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Buruli Ulcer Case Severity at Diagnosis in Benin and Cameroon: An Analysis of World Health Organization Surveillance Data, 2013

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An abstract of
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Abstract

Buruli Ulcer Case Severity at Diagnosis in Benin and Cameroon: An Analysis of World Health Organization Surveillance Data, 2013

By Shona R. Smith

Buruli ulcer (BU) is a necrotizing skin disease caused by *Mycobacterium ulcerans* infection. Annually, approximately 6,000 new cases are reported from more than 30 countries. To reduce BU burden, routine surveillance data can be analyzed to describe the epidemiologic situation and associations between demographic variables and severity of BU cases at diagnosis. Data were reported from the Benin and Cameroon national BU control programs and provided by the World Health Organization. Descriptive statistics were calculated by frequency analysis to assess the epidemiologic situation. Polytomous logistic and logistic regression analyses were used to assess associations between age, sex, and region of origin and clinical form, case Category, and localization of lesions. In 2013, Benin reported 378 new cases, and Cameroon reported 123. The overall distribution by sex was nearly balanced in each country. The median ages in Benin and Cameroon were 15 and 22 years, respectively. In Benin, we found a significant association between sex and clinical form at diagnosis. Males were more likely to present an ulcer while females were more likely to present a nodule, plaque, or edema. We also found a significant association in Benin between age and Category and lesion localization with cases 15+ years old more likely to be classified as Category III and have a lesion on a site other than lower limbs. We found several associations between region of origin in Benin and case severity; most regions were not significantly associated with any of the outcomes. In Cameroon, we found no significant associations between age, sex, or region and clinical form, case Category or lesion location. In some BU endemic areas, sex, age, and region are associated with one or more clinical disease severity outcomes. Our results indicate that national BU surveillance systems capture data that can be evaluated alongside data from other countries to describe the current disease burden, measure temporal changes, evaluate activities, and plan interventions. Until the exact mode of transmission is known, effective prevention methods are widely implemented, and treatment costs are reduced, the best strategies for reducing BU morbidity are early detection, consistent case reporting, and prompt treatment.

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Table of Contents

CHAPTER 1

Buruli Ulcer disease (BU) is a neglected but treatable tropical disease. It has been detected in at least 33 countries including sub-tropical Africa, Asia, and Australia (1). Almost 30,000 cases were reported in sub-Saharan Africa from 2005-2010 (2). After tuberculosis and leprosy, BU is the third most common mycobacterial disease that affects humans (3). In Africa, the most affected populations typically live in low-income rural areas where medical services are often expensive and limited in availability (4).

Transmission

Buruli Ulcer disease is caused by infection with the environmental bacterium, *Mycobacterium ulcerans. M. ulcerans* is endemic in certain aquatic environments and is believed to be prevalent in stagnant or slow-moving waters (2). As nearly all epidemiologic studies have found BU disease outbreaks to be associated with proximity to freshwater habitats that have been disturbed by humans, it is thought that *M. ulcerans* can become established in new areas after environmental disturbances perturb aquatic ecosystems and create a supportive environment. These disturbances include deforestation, agriculture, construction of dams and irrigation systems, and mining (5-8). However, even in areas where such disturbances have not occurred, reported cases of BU are still present.

The pattern of *M. ulcerans* transmission from the environment to infection of humans remains unknown (1). One hypothesis is that the bacteria colonize in aquatic environments, are ingested by aquatic insects, are biomagnified through the food chain, and infect humans through insect bites although this hypothesis is not universally accepted (9-10). Studies have been unable to detect *M. ulcerans* in the salivary glands

and mid-gut of mosquitoes, and other aquatic insects such as biting hemipterans rarely bite humans (11-12). It is more likely that larval forms of these vectors, known to filter feed on bacteria, simply aid in the bio magnification of *M. ulcerans* through the aquatic food chain (11). The more commonly accepted hypothesis is that infection occurs by *M. ulcerans*, present in aquatic environments, entering through the wound produced by a bite, small cut, or break in the skin (13). While some have found that the patterns of BU lesion distribution, often clustered around the ankles or at the back of the elbows, match neither mosquito bite patterns nor small skin injury patterns, this could be due to a delay between exposure and the onset of disease (8, 14).

Finally, though extremely rare, cases of person-to-person transmission have been reported. In one report, infection occurred after a child was bitten on the arm by another child. In cases such as these, it is possible that *M. ulcerans* was transmitted directly from the mouth of the biter into the wound. However, it is much more likely that either *M. ulcerans* was already present on the patient's skin when she was bitten, or it infected the body at a later time through the unhealed wound (15). All of this information taken together, multiple modes of transmission should be considered when determining and evaluating risk factors (8, 14).

Risk Factors

Historically, the primary population at risk has been reported to be children between four and fifteen years old with a general range of 1 to 50 years (16-18). Recent estimates indicate that those with the highest risk are aged 12 to 15 years (8, 19-20). Additionally, those 50 and older have also been found to be at high risk, especially in

Australia (20). In a 1991 case-control study in rural Côte d'Ivoire, the rate of illness was found to be highest among those aged 10-14 years with 143 cases per 1,000 (17).

BU is often more frequently found in males than in females (8, 19). However, many studies have not found a statistically significant difference (17-18, 20). Male BU cases are generally younger at diagnosis than female BU cases (14, 19, 21-22). However, many studies disregard this association after finding no statistically significant differences (6, 23-26). Hypotheses that have attempted to explain differences in risk by sex include differential exposure to *M. ulcerans* in the environment, differential host response, or both (19). Moreover, it has been hypothesized that adult women and children of both sexes are at increased risk due to shared behaviors and activities, but no significant association between BU and exposure to water sources when fetching water, washing dishes, or washing clothes has been found (14, 27).

Other risk factors such as proximity to rivers and wetlands, exposure to slow moving and stagnant waters, and not wearing long clothing during agricultural activities have been identified (10, 14, 17, 20-21, 23, 28-30). Moreover, it has been shown that patients are more likely to have had contact with a local river than with an artificial reservoir (8). Farming near major regional rivers was found to be associated with increased odds of infection in Côte d'Ivoire, with odds decreasing as walking distance from fields to a river increases. This study also found a protective effect of wearing long pants during farming (17). Wearing long-sleeves during agricultural activities, bathing with soap, and wearing shoes have also been found to be protective against *M. ulcerans* infection (7, 14). Human modification of landscape, such as deforestation and agriculture,

that likely facilitate the introduction of *M. ulcerans* into a new environment have also been identified as risk factors for infection (10).

In Benin, surveillance data have shown BU to be endemic only in the southern regions while the endemic levels assessed by prevalence vary by department, district, and village as a function of altitude. Findings suggest that low-land areas (<50 meters in elevation) are at higher risk than areas at a higher elevation (50-100 meters or > 100 meters). As low-lands tend to be wetter than higher areas, the conditions are more favorable to allow *M. ulcerans* proliferation and spread. Additionally, agricultural workers are more likely to spend time in these areas while planting, tending, and harvesting crops (31).

Having a family history of BU has also been found to be associated with higher odds of having the diseases when adjusting for daily contact with a natural water source. This suggests that there may be genetic factors that at least partially explain why some individuals, but not all, that are exposed to *M. ulcerans* develop Buruli ulcer disease. Thus, genetics in addition to acquired immune responses and behavioral risk factors can be considered when evaluating individual and group risk (28, 32).

Clinical Features

M. ulcerans produces a toxin (mycolactone) that causes extensive tissue damage through necrosis and immunosuppression that, untreated, produces ulcers and lesions (33-34). Accordingly, the clinical stages of Buruli Ulcer disease are classified into pre-ulcerative, ulcerative, and inactive disease. The pre-ulcerative form indicates the presence of a papule, nodule, plaque, or edema (35-36). The ulcerative form, developed through necrosis from mycolactone, has undermined edges and produces a peripheral induration.

In the absence of secondary infection the lesion is normally painless (36). This lack of pain as well as an absence of fever in cases is likely a result of the local immunosuppression by mycolactone (37). Cases can also present multiple lesions that may be a mix of clinical forms and sizes and may be located on one or more areas of the body (18). To advance to an inactive stage of BU, ulcers can spontaneously heal and produce a characteristic depressed scar (35).

Most cases reported from sub-Saharan Africa are diagnosed as an ulcerative form with most reports ranging from 65-90% of cases (19, 38-40). Ulcers most often form on the arms or legs but can also commonly form on the head and neck and are less frequent on the thorax (14, 17-18, 41). Approximately 50-60% of lesions are found on a lower limb, 30-40% of lesions are found on an upper limb, less than 10% are found on the trunk, head, or neck, and less than 1% are found in the perineal area (14, 19, 21, 38, 40, 42). While some studies have found that lesions on the trunk are significantly more likely among males than females, others have found no significant association between the distribution of lesions and the sex and age of case patients (14, 21).

BU disease lesions are classified by WHO into three categories. Category I indicates a single lesion less than 5 centimeters (cm) in diameter. Category II indicates a single lesion between 5 and 15 cm in diameter. Category III indicates a single lesion greater than 15 cm in diameter, multiple lesions, lesion(s) at a critical site such as an eye, breast, or genitals, or presenting osteomyelitis (43). Category I or II lesions are generally considered to be early lesions (44). Most studies have found that in approximately 10% of cases disease affects the bone causing osteomyelitis (16, 19). Osteomyelitis can be present beneath active BU lesions, at a distance from active or healed and inactive

lesions, or in patients without a confirmed history of lesions (19, 45). There may also be an association between clinical presentation of BU and age and sex with males more likely to present osteomyelitis than women (19).

