better access to protected water, could serve as a basis for effective disease control.

Methods

Study design and epidemiological data sources

We retrospectively obtained quantitative data. Epidemiological data for Buruli ulcer in Benin were collected from the annual statement of the four Centres for Leprosy and Buruli Ulcer Treatment and Diagnosis (CDTLUBs), as part of the National Control Program for Leprosy and Buruli Ulcer in Benin. The widespread locations of the four centres makes it possible to do continuous active surveys, to establish disease burden, and to provide the most appropriate care for each patient. Furthermore, it makes it possible to collect high-quality epidemiological data, as highlighted by many published epidemiological studies.[17,18]

Information about well drilling (dates and sites) was obtained from the Ministry of Energy, Water and Mines of Benin (Vouillamoz J-M, Institut de recherche pour le développement, personal communication). Coordinates were obtained for each well drilled in the departments of Ouémé and Plateau, with a hand-held global positioning system receiver (GarminGPSMAP-62sc; Olake, KS, USA).

Patient data were collected at the CDTLUB-Pobè, where all epidemiological records for the patients with Buruli ulcer from the Ouémé and Plateau departments (administrative areas approximately equivalent to a county) are archived. Data collection was approved by the institutional review board of the CDTLUB and the national Beninese Buruli ulcer control authorities (IRB00006860), as well as the ethics committee of the university hospital of Angers, France (Comite d'Ethique du CHU d'Angers). Buruli ulcer cases were defined on the basis of the WHO clinical definition, with a confirmation by PCR. Sociodemographic variables (ie, age, sex, village or city of residence) were also collected for the patients with Buruli ulcer. The annual incidence of the disease in the community and the ratio of the number of wells drilled to the number of inhabitants were calculated from the 2012 census data. Ethical approval to use these data was not required by the National Control Program for Leprosy and Buruli Ulcer.

Participants for the case-control study

Buruli ulcer cases for the case-control study were defined as any patient diagnosed with Buruli ulcer, treated at the CDTLUB-Pobè and living in Bonou at the time of diagnosis, between 2005 and 2015. Patients were not excluded from enrolment on the basis of age or sex.

The study protocol aimed to recruit two controls per case to obtain a case control ratio of 1:2. Neighbourhood controls were individually matched on the basis of age and sex. A door-to-door systematic procedure was implemented for control selection. For any given case, we took their house as a starting point; we then continued in a random direction. The random walking procedure described in our study was adapted from the methods detailed in a WHO survey[19] and is easy to implement during field investigations in rural areas. We then visited the nearest house and listed all members of the household to identify the potential controls who fulfilled the matching criteria. Whenever several suitable individuals were identified, we chose the individual closest in age (within 1 year) to the case. The procedure was repeated until two suitable controls were identified. Controls were examined to rule out the possibility of an active or healed Buruli ulcer.

All the individuals included, or their parents or guardians (for minors aged less than 18 years) received information about the aims of the study. Given the high proportion of illiteracy among the rural inhabitants of Bonou, oral informed consent was obtained from adults (cases and controls) or from the parents (or legal guardians) of minors (cases and controls), in the presence of the health worker (nurse) as a witness, before enrolment and interview. We also obtained authorisation for data collection from the medical officers and heads of the six districts in Bonou. Participation was voluntary and the individuals were free to withdraw from the study at any time.

Questionnaire design

The questionnaire developed for data collection in the case-control study was tested on a preliminary sample of patients and controls. After this preliminary testing, improvements were made and the questionnaire was then validated for the collection of data concerning sociodemographic status (eg, age, sex, education, region of residence, professional activities, among others), and activities involving contact with water (eg, washing, bathing, swimming, drinking, or cooking with river water or well water). The questionnaire was written in French, and then translated into local languages when read aloud to participants. During the interviews, questions were asked focusing on the time of the illness for the patient. The two controls were interviewed on their behaviour during this time. The questions also included water contact and changes in behaviour over time. The interviews were designed to identify the targeted periods and give benchmarks over time to minimise biases when doing the study.

