Buruli ulcer (BU) is a severe infection caused by Mycobacterium ulcerans. Most affected are rural populations living in tropical areas with abundant wetlands (1). BU causes extensive, damaging skin lesions and often results in severe disabilities. Of the 4,000 cases reported to the World Health Organization by 14 countries in 2011, >95% originated in African countries around the Gulf of Guinea (2).

Much remains unknown about the mode of M. ulcerans transmission and the epidemiology of BU (1). Specifically, although the spatial distribution of BU in several settings has been addressed (3,4), most studies have examined only temporal variations of BU incidence in terms of yearly trends (5). Several observational studies have reported seasonal changes in the monthly number of cases and have hypothesized that cases are linked with rainfall variation (6–8). One spatiotemporal study in Australia showed that BU incidence was associated with rainfall variability with a 5-month lag and with total rainfall with a 19-month lag (4). However, none of these studies provided quantitative evidence of seasonal changes in BU incidence and their relationship with seasonal environmental changes. Indeed, a formal demonstration of such evidence requires a sufficiently long time series, large numbers of cases from a defined source population, and use of signal analysis techniques adapted to the constraints of BU disease surveillance and environmental data (9) (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-1336-Techapp1.pdf). Therefore, we investigated the seasonality of BU case incidence during 2002–2012 in Akonolinga District, located in the highly BU-endemic region of the Nyong River valley in Centre Region, Cameroon.

**The Study**

Relying on previous spatial analysis of BU incidence in Akonolinga District, we analyzed a series of cases that occurred in the highest BU-risk area of the district, located along the Nyong River upstream of Akonolinga (3). This area includes 24,469 inhabitants of the town of Akonolinga and 24 surrounding villages. We analyzed 562 new cases of BU that originated in this area from January 2002 through May 2012, after aggregation by month of diagnosis. Biological confirmation was obtained from the National Reference Centre for Mycobacteria for 354 (63%) cases. The BU incidence rate remained stable over the 10-year period at 2.2 cases/1,000 person-years.

Median BU incidence peaked in March, and a second peak occurred in September (online Technical Appendix Figure 2), but monthly medians did not differ significantly (Kruskal-Wallis test, p = 0.149). Given the specificities (nonstationarity) of the BU case series, wavelet analysis was the appropriate method for analysis (online Technical Appendix). A 1-year periodic signal was identified in the BU-case time series from 2005 to 2011, and this periodicity was statistically significant from mid-2005 to the beginning of 2009 (Figure 1).

Next, we analyzed the links between BU and seasonal changes by using wavelet association and phase analyses between BU case incidence and total monthly rainfall (in mm) or mean Nyong River flow (in cubic meters per second). Strong seasonality was found in the series of monthly total rainfall and of monthly mean Nyong River flow; a 1-year period and a weaker 6-month period corresponded to the 2 rainy seasons separated by a period of lesser rainfall (the small dry season, mid-July to mid-August) (Figure 1; online Technical Appendix Figure 3). Because of
its shape, the wavelet detected yearly rainfall oscillations between a minimum in December (dry season) and a maximum in July (the middle of the rainy period) instead of the maximal rainfall months of October and November (online Technical Appendix Figure 3).

We assessed the association of the incident case signal with environmental variables (online Technical Appendix). The 1-year periodic signal of the BU case series was associated with Nyong River flow from the end of 2005 to the end of 2009 (Figure 1) and with rainfall from the end of 2005 to the beginning of 2011 (online Technical Appendix Figure 3). Under the assumption that changes in the environment preceded changes in BU incidence, phase analysis indicated that cases lagged 6 months behind Nyong River flow oscillations (Figure 1). When the 2 signals were associated, a 9-month lag behind rainfall oscillations was observed (online Technical Appendix Figure 3).