Functional Limitations

Even after ulcers are considered healed, functional disabilities may chronically persist as a result of range of motion limitations and contraction deformities that developed during the active stage of the disease. These disabilities can also result in a dramatic change in the activities that a patient is able to perform, including agricultural work, domestic activities, and school attendance. A 1995 study in Côte d'Ivoire found that 26% of cases with healed ulcers presented a chronic functional disability and 53% changed their activities as a result of *M. ulcerans* infection (17). A 2009 study in Uganda found that 43% of cases had a functional disability caused by a lesion before surgery was performed, and 22% of cases reported that their functional limitations hindered performing daily activities (46). A 2005 study of patients in Benin and Ghana found an even proportion of functional disability (58%) among patients years after treatment for BU (47). These disabilities are more commonly found among older female cases who had a lesion on a joint or who had a lesion on the distal part of a limb.

These independent risk factors for functional disabilities also often lead to patients stopping work or education. School dropout and job loss have also both been found to be significantly associated with having an amputation. It has been found that, among cases who either worked or went to school before developing BU, 53% had to stop working and 17% had to stop going to school as a result of the disease. This percentage increased with the severity of the functional limitation (47). Marston et al.

(1995), found that more than 63% of patients either stopped working or dropped out of school (17).

In order to assess the nature and severity of these long-term consequences faced by BU patients, Stienstra et al. (2005), developed and validated a BU functional limitation scoring system (BUFLS) (47, 48). This scoring system was developed based on studies conducted when the use of antibiotic treatment was less common. However, more recent studies have still found no significant difference in the frequency of functional limitations between patients who received either antibiotic treatment alone or both antibiotic and surgical treatments and patients who were only treated surgically (49).

Diagnosis

Most commonly, BU cases are diagnosed and reported based on the presentation of symptoms. However, several laboratory diagnostic tools are also available. *M. ulcerans* infection in humans is primarily laboratory confirmed through polymerase chain reaction (PCR) using swabs from case lesions or by Ziehl-Neelsen (ZN) staining of swab smears to confirm the presence acid-fast bacilli (50-52). As BU can present a wide range of clinical symptoms and can be confused with other tropical skin diseases, WHO recommends that at least 70% of cases be confirmed through PCR (53). The sensitivity of PCR is high (85-98%), but it is expensive and requires more advanced technical training and equipment (52, 54). A study by Raghunathan et al. (2005), used PCR to confirm 75% of probable BU cases in Ghana in 2000. Alternatively, the sensitivity of ZN staining is low (57%) and has been estimated to confirm the lowest number of probable BU cases (13%) compared to other methods (14, 52).

M. ulcerans can also be cultured from clinical samples; however, this method is difficult to perform as the bacterium takes around 6-8 weeks to grow, and like ZN staining it also has a low sensitivity (48%) and has been estimated to confirm only 38% of probable cases (14, 52, 54). Recent advances have been made to rapidly detect infection through the use of relatively inexpensive methods of detecting the presence of mycolactone even before the disease presents advanced symptoms, though this technology has not yet been implemented in the field (55).

Treatment

When detected early in the course of disease, BU is curable. However, treatment often requires multiple interventions including antimicrobial medications, surgery, and physiotherapy (56). Antibiotic treatment alone has been shown to cure 30-50% of cases and traditionally includes the use of Rifampin and Streptomycin in combination for eight weeks (39, 43). A recent study found that in Category I and II cases, Streptomycin can be replaced after two weeks of treatment with Clarithromycin to be used for the remaining six weeks. Using this strategy no recurrence was reported after 12 months of follow-up (57).

For many cases, especially those diagnosed as Category III, surgery and hospitalization are required in addition to antibiotics. In such cases it is still recommended that antibiotic treatment be administered for at least four weeks before surgery as it is believed to help minimize the amount of tissue that is required to be excised (39, 58). Surgery involves excision of the ulcer or lesion with or without skin grafting depending on the size of the lesion. Though rare, in very severe cases amputation must be performed. The duration of hospital stay depends on the severity of the case and

treatment required but has decreased over time. These factors directly affect treatment costs (29). Patients who present osteomyelitis are at a higher risk for a longer hospital stay, surgery, and crippling sequelae than BU cases without osteomyelitis (45).

Delays in treatment often occur because many patients live in rural areas and family member do not have the time to go with the patient to seek hospital treatment. These delays also result from limited or inaccurate knowledge regarding Buruli Ulcer disease (59-60). Patients may also not seek immediate treatment because of the lack of pain or fever that is common in the progression of most cases (37). Many cases seek help only when the disease has progressed to an ulcerative form. At this point mycolactone has produced advanced skin necrosis, and disfiguration may be present. Cases presenting these forms are limited in treatment options requiring extensive surgery and longer hospital stays (29, 61). Complications including persistence and relapse also may occur when treatment is begun at an advanced stage of the disease (62).

There is evidence that those suffering from BU seek medical treatment when traditional remedies used to treat the disease have failed (60). Renzaho et al. (2005), found that 71.8% of respondents reported that cases seek traditional treatment, and only 22.8% reported that cases seek help at a hospital or from a local doctor or nurse as the first source of treatment. Further only 7.7% of those who seek traditional medicine first go to the hospital as the case becomes more severe with 48.2% seeking care first from a local doctor or nurse (61). Delays in diagnosis and treatment lead to more complex patient management often necessitating longer hospital stays and riskier treatments such as surgery, and thus have been found to contribute to a high proportion of patients with long-term complications and chronic disabilities (45).

Cost

The cost of BU morbidity is an important factor for both treatment decision making and intervention programs. Direct costs include medical costs and nonmedical costs. Disability costs include both direct costs and intangible costs. Indirect costs are comprised of productivity loss, including job loss, and school days lost for children, including school dropout (47, 63-64). Intangible costs can be a result of stigma, pain, functional limitations, and social isolation. Taken together these costs contribute to the total morbidity cost of Buruli Ulcer disease (63-64). In 2012, Amoakoh et al. (2013), estimated that among a Ghanaian sub-population 65% of the total household economic cost of out-patient treatment was incurred by children at a mean cost of US$521.04. The mean annual household cost was calculated as US$570.09 with direct costs comprising 96% of this total. All but a small percentage of these direct costs were classified as non-medical (mean = US$529.27) including food and transport, and mean medical costs were estimated at US$18.94 (63).

The economic impact in this sub-population also varied among BU cases by the stage of the disease and the localization of the lesion. Dadzie et al. (2005), found that the average nine-month total cost incurred by a typical household in 2003 was US$23.44 if presenting a nodule, US$713.81 if presenting a plaque or edema, US$289 if presenting an ulcer located on a joint, US$80.66 if presenting an ulcer not on a joint, US$ 214.43 if presenting a scar with a visible contracture, and US$71.91 if presenting a scar without a visible contracture. The total average cost for an amputation with or without prosthesis was US$391.10 (64).

Financial difficulties as a result of BU have been found to be the main reason that children stop going to school (47). Moreover, while children also tend to feel more pain as a result of the disease, adults suffer from more functional limitations and loss of productivity. About 84% of children presenting BU disease reported social isolated, and an estimated 36% of these children reported that they were not accompanied during treatment likely as a result of additional costs that would have been incurred by the family. Moreover, it was estimated that, overall, only 11% of children were accompanied during treatment (63).

In impoverished populations like those studied by Amakoah et al. (2013), and Dadzie et al. (2005), where the per capita income was US$100 or less, Buruli Ulcer disease, regardless of the clinical form, case Category, localization of lesions, or treatment requirements, has a substantial economic impact on patients and families already facing considerable financial constraints (64).

Community-level perception

While research on local perceptions of Buruli Ulcer disease is limited, Stienstra et al. (2002), found that 57% of Ghanaian's interviewed mentioned witchcraft as a cause of BU (60). It appears that since that time the BU attribution to witchcraft has likely declined as a result of successful community education programs. In 2005, Renzaho et al., found that, while there is still some uncertainty among groups, only 5.2% of participants in a similar Ghanaian community believed the cause of BU to be witchcraft or curses. However, they also found that the majority of participants still did not know the cause of Buruli Ulcer disease. Sixteen percent attributed disease to drinking non-potable water, 8.1% to poor personal hygiene or dirty surroundings, and only 5.5% to

swimming or wading in ponds or rivers. It was also found that these perceptions and beliefs varied between children and adults with children being more likely to discriminate against those suffering from BU (61).

Prevention

While few studies have evaluated the effectiveness of BU prevention methods, a few are commonly promoted in BU programs. They include adequate clothing coverage, such as wearing long pants and sleeves while farming or fishing, footwear use, using soap when washing, treating small bites and wounds with soap or antibiotic powder, and using bed nets and insect repellent to prevent skin breaks from insect bites (7, 17, 21, 30, 65). A recent meta-analysis to assess whether footwear use was associated with a lower risk of various neglected tropical diseases found that footwear use was significantly associated with lower odds of Buruli Ulcer disease (66). Landier et al. (2011), estimated that using soap to clean wounds can prevent 32% of cases, and using bed nets could prevent 32% of cases if a causal link between insect vectors and disease were proven (7). Additionally, BCG vaccination has provided some short-term protection (67). No long-term protection has been found, but it has been suggested that BCG vaccination could protect younger populations from developing severe forms of BU such as osteomyelitis or presenting multiple lesions (68-69).

Limitations of Surveillance

Buruli ulcer surveillance is a challenge for multiple reasons. First, while *M. ulcerans* infection can be laboratory confirmed through PCR, the procedure requires laboratory facilities and cannot be performed in the field. Though new advances in field detection are promising, *M. ulcerans* infection is most commonly detected in

communities based primarily on the syndromic case definition and later confirmed through PCR (55). While experience has shown syndromic diagnosis to be relatively effective in endemic communities, it may occasionally lead to early or healed lesions being confused with other endemic diseases such as yaws (70).

Based on current literature, while current community-level passive surveillance systems are adequate for many infectious diseases and may be sufficient as part of a long-term plan, they have proven unreliable for BU in terms of accurate and timely case detection and reporting (29). Due to specific treatment requirements most patients travel to specific clinics that are specially trained to detect, treat, and report BU cases. This ultimately contributes to delays in treatment, need for more advanced forms of treatment, a greater burden on families of patients, and ultimately higher levels of adverse outcomes, recurrence, and disability (63).