Data collection for the case-control study

The limited number of patients in each district and the modifiable areal unit problem (aggregation) acted as major sources of statistical bias with a substantial effect on the analysis. Indeed, a correlation observed at the district level might not be visible at finer scales (depending on where area borders are drawn).[20] Consequently, in accordance with the incidence of Buruli ulcer in Bonou municipality, the distribution of cases in the various districts over the 10-year-period (2006–15) was proportional during recruitment, to attain the finest possible spatial scale with the highest possible resolution of incidence data. The sample was stratified as previously described,[21] and the data were analysed at the scale of the municipality. In each district of Bonou, the research team met the district medical officer, who introduced them to the heads of district and the nurses in charge of Buruli ulcer treatment in the villages. The research team and the nurse then went to meet the patients with Buruli ulcer at their place of residence or work. A questionnaire, either in French or translated into one of the local languages (ie, Goun or Nago), was read aloud to participants and completed in front of them. In the field, geographical coordinates were collected for the sites of human contact with water. These coordinates were obtained with the aforementioned hand-held global positioning system receiver. We calculated the Euclidean distance (shortest point-to-point distance) between the drilling site, the main bodies of water at which contact between humans and water occurred, and the places of residence and work of the patients, with ArcGis version 10. The various maps were constructed from field surveys and from diverse data sources (OpenStreetMaps, Google Earth, Shuttle Radar Topography Mission, Institut National de la Statistique et de l'Analyse Economique du Bénin).

Statistical analysis

Quality control was systematically done for all the data collected on questionnaires. Diagnosis of Buruli ulcer

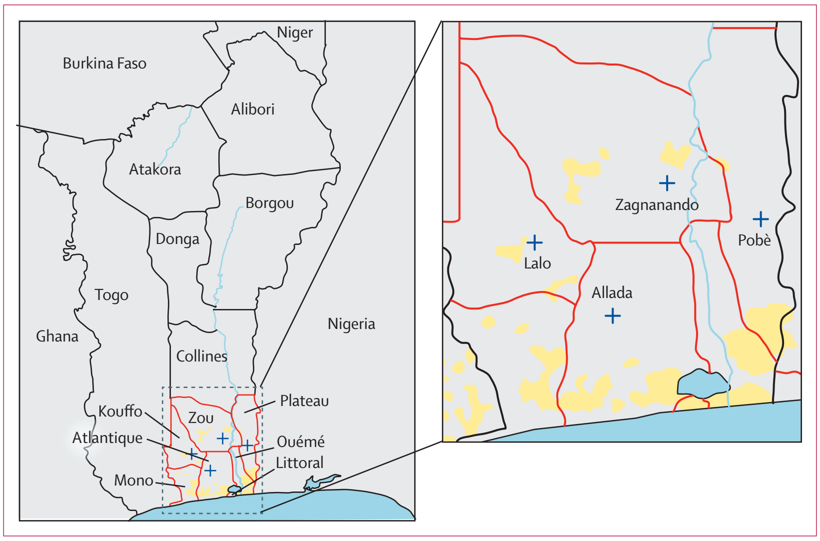
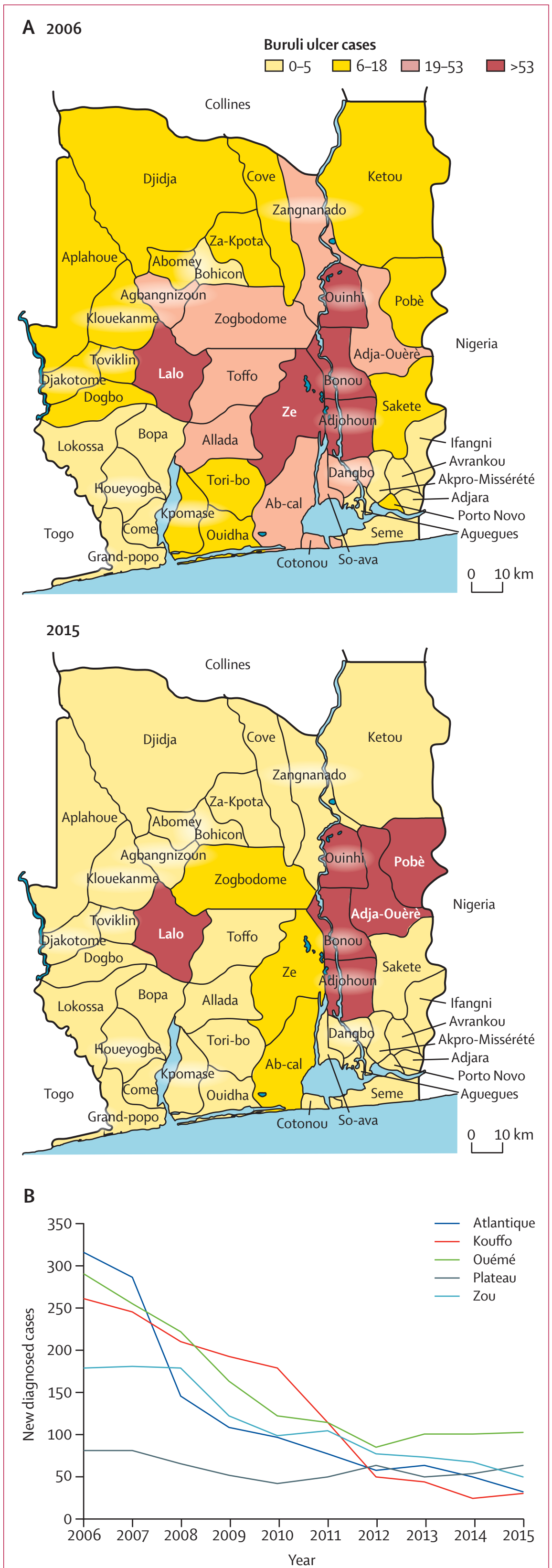