Conclusions
In the BU-endemic focus of Akonolinga, Cameroon, significant 1-year seasonal variations in BU incidence occur. The incubation period for BU has been estimated to be \( \approx 4.5 \) months when data from Australia are used (10) and \( \approx 3 \) months when data from Uganda are used (7). The median delay between symptom onset and health care seeking was reported to be 5 weeks in Akonolinga (interquartile range 3–12 weeks), yielding a delay between infection and diagnosis of 5–6 months. Given this delay and a finding of BU diagnosis peaks during March–April, the number of infections would therefore be highest from August through October (Figure 2). Such a pattern was observed in the 1970s in Uganda (6,7) and Cameroon (11) and more recently in Côte d’Ivoire (5). In low BU-endemicity French Guiana, an overseas territory located near Brazil at the same latitude as Cameroon, periodic peaks after the 2 rainy seasons have been reported (12).
Variations in BU incidence result from variations in population exposure (13) combined with variations in environmental presence of *M. ulcerans* (14). We hypothesize that one of the main drivers of these variations is the seasonal flooding of the Nyong River, which rises 3–5 m from April through November, creating temporary bodies of water and swamps on a vast surface, deeply affecting the ecosystem.

Although *M. ulcerans* was identified year-round in specific environments such as permanent swamps, its presence and abundance were maximal during the rainy months, July–October (14) (Figure 2). Prevalence of *M. ulcerans* in rivers was high at the beginning of the rainy season and was high in flooded areas during the following small dry season and high rainy season (14).

Human activity patterns follow these seasonal changes, resulting in seasonal variations in exposure to *M. ulcerans*. In Uganda, the contribution of permanent swamps to BU risk and the increased risk associated with temporary swamps during the rainy season have been documented (8). According to residence, age, and/or sex, the inhabitants of the Akonolinga district face varying exposures to aquatic environments; during the period identified as high risk, populations frequent seasonally flooded environments for water collection, fishing, and harvest of dry season cultures (Figure 2) (15).

During the study period, the intensity of the association between BU incidence and rainfall or Nyong River flow varied. The seasonal signal was detected over 5 consecutive years and was strongest when yearly variations in the Nyong River flow were lower (2005, 2006, 2008), which could indicate transient forcing of BU incidence by seasonal phenomena. Assessment of the effects of lower frequency climatic events, such as El Niño Southern Oscillation, is needed. In French Guiana, where BU endemicity is low, such events were shown to affect BU incidence dynamics (12).

We showed that BU incidence in this region varies significantly by season and linked these variations to the fluctuations of *M. ulcerans* occurrence in the environment, which are probably driven by the dynamics of freshwater ecosystems of the Nyong River. In Akonolinga, during the...
high rainy season when risk for *M. ulcerans* transmission seems to be highest, populations should increase their protective behaviors, and case detection efforts should be intensified in subsequent months to ensure early diagnosis and access to care.

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**References**


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**Investigating Listeria Outbreaks**

**Dr. Emily Cartwright,** Infectious Disease fellow at Emory University and former EIS Officer with CDC’s Division of Foodborne, Waterborne, and Environmental Diseases discusses foodborne *Listeria* outbreaks.

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Technical Appendix

Supplemental Material and Methods

Cases

This work was a registry-based study of the monthly number of Buruli ulcer cases who received a clinical diagnosis and treatment in Akonolinga Hospital, Centre Cameroon, from January 2002 to May 2012, as previously reported (1). A BU case was defined as a patient receiving a BU clinical diagnosis from a trained medical personnel in Akonolinga Hospital, which proved a reliable method in endemic areas (2). A confirmed case was defined as a patient for which laboratory identification of MU was obtained. Biologic confirmation of M. ulcerans infection was provided by the National Reference Centre for Mycobacteria in the Centre Pasteur du Cameroun in Yaoundé according to WHO guidelines (3). Detection of M. ulcerans (MU) was performed by microscopy, culture and PCR as previously described (4). PCR targeting MU-IS2404 has been available at the Centre Pasteur du Cameroun since 2001, and quantitative PCR was set up as early as 2009.