National surveillance data that yield estimates of incidence are usually reported at the national or district levels. While they convey the importance of disease in broad areas, they do not show potentially significant variations that exist at the village level and likely do not provide accurate estimates of true disease prevalence (31). Data collected from active surveillance programs can contribute to these gaps in knowledge and improve early detection of new cases. However, many local factors, lack of resources to perform active surveillance, difficulty getting to affected areas due to lack of roads or flooding, non-uniform training, lack of community knowledge of BU, unclear transmission pathways, and the prioritization of other diseases may impact the quality of local surveillance data and hinder more accurate estimations of disease burden (9, 44).

CHAPTER 2

Introduction

Buruli Ulcer disease (BU) is designated by the World Health Organization (WHO) as one of the 13 neglected tropical diseases. It has been detected in more than 30 countries with an estimated 5,000 – 6,000 new cases per year, and the majority of cases are reported from sub-Saharan Africa (1, 2, 35, 53). The disease begins as a non-ulcerative lesion appearing as a nodule, plaque, or edema (35-36). *Mycobacterium ulcerans*, the causative agent for BU, produces a necrotizing toxin called mycolactone.

When treatment is not begun at early stages of disease, the infection produces extensive skin necrosis resulting in an enlarging ulcer most often located on the limbs (14, 17-19, 21, 33-34, 38, 40-42). In the most advance stages of the disease, the bone and joints are affected often producing movement limitations, necessitating amputation, and resulting in permanent disability (16-17, 45-47). Current treatment methods often require more than one type of intervention including antibiotic use, surgery, and physiotherapy (29, 39, 43, 56, 58). As such there are considerable economic impacts on individuals, households, communities, and the health system (29, 47, 56, 63-64). Cases that are detected in the early stages of the disease have shown better treatment outcomes with lower need for surgery or hospitalization than those detected at later stages of disease (29, 61, 63).

Historically, in African countries children between the ages of four and fifteen years have been estimated to be at highest risk with an overall range of one to fifty years old (16-18). More recent estimates limit the highest risk to those between the ages of 12 and 15 years (8, 19-20). Generally, BU is more frequently reported among males than

females; however, most studies have not found this difference to be statistically significant (8, 17-20).

A major problem regarding BU is that despite many years and varied types of research, the exact mode of transmission remains unknown making the assessment of most predictive risk factors difficult (1, 9-10, 13). The most significant risk factors currently known are water-related, including washing, wading, swimming, fishing, and farming in or near rivers or swamps (10, 14, 17, 20-21, 23, 28-30). Accordingly, the most commonly evaluated prevention measures include adequate clothing covering, footwear, bathing with soap, cleaning small wounds and insect bites with soap or antibiotic powder, and using bed-nets to prevent insect bites (7, 17, 21, 30, 65). The BCG vaccine has also been shown to provide some short-term protection against *M. ulcerans* infection, specifically by protecting younger groups from developing advanced forms of BU; however, no long term protection has been found in any age group (68-69).

Despite successful community education efforts individuals living in affected communities still express uncertainty about the cause of the disease (60-61). In some groups, infection is still considered to be associated with sorcery (9, 60-61). Further, most cases of BU occur in poor rural communities where individuals commonly visit traditional healers before seeking Western medical treatment and may not access available healthcare resources, yielding limited data to guide effective BU prevention, treatment, and control activities (59-61, 71).

Surveillance activities in most affected countries consistently provide guiding knowledge on the current extent of Buruli Ulcer disease. However, they are usually reported at the national or district levels and include only cases detected when treatment

is sought. Regardless, the utilization of this routinely available data provides current estimates of disease incidence and should be cumulatively used as the basis for more in-depth investigation and estimation of BU incidence and prevalence (31).

In order to improve BU detection, prevention, and treatment it is beneficial to analyze recent routinely collected surveillance data to determine associations by demographic risk factors. It has been widely hypothesized that risk or odds of disease and severity of disease at diagnosis vary by age, sex, and region, and most studies present these characteristics commonly reported to national surveillance programs as descriptive factors, but few studies have further investigated these associations (14, 19, 21). No studies from Benin or Cameroon have used national level data or compared more than one country in these investigations. Therefore, the goal of this study is to explore associations between age, sex, and region/department of case origin and clinical form, case Category, and localization of lesions on the body among new histologically diagnosed cases using the 2013 BU surveillance data from Benin and Cameroon.

Methods

Ethics Statement

This study utilizing secondary de-identified data did not meet the definition for human subject research as specified by Emory University's Institutional Review Board.

Research Questions

Our primary research question addresses the use of demographic data captured through community-based surveillance to explore differences in the clinical characteristics of incident Buruli Ulcer disease cases. Which characteristics, captured by

demographic information, most influence the severity of reported new cases of BU in Benin and Cameroon?

- What is the association between age and/or sex and clinical disease form?
- What is the association between age and/or sex and case Category?
- Are age and/or sex associated with the localization of a lesion(s) on the body?
- What are the associations between region and clinical form, case Category, or localization of lesion, controlling for age and sex?

Data Source

Buruli ulcer national control programs in Benin and Cameroon provide incidence data to the World Health Organization (WHO), and WHO shared country-level summaries with us for analysis. Our data include information collected using the standardized WHO BU02 and BU03 Buruli Ulcer case reporting forms (72). Cases report at regional treatment centers or hospitals, and these centers report cases through the BU02 form at the time the individuals seek treatment, often after being identified through community-based surveillance conducted by local health facilities or through community health workers (44, 73). Local treatment centers then report cases to the regional level and national control program for the country. Treatment facilities also complete the BU03 form and send it to a laboratory with a specimen for diagnostic testing, as needed. After completing testing, the laboratory sends the results back to the treatment facilities and to the national control program, and the results are added to the patient record. Each country's Ministry of Health enters data into databases for analysis and mapping (56). The country specific national control programs then report case summaries to the World Health Organization. The Global Buruli Ulcer Initiative of WHO, led by Dr. Kingsley

Asiedu, MD MPH, provided the compiled reports, with the removal of all personal identifiers, for our analysis. Our data include all histologically diagnosed Buruli Ulcer cases reported in 2013. General data on annual BU incidence by country are publicly available on the World Health Organization website at http://apps.who.int/gho/data/node.main.A1631.

Variables

Each country level dataset included data on treatment center district and region where the information was collected, age, sex, region or department where the case originated, clinical form, localization of lesion, case Category, limitation of joint movement, whether or not a specimen was collected for laboratory testing, use of antibiotic treatment, month of diagnosis and results of laboratory confirmation through polymerase chain reaction (PCR). The dataset from Cameroon also includes the result of laboratory testing through Ziehl-Neelsen (ZH) smear examination.

Independent Variables. The WHO data reports age in years, and we considered this as a categorical variable for bivariate and multivariate analyses. Based on previous research indicating higher incidence in children younger than 15 years of age, we classified age into two categories: less than 15 years old and 15 or more years old (8, 14, 16-17, 19-20, 27). We recoded age values for infants less than one year old that were originally reported in months as 0. WHO data reports any age greater than 90 as a maximum value of 90 to protect individual identification among the very few cases in older adults. The reports classify sex as male or female. WHO defines the region and department of case origin as the region or department of residence. We grouped departments of case origin in Benin that reported less than five cases with a neighboring department for bivariate and

multivariate analysis. Collines was grouped with Plateau, and Mono and Littoral were grouped with Atlantique. To compare characteristics of cases originating nearer to the capital and have a larger number of observations in each group, we categorized the regions of Cameroon as Centre vs. all others for bivariate and multivariate analysis.

Dependent Variables. Treatment centers reported clinical lesion form as nodule, plaque/placard, edema, papule, ulcer, osteomyelitis, or scar. If the clinical form for more than one lesion was reported, we recoded the form as either mixed lesion forms without an ulcer or osteomyelitis, mixed lesions with ulcer and no osteomyelitis, or mixed lesion forms with osteomyelitis. We grouped clinical forms as pre-ulcer (including mixed form without ulcer), ulcer (including mixed form with ulcer), or osteomyelitis (including mixed form with osteomyelitis) to define our clinical disease form states for logistic regression analysis. We excluded cases presenting only an inactive form, indicated by the presence of a scar, from bivariate and multivariate analyses.

Treatment centers also classified case Category upon detection as I, II, or III according to the definitions established by WHO (72). A single lesion of less than 5 cm in diameter defines Category I. A single lesion of between 5 cm and 15 cm in diameter defines Category II. A single lesion of more than 15 cm in diameter, multiple lesions, or one or more lesions at a critical site (eye, breast, or genitalia) defines Category III.

The locations of lesions on the body were recorded as upper limb, lower limb, abdomen, back, thorax, buttocks and perineum, and head and neck. For our analysis, we group lesions on the abdomen, back, or thorax as located on the trunk. We also categorized cases with multiple lesions that were reported in more than one of categories listed above according to the multiple locations reported. These new multi-location

categories were: both upper and lower limb, lower limb and trunk, or upper limb and trunk. Each observation was only included in one localization of lesion group. We dichotomized lesion locations as exclusively lower limb versus all other location categories, including multi-location, for bivariate and multivariate analyses in order to stabilize estimates.

Descriptive Variables. We provided descriptive statistics for additional variables collected by the National Control Programs and reported to WHO. WHO defines limitation of movement as "the inability of the patient to move an affected joint over the normal range of movement at the time of diagnosis." The WHO BU02 form defines specimen collected as whether or not a specimen was collected for laboratory diagnosis. The WHO data classified PCR confirmation as positive, negative, not tested, or not available. The ZN confirmation was classified in the data as positive, negative, or not tested. The case reports indicated antibiotic treatment when a patient was or would be treated with antibiotics. WHO defines region and/or district of treatment center as the region and/or district in which the reporting facility is located. WHO defines month of diagnosis as the month in which the BU02 form was completed.