Figure 1: **Departments of Benin in which Buruli ulcer is endemic and locations of the four CDTLUBs**
Buruli ulcer is endemic to seven (outlined in red) of the 12 departments of Benin. All seven are located in the south of the country (Zou, Mono, Kouffo, Atlantique, Littoral, Ouémé, and Plateau). Four specialised CDTLUBs (CDTLUB-Lalo, CDTLUB-Allada, CDTLUB-Pobè, and CDTLUB-Zagnanado) have been set up in the areas of endemic disease, for the diagnosis and treatment of Buruli ulcer. Maps were created from Buruli ulcer data collected from the National Control Program for Leprosy and Buruli Ulcer. Blue crosses indicate CDTLUBs. CDTLUBs=Centres for Leprosy and Buruli Ulcer Treatment and Diagnosis.



was the dependent variable, and sociodemographic status and activities leading to contact with water were independent variables. All variables are expressed as proportions.

Univariate odds ratios (ORs) and 95% CIs were estimated for each variable using a conditional logistic regression model (to account for the matched design) and significance was assessed with the Wald test. Interactions were tested by introducing interaction terms in the model. Variables with $p<0\cdot20$, except informal workers and frequency of river visit variables, were entered into a multivariate conditional logistic regression model to simultaneously examine their independent effect. The final model was obtained through stepwise deletion of variables until all predictors left had $p<0\cdot05$. The two variables of informal workers and frequency of river visits were not included to avoid potential confounding effects because these variables change according to several factors (eg, rainy season or dry season, flooding or recession of river water, and professional activities such as the type of employment). Data were processed and analysed using EpiInfo version 7.1.3.3.3 and Stata/SE version 11.0.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, writing of the report, or in the decision to submit the paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The fight against Buruli ulcer in Benin is organised around four Buruli ulcer hospitals or CDTLUBs (figure 1). Between 2006 and 2015, 6051 Buruli ulcer cases were reported in Benin with the highest endemicity in Ouémé (1555 patients, 26% of total cases; appendix p 3). The number of new cases reported per year has decreased by 74%, from 1195 in 2006 to 311 in 2015 (figure 2). After 2012, the decrease slowed and the number of new cases stabilised. By contrast, the number of new cases in Plateau remained low and stable, and thus displayed a different pattern of change over the 2006 to 2015 period (figure 2B).

The Ouémé and Plateau departments are covered by the CDTLUB-Pobè. Between 2006 and 2015, the hospital diagnosed and treated 1365 patients: 946 (69%) patients from Ouémé and 419 (31%) patients from Plateau. Three of the nine municipalities in Ouémé (Adjohoun,

Figure 2: **Survey of Buruli ulcer in Benin (2006–15)**
Incidence of Buruli ulcer in all the areas in which this disease is endemic (A). New Buruli ulcer cases reported between 2006 and 2015 in the five departments with the highest levels of endemicity (B). Maps were created from Buruli ulcer data collected from the National Control Program for Leprosy and Buruli Ulcer.