We restricted our analysis of BU incidence to villages located in the high BU-risk area previously identified (1). This high risk area was located along the Nyong River upstream and downstream from Akonolinga town (Technical Appendix Figure 1).

All incident new BU cases were included and aggregated by month of diagnosis at Akonolinga Hospital.

Environmental data

Because of a suspected link with water, water-related environmental variables included in the analysis. We used monthly total rainfall obtained from validated remote-sensing data (MARS project) which combines satellite data and rainfall measurements (5). We extracted the data from the 25x25km cell that overlapped with our study area. We also used the monthly average of Nyong river flow measured in the hydrologic station of
Mbalmayo, a city located ≈50 km downstream from Akonolinga area. This data was only available from January 2002 to December 2010 (6).

Ethics Statement

This study used anonymised case data, aggregated by village and by month, which were collected by the Service de Mycobactériologie of the Centre Pasteur du Cameroun as part of the surveillance activity of the National Reference Laboratory for BU in Cameroon, within the National BU Control Program. In this study, no intervention was performed (either diagnostic or therapeutic) and we only relied on a retrospective collection of anonymous cases authorized by the Cameroonian Ministry of Health.

Statistical Methods

Wavelet analysis was performed on the time-series of monthly BU cases to determine the significant oscillating modes. This method has been validated in epidemiology and was employed because epidemiologic and environmental time-series are typically nonstationary, i.e., their dominant periodic components change over time (7–10). These characteristics may render traditional spectral techniques, such as Fourier analysis, inappropriate to analyze the temporal trend of local variations in frequency and periodicity (7). We selected the Morlet wavelet for the wavelet decomposition in the periodic band between 0.3 (4 months) and 2 years over the 125 months duration (January 2002 to May 2012). Detection of periodic signals is performed within a confidence cone, which excludes the beginning and the end of the series where edge effects would be too likely (7). Statistical significance of the signal was computed on 1,000 simple bootstrapped series and the $\alpha = 5\%$ significance level zones are circled with black dashed lines (8). The right panel of the wavelet spectrum corresponds to the global wavelet spectrum (black line) with its significant threshold value of 5% represented by the black dashed line (8).

Wavelet coherency was then used to identify and quantify possible statistical associations between two time series, e.g., the BU case time series and one of the environmental variables. Coherency is roughly similar to a classical correlation, but pertains to oscillating components in a given frequency mode for a given time period (7). Statistical significance of coherency was computed on 1,000 simple bootstrapped series and the $\alpha = 10\%$ and $\alpha = 5\%$ significance level zones are circled with black dashed lines. We also analyzed the phases in the time-series which enabled us to obtain information about the possible delay between incidence and environmental variables (i.e., in phase or out of phase relations). Phase analysis was complemented with the computation of the evolution of periodic components in
the 1-year mode. The phase difference between the two oscillating components (ΔT) was represented by the black dotted line. The right panel of the phase analysis corresponds to the phase difference histogram over the whole time period (black line). The phase difference can be converted into months (by multiplying by 12/2π): when investigating oscillations with a 12-month period, a phase difference of π corresponds to a 6-month time delay. Statistical analyses were performed using Matlab (version R2013a, The MathWorks, Naticks, Massachusetts, United States).

Supplementary Results

**Technical Appendix Figure 1.** Presentation of the study area displaying the location of Akonolinga Hospital, of the Health Centers and of the main roads. The boundaries of each village are displayed in dark gray.
Technical Appendix Figure 2. Boxplot of monthly BU incidence data over the study period, and mean monthly rainfall and Nyong River flow.
Technical Appendix Figure 3. Wavelet analysis of BU incidence and rainfall.

Panel 1: analysis for the time series of BU cases,

Panel 2: Nyong River flow

Panel 3: coherency between the two signals,

Panel 4: phase analysis for the 1-year period, BU cases are represented in blue and nyong flow variables in red.
Technical Appendix Figure 4. Original time-series included in the analysis. From top to bottom: rainfall, Nyong River flow, and BU-cases time monthly series.

References