Statistical Analysis

We performed all statistical analysis using Statistical Analysis Software (SAS, version 9.4, Cary, NC). We limited our analysis to new cases to explore incidence rather than prevalence. We performed frequency calculations on all categorical variables and calculated the median and range for age. We also assessed bivariate associations among the independent variables of age, sex, and region.

We used multivariate analysis of associations between independent variables and levels of dependent outcome variables indicating case severity to determine odds ratios (OR) and 95% confidence intervals (95% CI). We used polytomous logistic regression on the dataset from Benin to estimate the odds of presenting osteomyelitis compared with pre-ulcer and the odds of presenting an ulcer compared with pre-ulcer by age, sex, age and sex, and region, adjusting for age and sex, respectively. We used logistic regression models on the Cameroon data to estimate the odds of clinical form (pre-ulcer vs. ulcer) by age, sex, age and sex, and region of case origin, controlling for age and sex. We used polytomous logistic regression to assess associations between WHO case Category (I, II, or III) and independent variables in each dataset with age, sex, and both age and sex. The fourth model assessed the associations between incidence and region of case origin after adjusting for age and sex. Finally, we also used logistic regression models to assess associations between the dichotomous outcome of localization of lesion (lower limb vs. other) and age, sex, age and sex, and region adjusting for age and sex, respectively, in each dataset.

Polytomous logistic regression uses a generalized logit model for outcomes having three or more levels that are either not assumed to be ordered or do not satisfy the proportional odds assumption indicating that there is not a significant difference in odds for each one unit change in outcome. Odds ratios are calculated for each outcome level against the reference level. In SAS we used the logistic procedure with link = glogit to perform this analysis. We also used the logistic procedure without the link option for the other two-level outcome logistic regression models. The procedures generated point

estimates, 95% confidence intervals, Wald χ^2 test statistics, and probability values (*P*). We considered associations having a p-value less than 0.05 as statistically significant.

Results

Benin reported 378 new cases of Buruli Ulcer from four treatment and detection centers in 2013. One case missing a value for age, a second case missing values for age and all three outcome variables, and three additional cases each missing one of the outcome variables were excluded from the analysis. Cameroon reported 133 cases of BU from six treatment centers in 2013. Six patients classified as recurrent cases, four classified as old cases, and one that was missing more than two key variables were excluded from the analysis. The characteristics of newly reported cases included in analysis are shown in Table 1 by country.

Benin

The median age was 15 years with a reported range of 0-90 years (Figure 1). Among those less than 15 years old, 61% were male, while 44% of those 15 and older were male. The distribution of cases by sex were similar with 52% males and 48% females. One hundred cases (27%) resided in the department of Ouémé, 74 (20%) were from the department of Zou, 62 (17%) were from the department of Atlantique, 45 (12%) were from the department of Plateau, 44 (12%) were from the department of Couffo, four (1%) were from the department of Mono, and the departments of Littoral and Collines each reported only one case (Figure 2). Forty-two (11%) of all new cases originated in Nigeria. A further description of these Nigerian cases is provided below.

Nodules were found in 2% of cases, and edema was also described in 2% of cases. Eighty-four cases (23%) had a plaque detected. Exclusively ulcerative forms

accounted for 42% of the reports. Osteomyelitis was reported as the only form present in 3% of cases. Mixed clinical forms were present in over 25% of the cases. Only three cases presented a scar. The majority of cases (53%) presented with Category III lesions. Forty-two cases (11%) were Category I, and 134 (36%) were Category II. The most common sites for lesions were the limbs with 64% on a lower limb and 24% on an upper limb. Twenty-two cases (6%) reported lesions exclusively on the trunk. Lesions on the head and neck were reported in less than 1% of cases, and lesions on the buttocks or in the perineum were reported in 2% of patients. Twelve patients (3%) had lesions in more than one area. Over 25% of cases presented a movement limitation as a result of the disease.

Specimens were collected for laboratory analysis from 83% of the cases. While PCR was performed for 295 cases (79%) identified through histopathological examination, 80% of those with a PCR result were positive, and 63% of all new case reports were positively confirmed through PCR. Only five cases did not or were not expected to receive antibiotic treatment. The detection and treatment centers in Pobè and Zagnanado each reported 36% of the cases (136 and 135 cases, respectively). CDTUB Allada reported 62 (17%), and CDTUB Lalo reported 40 (11%). The highest number of case reports appeared in June (11%) and the lowest in September (5%).

Nigeria. The median age of the 42 cases that originated in Nigeria was 13 years with a range of 3-65 years. Fifty-five percent were under the age of 15, and 60% were males. Pre-ulcerative lesion forms were described in six cases (14%). Exclusively ulcerative forms accounted for 31% of the reports. Osteomyelitis was reported as the only form present in 5% of cases. Mixed clinical forms were present in 50% of the cases with 15

(36%) presenting mixed forms with ulcers and no osteomyelitis. The majority of cases (57%) presented with Category III lesions. Four (10%) were classified Category I, and fourteen (33%) were classified as Category II. The most common sites for lesions were the limbs with 62% on a lower limb and 21% on an upper limb. Two cases (5%) reported lesions exclusively on the trunk, and lesions on the buttocks or in the perineum were reported in one patient. Four patients (10%) had lesions in more than one area. Over 65% of cases presented a movement limitation as a result of the disease.

Specimens were collected for laboratory analysis from 93% of the cases. PCR was performed for 42 cases (90%) identified through histopathological examination, and all but four were positive. Overall, 81% of all new case reports were positively confirmed through PCR. All cases did or were expected to receive antibiotic treatment. The highest number of case reports were in March and May (19% each) and the lowest in February and September (2% each). The CDTUB in Pobè diagnosed 81% of these cases. The others were diagnosed at the CDTUBs in Zagnanado (14%) and Allada (5%).

Cameroon

The median age was 22 years with a range of 1-84 years and 37% of cases less than 15 years old (Figure 3). The distributions of males and females in each age group were somewhat different with 60% of those under 15 years of age being female; males accounted for 58% of those 15 and older. The overall distribution of cases by sex is nearly balanced with 52% males and 48% females. The majority of cases (60%) resided in the Centre region, 32 (26%) were from the Adamaoua region, seven (6%) were from the Ouest region, and the Nord Ouest, Sud, Sud Ouest, and Littoral regions each reported 2% of the cases (Figure 4). No cases were reported from the Nord or Extreme Nord

regions. While there were differences in the proportions of cases in each age group per region, region was not significantly associated with age or sex.

In contrast to Benin, Table 1 shows that, in Cameroon, ulcerative forms accounted for 90% of the reports. None of the cases were reported as presenting osteomyelitis or mixed clinical forms. Also in contrast to Benin the highest number of cases (40%) were Category I. Forty-one cases (37%) were Category III, 32 (26%) were Category II. The most common sites for lesions were the limbs with 62% on a lower limb and 25% on an upper limb. One case had lesions on both the upper and lower limbs. Twenty-three (19%) cases presented a movement limitation as a result of the disease.

Specimens were collected for laboratory analysis from 88% of the cases. PCR was performed for 79 cases (65%), and 49% of those with a PCR result were positive. Specimens from 85% of reported cases underwent ZN smear staining. Fifty-four of those cases (52%) were positively confirmed. One hundred and eight cases (89%) received or were expected to receive antibiotic treatment. The treatment centers in Ankonolinga and Bankim each reported 36% of the new cases. The center in Ngoantet reported 20 (16%), the center in Ayos reported 12 (10%), and the centers in Ekonditi and Mbonge reported one case each. The number of case reports was highest in November (18%), and lowest in August (4%).

Clinical Form

In Benin, age was not significantly associated with clinical form (Table 2). As shown in Figure 1, the number of cases in presenting each clinical disease form (pre-ulcer, ulcer, or osteomyelitis) appear to follow the same trend as the total number of cases. The odds of presenting an ulcerative form compared with a pre-ulcerative form

(nodule, plaque, or edema) were lower among female cases than among male cases (OR = 0.63, 95% CI 0.40-0.98, P = 0.042), and this association remained significant when controlling for age (OR = 0.63, 95% CI 0.40-0.99, P = 0.048) (Table 2). The odds of presenting an ulcerative lesion rather than a pre-ulcerative lesion among those residing in Zou were lower than among cases residing in Collines or Plateau (reference group) (OR = 0.12, 95% CI 0.05-0.29, P < 0.001). The odds of presenting osteomyelitis compared with a pre-ulcerative form were significantly greater among cases originating in Collines or Plateau than those living in Zou (OR = 0.15, 95% CI 0.03-0.69, P = 0.015) or Ouémé (OR = 0.19, 95% CI 0.04-0.94, P = 0.042). There were no other significant associations between region and clinical form among cases in Benin, adjusting for age and sex (Table 2). Among new cases in Cameroon, we did not find any significant differences between age, sex, or region of origin of a case and the clinical form presented (Table 3). The lack of association between age and clinical form is also demonstrated in Figure 3. The trends in the numbers of cases presenting each clinical form per age group closely followed the trend in the number of total cases diagnosed per age group.

Case Category at Diagnosis

Among cases in Benin we found a significant association between being younger than 15 years and the unadjusted odds of a case being classified as Category III compared with Category I (OR = 2.18, 95% CI 1.10-4.36, P = 0.027) (Table 2). This association became stronger when controlling for sex (OR = 2.44, 95% CI 1.20-4.94, P = 0.013). Figure 1 shows a greater difference in the number of Category III compared with Category I cases among younger age groups than among older age groups. The odds of being classified as case Category III compared with case Category I among females was

1.60 times greater than among males; however, this association was not significant (95% CI 0.82-3.12, *P* = 0.172). Adjusting for age, this association was stronger but not still not significant (OR = 1.86, 95% CI 0.94-3.70, *P* = 0.076). Figure 2 shows the proportion of Category III cases per region. Of those departments reporting cases, only Atlantique had less than 50% of patients diagnosed as Category III. Region was significantly association with the odds of presenting as Category I compared with Category III for cases originating in Atlantique, Littoral, or Mono and cases originating in Ouémé compared with Collines and Plateau, controlling for age and sex (OR = 14.27, 95% CI 1.73-117.72, *P* = 0.014 and OR = 10.54, 95% CI 1.33-83.59, *P* = 0.026, respectively). No significant associations were found between age, sex, or region and the odds of being classified as Category II compared with Category III in Benin (Table 2, Figure 1).