Bonou, and Dangbo, which are located along the river) and two of the five in Plateau (Pobè and Adja-Ouèrè) were retained for analysis as they accounted for 94% of all cases (1283 patients; appendix p 4). During the 2006–15 period, the decrease in Buruli ulcer incidence in Ouémé was due to decreases in the two municipalities in which endemicity for Buruli ulcer was highest: Bonou and Adjohoun (appendix pp 3, 6). By contrast, Buruli ulcer incidence in Pobè and Adja-Ouèrè in Plateau was stable or fluctuated only slightly. We noted some differences in the epidemiological profile between municipalities, despite all the municipalities in these two departments having access to similar care and control measures for Buruli ulcer. This finding suggests that an environmental factor might underlie the decrease in the number of new Buruli ulcer cases in Ouémé.

During the 2006–12 period, substantially more new wells were drilled in Ouémé than in Plateau. The number of wells in Ouémé increased from 10·9 per 10 000 inhabitants in 2006 to 22·5 per 10 000 inhabitants in 2012, whereas the number of wells remained relatively stable in Plateau (4·2 wells per 10 000 inhabitants in 2006 and 5·2 per 10 000 inhabitants in 2012). Most of the wells drilled after 2006 were located in endemic foci for Buruli ulcer. In Ouémé, the increase in the number of wells was particularly large in the municipality of Bonou (from five wells per 10 000 inhabitants in 2006 to nine wells per 10 000 inhabitants in 2012) and Adjohoun (from four wells per 10 000 inhabitants in 2006 to 12 wells per 10 000 inhabitants in 2012), whereas the number of wells remained relatively stable (<1·5 times change) in the other three municipalities (Dangbo in Ouémé and Adja-Ouèrè and Pobè in Plateau; figure 3).

The increase in the number of new wells drilled and the decrease in the number of Buruli ulcer cases in Ouémé were strongly correlated in Bonou and Adjohoun (figure 3). By contrast, this correlation was almost non-existent when the number of wells drilled remained more stable. On the basis of this correlation, we hypothesise that the drilling of new wells provides the population with a source of protected water, thereby limiting contact with contaminated sources of water, favouring a decrease in the number of Buruli ulcer cases.

To investigate whether the drilling of new wells was a protective factor against Buruli ulcer, we did a case-control study in Bonou, which is the municipality with the highest level of endemicity for Buruli ulcer, and presented the strongest correlation between Buruli ulcer incidence and the number of wells drilled (figure 3, appendix p 3). Cases and controls were recruited from Sept 12, 2016, to Jan 20, 2017. Bonou consists of five districts (Damè-Wogon, Bonou, Atchonsa, Affamè, and Houinviguè), each of which is crossed by the large Ouémé river (figure 4). The sociodemographic characteristics of the cohort are shown in the appendix (p 5). 470 patients living in Bonou were identified. Their areas of residence were distributed as follows: 153 (33%) were living in Atchonsa