For reported cases in Cameroon, we did not observe an association between age and disease case Category classification, even when controlling for sex (Table 3). Figure 3 supports this finding. The distributions of cases per case Category in each age group appear to follow the same trend as the total number of cases in each age group. We also did not observe a difference between sex and case Category. Region, when grouped as Centre compared to all other regions, was also not significantly associated with case Category, adjusting for age and sex (Table 3). Figure 4 demonstrates that in Centre less than 50% of cases were diagnosed as Category III. In other endemic regions the percentages of Category III cases varied; some regions reporting no Category III cases.

Localization of BU lesion

Among the new cases of BU reported from Benin, cases younger than 15 years were significantly less likely to present with a lesion on a location other than the lower

limbs lower limbs compared with those 15 years and older (OR = 0.48, 95% CI 0.31-0.74, $P < 0.001$) (Table 2). This association remained significant and became slightly stronger when controlling for sex (OR = 0.46, 95% CI 0.30-0.72, $P < 0.001$). Figure 1 supports this finding as the differences in numbers of cases with lower limb lesions compared to other body locations is greater in the younger age groups than the older age groups. However, we did not find a significant association between sex and presenting a lesion on an area other than a lower limb. Cases reports from those residing in Atlantique, Littoral, Mono, Ouémé, Zou, or Nigeria did not show a significant association between region of origin and localization of lesion, controlling for age and sex (Table 2). Among new cases in Cameroon, we found no significant associations between age, sex, or region, and the localization of lesions presented (Table 3, Figure 3).

Discussion

This study analyzed data on all histologically confirmed BU cases in 2013 reported from the Buruli Ulcer national control programs of Benin and Cameroon to WHO to calculate descriptive statistics and measures for associations of interest. For cases in Benin, we found several statistically significant associations between sex, age at diagnosis, and region of origin and clinical characteristics of Buruli Ulcer disease, including clinical form, case Category, and localization of lesion(s). For cases in Cameroon we found no significant associations.

In Benin, female sex was associated with decreased odds of presenting an ulcer compared with a nodule, plaque, or edema. We found no association between sex and the odds of having osteomyelitis compared to a pre-ulcerative form, and we found no association between age and clinical form. An age of 15 years or older was associated

with increased odds of being diagnosed as Category III compared with Category I and with increased odds of having at least one lesion on the mid and upper body compared with a lower limb, and sex was not associated with case Category nor lesion location as found in other studies (14, 21). Originating from Zou compared with Collines or Plateau was associated with decreased odds of presenting an ulcer compared with a nodule, plaque, or edema. Originating from Zou or Ouémé compared with Collines or Plateau was also associated with decreased odds of presenting osteomyelitis compared with a nodule, plaque, or edema. Originating from Atlantique, Mono, Littoral, or Ouémé compared with Collines or Plateau was associated with increased odds of being diagnosed as Category I rather than Category III and may be an indicator of outreach efforts and community awareness in these areas.

This study validates many of the common demographic and clinical features of cases found in many other studies from Benin, Cameroon, and other countries in sub-Saharan Africa. The median age in Benin was similar to that reported previously, but the distribution of cases by age in either country differed from many earlier reports that identified young children, usually those under 15 year of age, at highest risk for infection (16-18, 56, 65, 74-78). These differences by age also depend on the cut-off age used to define two or more groups and could be a result of reports from community-based surveillance rather than active case finding for study enrollment (14). The distribution of cases by sex was almost balanced in each country as has been found in most studies (17-18, 20). In our study more cases originated from the Southern coastal areas of Benin than northern areas as has been found in previous reports, and Ouémé and Zou remain the regions with the highest endemicity (56, 31).

Ulcerative forms of the disease with no osteomyelitis were presented in the majority of all cases (63% in Benin and 90% in Cameroon), and the percentage of cases in Benin with osteomyelitis was lower than those found from 1997-2001 and 2003-2005 (16, 19, 38-40, 56). The small number of cases from Benin diagnosed when presenting a nodule (2%) and the large number of cases diagnosed when presenting a plaque (23%) are consistent with Category I cases being the least commonly reported (Table 1). This result and the finding that 28% of cases had limited movement indicate that most patients in Benin were diagnosed late in the disease progression but before resulting in osteomyelitis.

The percentage of ulcerative forms in Cameroon is inconsistent across different types of studies, but the high percentage of ulcers in our study is consistent with other cross-sectional studies (21, 69, 78). This may explain the high median age of the cases reported in Cameroon, but does not explain why more Category I cases (40%) were reported than categories II (26%) or III (34%). The percentage of Category I cases may validate hypotheses that BU is still newly emerging in some regions and may have influenced our measures of association, but it also indicates that Cameroon is achieving the WHO goal of detecting more Category I cases (79). As 19% of reports indicated a movement limitation, it appears that, in general, cases are being detected after an ulcer forms but before necrosis increases the lesion size to Category II or III and before movement becomes limited. Also, consistent with other studies, approximately 60% of the case patients from both Benin and Cameroon had lesions exclusively on their lower limbs indicating that infection likely occurred while performing routine outdoor activities (14, 17-19, 21, 38, 40-42, 56, 69, 78).

Disease detection appears to be happening at earlier stages of BU progression in Cameroon than Benin. Cameroon is likely detecting very small ulcers and providing treatment before the disease progresses enough to limit movement. Benin is likely detecting larger ulcers thus reducing the ability to prevent long-term complications. However, the percentages of patients with movement limitations in both countries still indicate that there is a need for increased training of patients, families, and healthcare workers to prevent chronic disability and other long-term physical, financial, and social consequences of advanced disease from *M. ulcerans* infection.

The regional associations that we found in Benin could be due to a greater awareness of disease presence among some communities and may be confounded by treatment center and method of patient identification (23, 29, 44, 73, 80). For cases from Cameroon, the lack of association with region indicates that overall outreach efforts by healthcare facilities, community education, case detection, and access to treatment centers is likely comparable across the regions reporting cases. It is possible that there are variations in case severity at diagnosis among villages that our study was unable to investigate, but due to relatively small case numbers in most regions it would be difficult to assess these variations with sufficient statistical power. The lack of association between age, sex, or region and case severity in Cameroon could be due to a small overall number of pre-ulcerative forms and the emergence of BU in some areas and historic endemicity in others (79).

There is no health center in Nigeria dedicated to diagnose and treat BU; patients residing close to the southwestern border rely on the health system of Benin (81). Our study included 42 incident cases from Nigeria among the Benin data. The percentages in

most demographic and clinical categories were very similar to those calculated from total cases detected in Benin except for 67% presenting movement limitations. As such, the inclusion of these cases likely did not greatly skew the overall results or measures of association from Benin. However, this study and previous reports do indicate that BU is likely emerging, if not already endemic, in a least some areas of Nigeria, especially given that it is bordered by BU endemic Benin and Cameroon. Therefore, there is need for a Nigerian BU surveillance system, training of health workers in regions where cases have been reported, and local treatment options in order to further investigate BU burden in this area and reduce morbidity and high direct and indirect costs in this unique group of patients (81).

The lack of apparent seasonality found in our analysis is also consistent with current knowledge. The incubation period for *M. ulcerans* has been found to be quite long and highly variable making it unlikely that an increased incidence of reported cases can be attributed to a particular time of year or weather pattern until a more conclusive mode of transmission and incubation period are identified (82). Alternatively, this variation in reporting could be due to other seasonal barriers such as flooding during the rainy season. The rainy months in Benin are from April to July and September to October with a long dry season from November to March. The rainy months in Cameroon are from March to June and August to October with a long dry season from November to February. However, the percentages of cases reported each month do not closely follow these patterns with Benin reports only slightly peaking in June and October and peaking in Cameroon in March, May, July, and November. Another possible explanation is that

among rural and poor populations substance farming and other agricultural activities keep patients from seeking treatment during harvest season.

Laboratory confirmation methods differed between the two countries in that Benin only reported PCR confirmation, and Cameroon reported testing by both PCR and ZN staining. Independent of diagnostic method used, specimens were collected 83% of reports from Benin and 88% from Cameroon indicating that laboratory confirmation was planned for a large proportion of cases at initial diagnosis. WHO recommends that at least 70% of all cases be confirmed by PCR. Our results (Table 1) show that Benin has reached a high proportion of tested cases with 79% reporting a PCR result but only 63% of all histologically diagnosed cases tested positive.

Cameroon had a lower proportion of cases that had a reported result for PCR lab testing (61%), and only 30% of all cases were reported as PCR positive for *M. ulcerans* infection (53). The true number of PCR confirmations may be slightly higher in both countries if some results that were reported as not available or missing were actually completed but not properly linked to the patient report when sent to the national control programs. The level of laboratory diagnosis through Ziehl-Neelsen staining remains high in Cameroon (89% tested and 46% positive) even though previous studies have shown this method to have low sensitivity (14, 52). Likely, this method is still widely used in Cameroon as many of the laboratories that test specimens from BU cases may not have the capacity to perform PCR to the levels recommended by WHO. Cheaper and faster diagnostic methods that are continuing to be developed could be tested and utilized in Cameroon to validate their effectiveness as well as improve levels of laboratory confirmation until PCR can be used more widely (55).

Antibiotic treatment was planned or provided for 99% of cases in Benin and 89% of cases in Cameroon. These proportions seem extremely high considering the lower levels of laboratory confirmation that were reported. However, due to the high proportion of ulcerative forms among reports from each country it is likely that antibiotics were deemed necessary to treat infection even if *M. ulcerans* infection was not positively confirmed. Surgical treatment was not reported but would be useful to investigate the effect of treatment decision making on the association between age, sex, region, and the outcomes of clinical form, case Category, and localization as well as movement limitations.