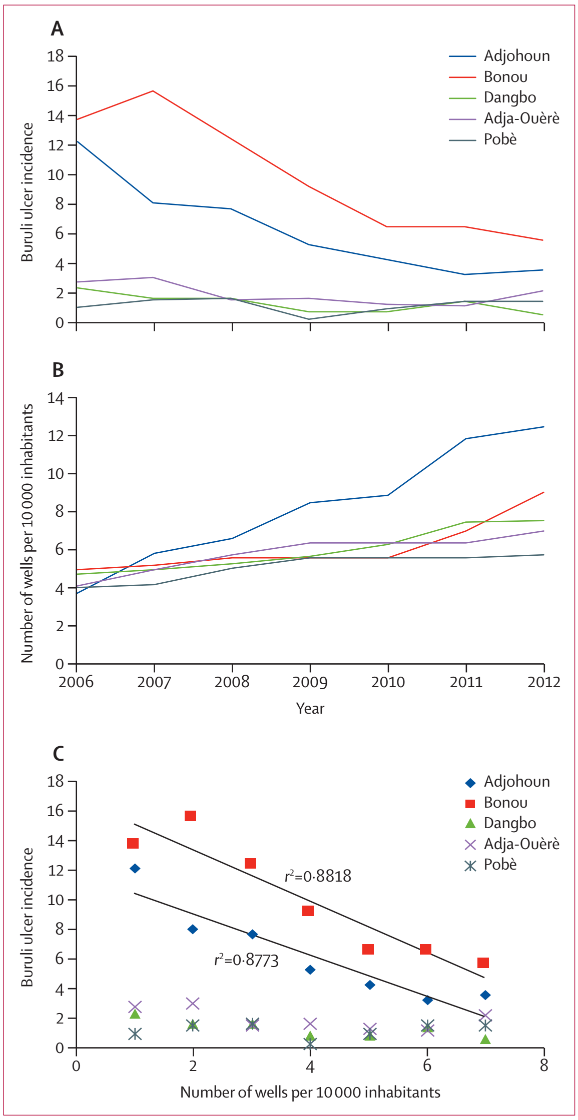


Figure 3: **Correlation between decrease of Buruli ulcer incidence and increase of new wells in municipalities of Ouémé and Plateau**
Incidence of Buruli ulcer in the municipalities of Ouémé (Bounou, Adjohoun, Dangbo) and Plateau (Pobè and Adja-Ouèrè) in which endemicity was highest (A). Only cases diagnosed (PCR-positive) by Centre for Leprosy and Buruli Ulcer Treatment and Diagnosis-Pobè from 2006 to 2012 are reported here. Number of wells per 10 000 inhabitants in the municipalities of Ouémé (Bounou, Adjohoun, Dangbo) and Plateau (Pobè and Adja-Ouèrè) with the highest levels of endemicity for 2006–12 (B). Linear correlation between Buruli ulcer incidence and the number of wells drilled, from 2006 to 2012, in the municipalities of Ouémé and Plateau with the highest endemicities (C). In Ouémé, the increase in the number of wells drilled was strongly correlated with the decrease in the number of Buruli ulcer cases, particularly in Bonou and Adjohoun. This correlation was very weak (almost non-existent) in the municipalities of Plateau and Ouémé in which the number of wells drilled remained more stable during the study period.

district, 110 (23%) in Damè-Wogon district, 93 (20%) in Affamè district, 90 (19%) in Bonou district and 24 (5%) in Houinviguè district. The proportions of patients recruited from the various districts reflected the population densities of those districts. There was no exclusion

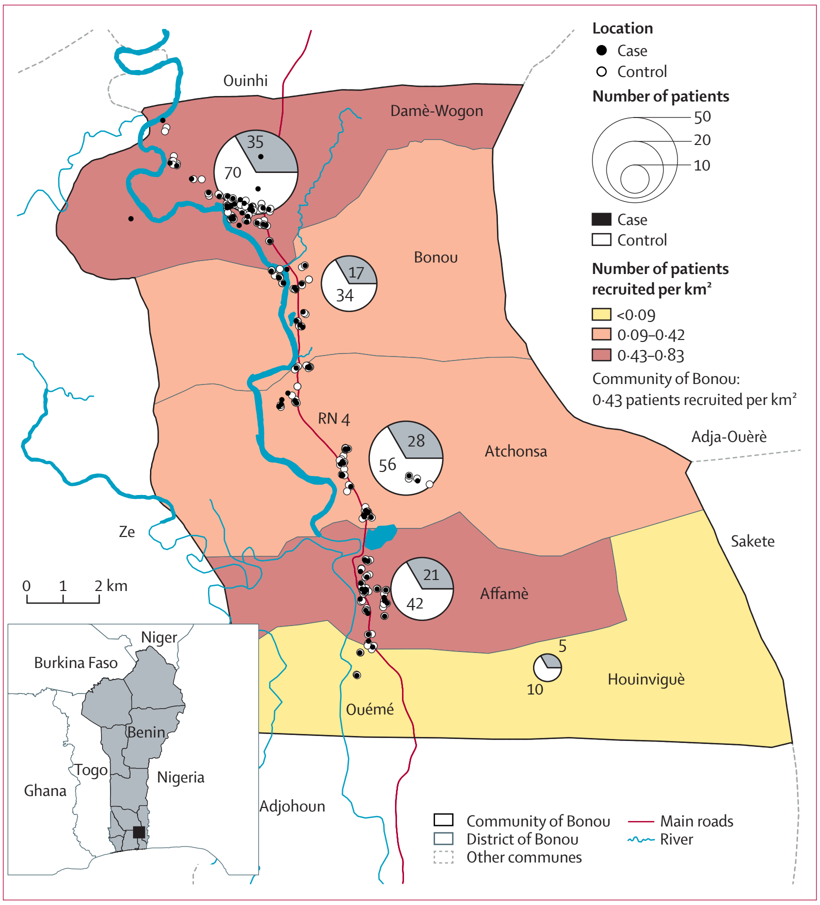


Figure 4: **Map showing the number of patients recruited per 1000 inhabitants**
Most of the patients recruited lived in Damè-Wogon district (35 [33%] patients). The distribution of cases in the different districts over the 2006–15 period was proportional during recruitment. Only patients diagnosed (PCR-positive) by Centre for Leprosy and Buruli Ulcer Treatment and Diagnosis-Pobè are reported here.