We found significant associations only among cases from Benin and not Cameroon, indicating that these findings are likely not immediately generalizable to other countries where BU is endemic. However, as the cases were identified through a passive surveillance program rather than by study recruitment these findings are likely internally valid for the population in southern Benin where BU is most commonly found (31, 56). Active case finding in case-control and cohort studies may result in case detection at earlier stages in disease progression than passive surveillance that is often used in cross-sectional studies such as ours only detecting cases when they seek treatment.

Most studies have presented these demographic and clinical characteristics as descriptive factors and have not investigated associations between them. Only a few studies have investigated similar associations to ours (14, 19, 21, 65). None have used national data nor have used the same methodology in more than one country.

There are several strengths to our study. The level of completeness of the data used in our analyses was very high. Overall, only six cases (1%) were excluded from

analysis due to having more than two key variables missing. Moreover, our data were obtained from the national surveillance reports of each country and are thus thought to be representative of the known burden of Buruli Ulcer disease in those countries. As our study includes surveillance reports from two countries we can draw comparisons between case characteristics and differences in clinical presentation in two different groups for the same time period.

Our study also had some weaknesses. The small number of cases in several strata used in multivariate analysis produced very wide confidence intervals and greatly reduced the precision of our estimates. We were also limited by the accuracy of case report form completion. Although the community health workers, nurses, and doctors typically received training in how to complete the forms, there is no way to check for accuracy. We also found apparent variations in how forms were completed in each country. While the case reports from Benin often included more than one clinical form and localization of lesions as indicated in the instructions for the BU02 form, the case reports from Cameroon were primarily limited to presenting only one lesion form and one lesion location for each case (Table 1). The small number of cases originating in several of region also limited the statistical power to make more meaningful comparisons by region, especially in Cameroon. This may be the true nature of the cases, but it likely reduced the accuracy of our estimations as well as our ability to make more meaningful comparisons between the countries.

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TABLES

Table 1. Demographic and clinical characteristics of new histologically diagnosed Buruli Ulcer cases reported to Buruli Ulcer national control programs, Benin and Cameroon, 2013.

	Benin (N=373)		Cameroon (N=122)	
Variable	**n (%)**	**Median (range)**	**n (%)**	**Median (range)**
Age				
Continuous		15 (0-90)		22 (1-84)
< 15 years	181 (48.5)		45 (36.9)	
≥ 15 years	192 (51.5)		77 (63.1)	
Male sex	195 (52.3)		63 (51.6)	
Clinical form				
Nodule	9 (2.43)		1 (0.82)	
Plaque	84 (22.7)		5 (4.10)	
Edema	7 (1.89)		6 (4.92)	
Ulcer	156 (42.7)		110 (90.2)	
Osteomyelitis	11 (2.97)			
Mixed without ulcer	15 (4.05)			
Mixed with ulcer	78 (21.1)			
Mixed with osteomyelitis	10 (2.70)			
Scar	3 (0.80)			
Category				
I	42 (11.3)		49 (40.2)	
II	134 (35.9)		32 (26.2)	
III	197 (52.8)		41 (33.6)	
Localization				
Lower limb	238 (63.8)		76 (62.3)	
Upper limb	91 (24.4)		31 (25.4)	
Trunk	22 (5.90)		2 (1.64)	
Buttocks & perineum	7 (1.88)		5 (4.10)	
Head & neck	3 (0.80)		7 (5.74)	
Upper & lower limb	6 (1.61)		1 (0.82)	
Lower limb & trunk	4 (1.07)			
Upper limb & trunk	2 (0.54)			
Limitation of movement	105 (28.2)		23 (18.9)	
Specimen collected	307 (82.5)		107 (87.8)	
PCR confirmation[a]	235 (63.0)		35 (29.9)	
ZN confirmation[b]			54 (46.2)	
Received treatment	368 (98.7)		108 (88.5)	

[a] positive case from laboratory testing through polymerase chain reaction
[b] positive case from laboratory testing through Ziehl-Neelsen swab smear staining

Table 2. Association between age, sex, and region and clinical form, case category, and localization of lesions of histologically diagnosed Buruli Ulcer cases reported to the national BU Control Program, Benin, 2013 (N=373).

	Clinical Form (n=370)				Case Category (n=373)				Localization of Lesion (n=373)	
	Ulcerative compared with pre-ulcerative		Osteomyelitis compared with pre-ulcerative		Category I compared with category III		Category II compared with category III		Mid and upper body compared with lower limbs	
	OR[a] (95% CI)	p	OR[a] (95% CI)	p	OR[a] (95% CI)	p	OR[a] (95% CI)	p	OR[a] (95% CI)	p
Crude associations										
Age (<15 years)	1.13 (0.72-1.77)	0.591	1.90 (0.73-4.94)	0.187	2.18 (1.10-4.36)	0.027	1.14 (0.74-1.78)	0.551	0.48 (0.31-0.74)	<0.001
Sex (female)	0.63 (0.40-0.98)	0.042	0.49 (0.19-1.27)	0.143	1.60 (0.82-3.12)	0.172	1.44 (0.93-2.24)	0.104	0.89 (0.58-1.35)	0.578
Adjusted associations [b]										
Age (<15 years)	1.05 (0.67-1.66)	0.824	1.73 (0.66-4.54)	0.268	2.44 (1.20-4.94)	0.013	1.23 (0.78-1.92)	0.371	0.46 (0.30-0.72)	<0.001
Sex (female)	0.63 (0.40-0.99)	0.048	0.53 (0.20-1.40)	0.202	1.86 (0.94-3.70)	0.076	1.50 (0.95-2.34)	0.079	0.78 (0.50-1.20)	0.255
Fully adjusted associations [c]										
Collines & Plateau	reference	-	reference	-	reference	-	reference	-	reference	-
Zou	0.12 (0.05-0.29)	<0.001	0.15 (0.03-0.69)	0.015	1.36 (0.12-15.84)	0.804	1.10 (0.51-2.41)	0.803	2.02 (0.92-4.41)	0.709
Couffo	1.12 (0.39-3.23)	0.828	0.39 (0.06-2.59)	0.329	4.32 (0.45-41.67)	0.205	1.18 (0.49-2.85)	0.718	1.24 (0.53-2.89)	0.626
Atlantique, Littoral, & Mono	1.60 (0.58-4.41)	0.364	0.35 (0.05-2.27)	0.269	14.27 (1.73-117.72)	0.014	2.10 (0.93-4.76)	0.074	1.56 (0.71-3.38)	0.264
Ouémé	0.69 (0.29-1.63)	0.394	0.19 (0.04-0.94)	0.042	10.54 (1.33-83.59)	0.026	1.16 (0.55-2.48)	0.695	1.76 (0.85-3.63)	0.128
Nigeria	0.89 (0.31-2.57)	0.826	0.93 (0.20-4.41)	0.924	4.83 (0.50-46.48)	0.173	1.08 (0.44-2.66)	0.873	1.45 (0.61-3.44)	0.403
Age (<15 years)	0.76 (0.46-1.27)	0.301	1.47 (0.54-4.01)	0.457	2.25 (1.08-4.69)	0.030	1.19 (0.75-1.88)	0.459	0.47 (0.30-0.74)	0.001
Sex (female)	0.57 (0.34-0.93)	0.025	0.53 (0.20-1.44)	0.214	1.91 (0.94-3.90)	0.074	1.50 (0.85-2.35)	0.080	0.77 (0.50-1.20)	2.515

[a] Odds Ratio and 95% Confidence Interval; [b] Age adjusted for sex, sex adjusted for age; [c] Region, age, and sex each adjusted for the others

Table 3. Association between age, sex, and region and clinical form, case category. and localization of lesions of histologically diagnosed Buruli Ulcer cases reported to the national BU Control Program, Cameroon, 2013 (N=122).

	Clinical Form		Case Category				Localization of Lesion	
	Ulcerative compared with pre-ulcerative		Category I compared with category III		Category II compared with category III		Mid and upper body compared with lower limbs	
	OR[a] (95% CI)	p	OR[a] (95% CI)	p	OR[a] (95% CI)	p	OR[a] (95% CI)	p
Crude associations								
Age (<15 years)	1.25 (0.37-4.20)	0.718	1.03 (0.43-2.45)	0.957	1.50 (0.58-3.88)	0.403	0.74 (0.35-1.57)	0.432
Sex (female)	1.08 (0.33-3.54)	0.905	1.50 (0.65-3.48)	0.345	2.28 (0.89-5.87)	0.087	0.68 (0.33-1.42)	0.304
Adjusted associations[b]								
Age (<15 years)	1.24 (0.36-4.26)	0.730	0.95 (0.39-2.32)	0.915	1.31 (0.49-3.47)	0.589	0.79 (0.37-1.70)	0.539
Sex (female)	1.04 (0.31-3.48)	0.955	1.51 (0.64-3.55)	0.343	2.18 (0.84-5.69)	0.111	0.71 (0.36-1.50)	0.366
Fully adjusted associations[c]								
Centre region	reference	-	reference	-	reference	-	reference	-
All other regions	1.07 (0.31-3.64)	0.916	0.44 (0.18-1.04)	0.060	0.44 (0.17-1.18)	0.104	0.52 (0.24-1.12)	0.095
Age (< 15 years)	1.26 (0.36-4.38)	0.721	0.83 (0.33-2.08)	0.694	1.14 (0.42-3.11)	0.794	0.70 (0.32-1.54)	0.373
Sex (female)	1.03 (0.31-3.47)	0.959	1.62 (0.68-3.86)	0.278	2.33 (0.88-6.17)	0.089	0.73 (0.34-1.55)	0.411

[a] Odds Ratio and 95% Confidence Interval; [b] Age adjusted for sex, sex adjusted for age; [c] Region, age, and sex each adjusted for the others

FIGURES

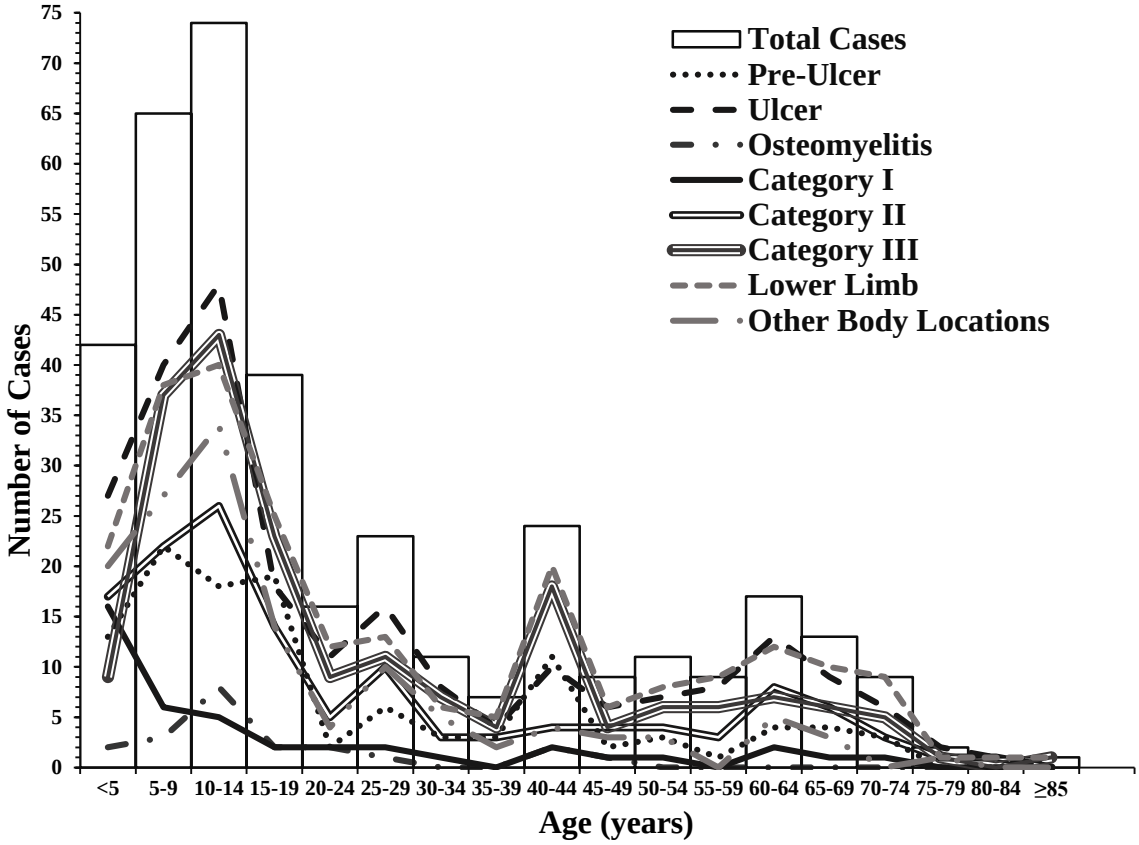

Figure 1. Distribution of total new histologically diagnosed Buruli Ulcer disease cases and case numbers in each level of clinical characteristics by age at diagnosis and reported to the Buruli Ulcer national control program, Benin, 2013.

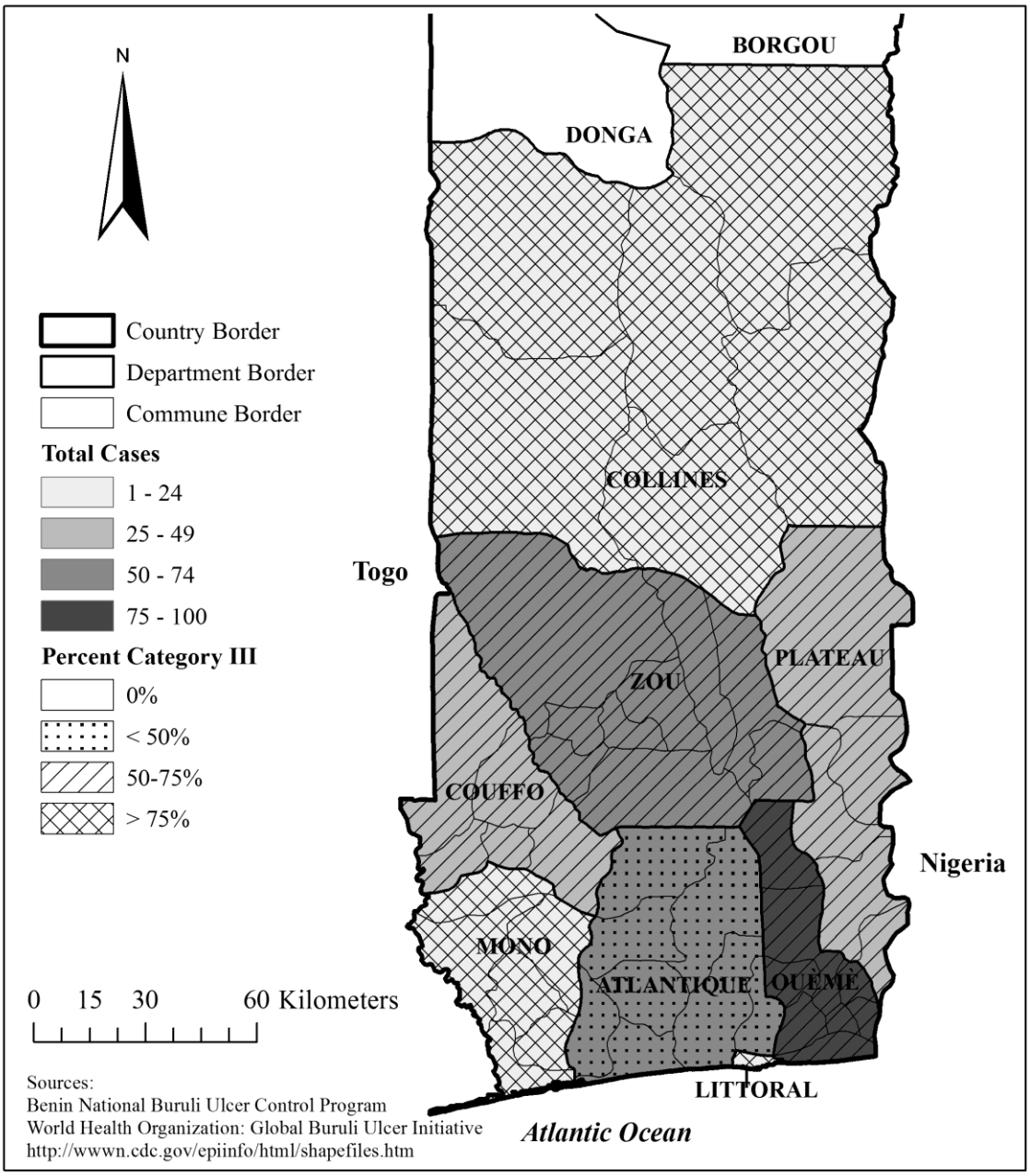

Figure 2. Distribution of total new histologically diagnosed Buruli Ulcer (BU) disease cases and percent of Category III cases at diagnosis by department of case origin reported to the BU national control program, Benin, 2013. This map was created using ESRI ArcMap, version 10.2.2.3553, Redlands, CA. Shading represents level of total case numbers per department reported as case origin. Gradient represents level of percentage of cases diagnosed as Category III per department reported as case origin.

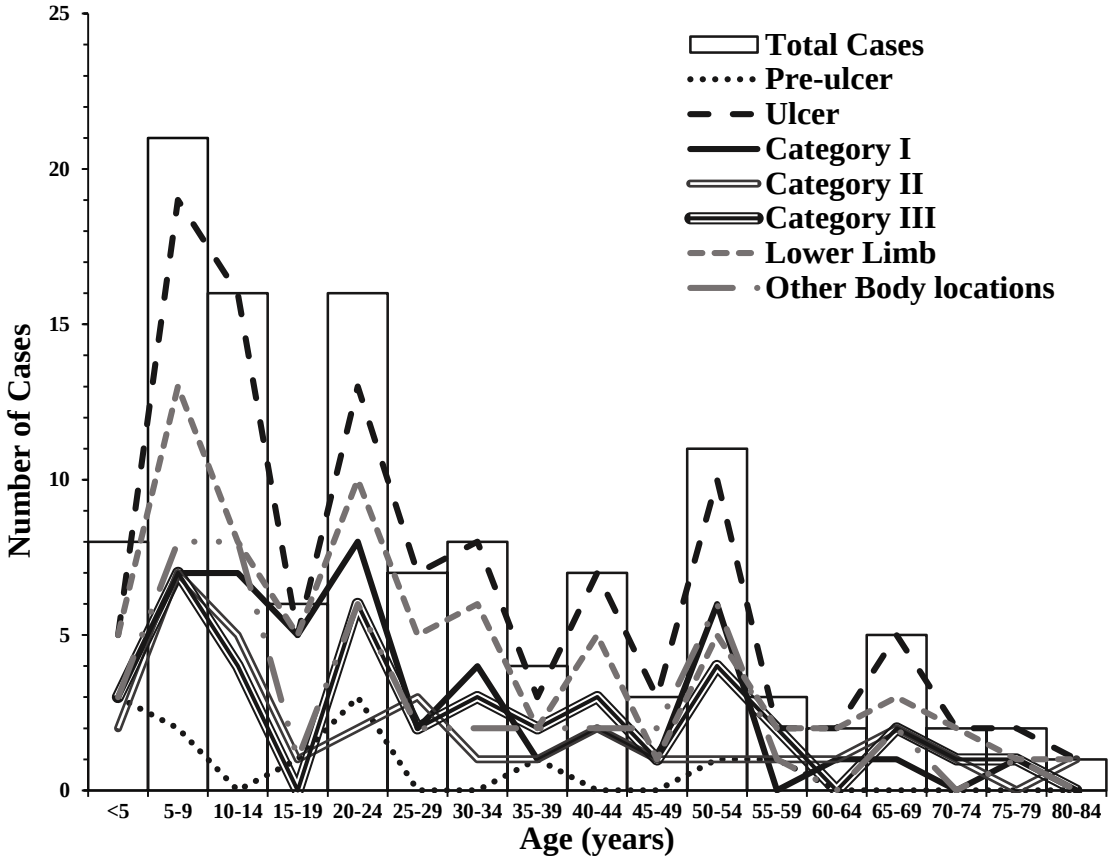

Figure 3. Distribution of total new histologically diagnosed Buruli Ulcer disease cases and case numbers in each level of clinical characteristics by age at diagnosis and reported to the Buruli Ulcer national control program, Cameroon, 2013.