criteria, but some patients refused to participate, were untraceable or moved out, or were deceased. We enrolled 106 individuals with previously diagnosed Buruli ulcer (diagnosed by the CDTLUB-Pobè) and 212 controls in this case-control study. Most patients (69 [65%] patients) were younger than 15 years old with a median age of 10 years (IQR 6·0–29·5). The sex ratio of male to female participants was 0·77 at the time of Buruli ulcer diagnosis. Most of the patients were young, accounting for the large proportion of schoolchildren and students among the recruited cases (49 [46%] patients). As ascertained from the questionnaire, the main socioeconomic activity in adult Buruli ulcer cases was subsistence farming (data not shown). We first did a univariate analysis to investigate the association between Buruli ulcer incidence and independent variables, such as sociodemographic factors and activities involving contact with water (table). Our analysis showed that regular contact with river water, and its use for washing, bathing, drinking, swimming, or cooking (OR 3·4, 95% CI 1·70–6·99; p=0·0013) and being a schoolchild or student (OR 9·2, 95% CI 2·01–41·76; p=0·0041) were associated with a higher risk of contracting Buruli ulcer. These factors have been identified as the main risk factors in many epidemiological studies,[9,10,12] supporting the quality of our case-control study. Our analysis also clearly showed that the use of well water (for washing, bathing, drinking, or cooking) protected against Buruli ulcer (OR 0·2, 95% CI 0·06–0·55; p=0·0032; table). We controlled for confounding factors by doing a multiple conditional logistic regression analysis including several variables known to be associated with Buruli ulcer (table). Variables identified in univariate analysis as of potential importance (p≤0·20) were introduced into the logistic regression model. We found that regular use of river water (washing, bathing, drinking, swimming, or cooking) was strongly associated with the risk of contracting Buruli ulcer (adjusted OR 3·8, 95% CI 1·73– 8·38; p=0·0011; table). Regular use of well water for washing, bathing, drinking, or cooking reduced amounts of *M ulcerans* infection (adjusted OR 0·1, 95% CI 0·04–0·44; p=0·0012).

Discussion

As for other neglected tropical diseases, the fight against Buruli ulcer will require integrated approaches to reduce transmission and ensure earlier patient management.[22] Two major strategies can be used to reduce pathogen transmission. One strategy is mass vaccination of the exposed population to eradicate or eliminate the disease, as has already been done for various infectious diseases, including smallpox. However, this strategy has been unsuccessful for mycobacterial diseases (eg, the BCG vaccination and the elimination of tuberculosis). A second strategy is to use preventive approaches such as reducing contact between humans and the bacterium. This strategy has proved effective for various diseases, including cholera and various types of diarrhoea, but precise knowledge of the ecology and transmission routes of the causal microorganism is required.[23–26]

The beneficial effect of access to protected water sources has been established for several water-borne diseases (eg, typhoid and cholera), which are a major cause of morbidity and mortality worldwide. However, few studies have assessed the effect of well drilling on environmental microbial infections (caused by contact with, but not the ingestion of contaminated water, such as leptospirosis and Buruli ulcer). Previous epidemiological studies on Buruli ulcer in Benin reported case numbers at the national or regional level. However, Buruli ulcer has a highly focused distribution in endemic areas, with the coexistence of endemic and non-endemic subdistricts and districts within the same region.[18] At the scale of the municipality, we showed the existence of a strong correlation between the increase in the number of wells and the decrease in Buruli ulcer incidence, and we identified the use of well water as a factor clearly reducing the risk of *M ulcerans* infection. This study provides the first evidence for a factor potentially accounting for the decrease in Buruli ulcer