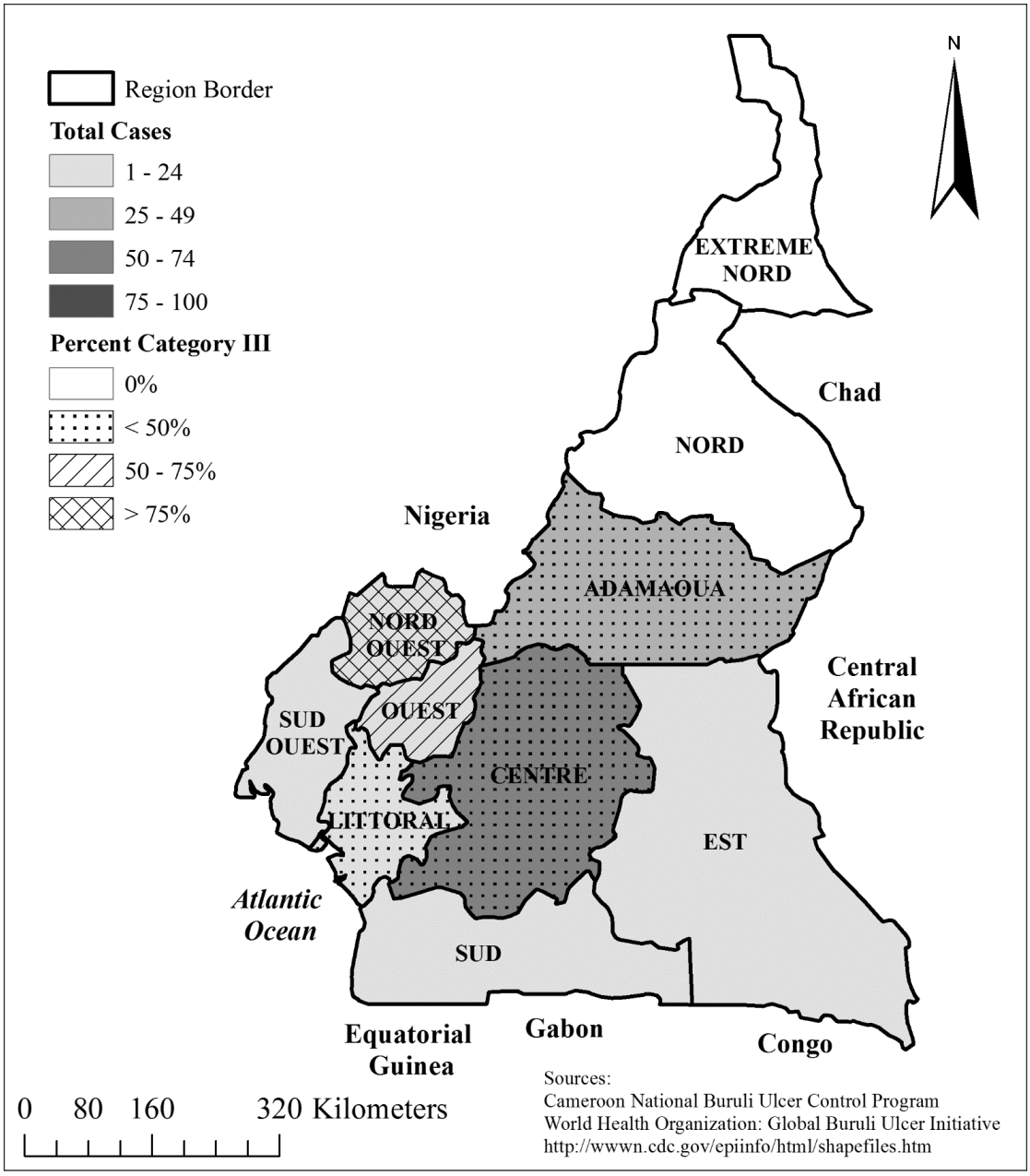

Figure 4. Distribution of total new histologically diagnosed Buruli Ulcer (BU) disease cases and percent of Category III cases at diagnosis by department of case origin reported to the BU national control program, Cameroon, 2013. This map was created using ESRI ArcMap, version 10.2.2.3553, Redlands, CA. Shading represents level of total case numbers per region reported as case origin. Gradient represents level of percentage of cases diagnosed as Category III per region reported as case origin.

CHAPTER 3

Summary

In 2013, the national surveillance system in Benin reported 378 new cases of Buruli Ulcer disease to the World Health Organization, and the national surveillance system in Cameroon reported 123 new cases. While the overall distribution of cases by sex was nearly balanced in each country, in Benin, we found a significant association between sex and clinical form at diagnosis. Males of all ages are significantly more likely to present an ulcerative form of Buruli Ulcer disease at diagnosis than females. Females have a greater odds of presenting a nodule, plaque or edema than rather than an ulcer compared with males. We also found a significant association between age and case Category at diagnosis. Both male and female cases 15 years of age or older were also more likely to be classified as Category III indicating a lesion of more than 15 cm in diameter, multiple lesions, or one or more lesions at a critical site and were more likely to have a lesion on a site other than the lower limbs. While we did find several significant associations between case severity and region of case origin, the majority of comparisons did not show significant differences in odds for any of the outcomes. Originating in Atlantique, Mono, Littoral, or Ouémé was significantly associated with increased odds of having a Category III lesion rather than a Category I lesion, compared with cases originating in Collines or Plateau. Originating in Zou was significantly associated with increased odds of presenting a nodule, plaque or edema rather than an ulcerative form compared with cases originating in Collines or Plateau. For cases in Cameroon, no significant associations were found between age, sex, or region and clinical form, case Category, or localization of lesion.

Public Health Implications

Limited published research exists that examines and analyses the characteristics of incident cases detected per year through county level surveillance systems. Moreover, few studies have compared case characteristics of more than one region or country. None have compared associations between demographic characteristics and clinical outcomes in more than one country. Despite having population sample sizes smaller than most previous studies that grouped several years of data into one dataset, our findings were relatively consistent with current epidemiologic and clinical knowledge of Buruli Ulcer disease and add to the body of evidence supporting current research priorities.

As patients who present osteomyelitis are at higher risk for a longer surgery or lifetime complications it is important to note that in Benin the incidence of cases presenting osteomyelitis is decreasing from previous reports. However, the proportion of cases diagnosed as Category III and ulcerative remains higher than other categories and forms. Our findings from Cameroon are also consistent with previous studies noting a high percentage of ulcerative cases and no reports of osteomyelitis. A lack of cases of reported as presenting osteomyelitis and a low level of any presentation meeting Category III requirements could indicate fewer cases requiring more expensive treatment procedures in Cameroon compared with Benin.

Moreover, cases from Cameroon included in this report do not follow the same distributions in age and sex as cases from many other studied countries and regions indicating that risk factors and case detection should continue to be uniquely assessed in each country and compared to countries in the same geographic area. As such

surveillance and case detection efforts in all countries should be more critically evaluated to ensure that proper comparisons can be made.

The information obtained through data analysis in this study can be used in the improved detection of early stage infections by assisting in identifying areas and groups that are found to have higher rates of late stage infection. Increased awareness of which groups in each country have the greatest odds of being diagnosed and reported at a more advance BU disease stage is the first step to gaining local support, providing educational awareness opportunities, and motivating the promotion early detection and treatment. This analysis synthesized with results from similar recent country and region analyses will guide the fortification of more thorough and reliable public health surveillance programs for BU. Moreover, our investigation increases the understanding of the current BU burden and encourages implementation of improved intervention activities to control the resulting physiological, psychological, and economic impact of the disease, especially in Benin, Cameroon, and Nigeria.

Possible Future Directions

The methods used in this study can be replicated annually with national datasets from various countries to assist in overall monitoring and estimation of worldwide Buruli ulcer burden. While the same BU reporting form is used by most countries with an established surveillance system, it is important that the instructions that accompany the forms be followed closely to allow for more accurate estimates and comparisons among regions and countries. As local reporting becomes more accurate, we can better estimate disease burden to understand disease transmission mechanisms, target educational efforts and case finding, and ultimately provide early and effective treatments.

Since the quality of surveillance systems can vary greatly by region due to the above mentioned local factors, it is beneficial to continue to use available surveillance data to determine regions and groups that should be prioritized for increased active case detection. After meaningful analysis, these national surveillance summaries should be shared at all levels of the government and with all BU treatment and detection centers and affected communities. The summaries should also continue to be provided to WHO each year in a timely matter and should be shared with all other countries in the region known or suspected to be BU endemic. Most importantly, it is important to continue country-wide community-level education and improved ability for sensitive and lower cost laboratory diagnosis capabilities in order to decrease overall new case incidence and proportions of late stage and advanced forms of Buruli Ulcer. Until transmission is more clearly defined, consistent risk factors are identified and reduced, effective prevention methods are widely implemented, and treatment costs are reduced, the best strategies for reducing Buruli ulcer morbidity are still early detection and prompt treatment.