	Cases (n=106)	Controls (n=212)	Univariate odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Professional activities						
Informal workers	46 (43%)	109 (51%)	0·2 (0·05–0·59)	0·0063*	..	..
Not informal workers	60 (57%)	103 (49%)	1	..	..	..
Schoolchildren	49 (46%)	80 (38%)	9·2 (2·01–41·76)	0·0041*	8·8 (1·89–41·26)	0·0064*
Not schoolchildren	57 (54%)	132 (62%)	1	..	..	..
Formal workers	2 (2%)	2 (1%)	2·7 (0·23–33·00)	0·43	..	..
Not formal workers	104 (98%)	210 (99%)	1	..	..	..
Washing, bathing, drinking, or cooking with well water						
Yes	30 (28%)	81 (38%)	0·2 (0·06–0·55)	0·0032*	0·1 (0·04–0·44)	0·0012*
No	76 (72%)	131 (62%)	1	..	..	..
Washing, bathing, drinking, or cooking with river water						
Yes	87 (82%)	140 (66%)	3·4 (1·70–6·99)	0·0013*	3·8 (1·73–8·38)	0·0011*
No	19 (18%)	72 (34%)	1	..	..	..
Frequency of river visits						
Regular	54 (51%)	82 (39%)	2·2 (1·19–4·01)	0·012*	..	..
Occasional	52 (49%)	130 (61%)	1	..	..	..

Data are n (%) unless stated otherwise. The unadjusted odds ratio compared cases to the control group. The adjusted odds ratio adjusted for the effects of all variables included in the model. Officials are regular employees and workers refers to unreported employees having no contracts (eg, subsistence farming). *Statistically significant.

Table: **Univariate analysis and conditional logistic regression of risk factors for Buruli ulcer in 318 people**

incidence. Our data suggest that the drilling of new wells could be crucial for decreasing the incidence of Buruli ulcer. However, the use of well water does not prevent the use of river water, and some types of behaviour or activity might persist and confer a high risk of developing Buruli ulcer. The identification of these types of behaviour and activities is essential to improve preventive strategies. Furthermore, to limit the incidence of Buruli ulcer in various endemic countries, it is crucial to identify the precise reservoirs and routes of transmission of the bacilli. In this respect, it has been proposed that humans are a main reservoir of the bacilli,[16,27] and that antibiotic treatment could break the epidemic cycle. However, the outbreak of Buruli ulcer in Australia since 2011 does not fully support such hypotheses because Australia is a country with a well developed health system, as well as the extinction of outbreaks before the introduction of antibiotics.[28,29] Epidemiological studies have identified aquatic environments as major areas of contamination.[6,7,9–11] Other factors could also be involved in the decrease of Buruli ulcer incidence in most endemic countries, such as changes in land use planning, including urban development and changes in agricultural practice. However, in the past 30 years, no noticeable or relevant improvements occurred in our study area, except for the improved access to drinking water. Such possible additional factors could be further investigated in larger scale studies grouping together several endemic sites and also resorting to relevant expertise in human and social sciences and geographical information systems.

One of the limitations of the study was the use of a retrospective case-control design with the associated possibility of bias. Nevertheless, the questionnaire was specifically designed to minimise such bias. Notably, the questionnaire required the participant to report in detail all behaviours connected to water activities. In rural areas of Benin, the activities connected to water are crucial for the household and repeated on a daily basis. In this context, it is expected that reports related to water activity are accurate. In addition, we did not include several confounding factors in the questionnaire, for which less trustworthy answers could have been expected (eg, duration of activities). A prospective case-control study that detailed all activities involving contact with water would improve our understanding as to which precise behaviours might be associated with a high risk of infection, further paving the way to the identification of contamination sites. However, with a small number of new cases per year, in a small area, implementation of such prospective studies is not expected in the immediate future.

Neglected tropical diseases affect more than 1 billion of the poorest people in the world,[30] populations who also often do not have ready access to protected water sources. This situation sustains a vicious cycle of poverty and disease, in a context in which it has already been established that access to protected water is crucial for the prevention and management of many neglected tropical diseases.[31] Our findings highlight the need to develop an integrated approach to fight Buruli ulcer within a One Health framework, to render patient management and disease prevention more efficient in exposed populations.

Contributors

HD, EM, SF, and RCJ conceived and designed the experiments. HD, RG, and EM collected the data. HD, EM, and SG analysed the data. HD and EM wrote the first draft of the Article. SF, RCJ, and EY made a substantial intellectual contribution to the Article. All authors reviewed the manuscript and are in agreement with regard to the contents.

Declaration of interests

We declare no competing interests.

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