Seasonal Patterns of Buruli Ulcer Incidence, Central Africa, 2002–2012

Jordi Landier, Guillaume Constantin de Magny, Andres Garchitorena, Jean-François Guégan, Jean Gaudart, Laurent Marsollier, Philippe Le Gall, Tamara Giles-Vernick, Sara Eyangoh, Arnaud Fontanet, Gaëtan Texier

To determine when risk for Buruli ulcer is highest, we examined seasonal patterns in a highly disease-endemic area of Cameroon during 2002–2012. Cases peaked in March, suggesting that risk is highest during the high rainy season. During and after this season, populations should increase protective behaviors, and case detection efforts should be intensified.

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.

Author affiliations: Institut Pasteur, Paris, France (J. Landier, T. Giles-Vernick, A. Fontanet); Centre Pasteur du Cameroun, Réseau International des Instituts Pasteur, Yaoundé, Cameroon (J. Landier, S. Eyangoh, G. Texier); UMR MIVEGEC, Montpellier, France (G. Constantin de Magny, A. Garchitorena, J.-F. Guégan); Ecole des Hautes Etudes en Santé Publique, Rennes, France (A. Garchitorena, J.-F. Guégan); UMR912-SESSTIM, Marseille, France (J. Gaudart, G. Texier); INSERM and CHU et Université d'Angers, Angers, France (L. Marsollier); Institut de Recherche pour le Développement, Gif sur Yvette, France (P. Le Gall); Université Paris-Sud 11, Orsay, France. (P. Le Gall); Chaire Santé et Développement, Conservatoire National des Arts et Métiers, Paris (A. Fontanet)

DOI: http://dx.doi.org/10.3201/eid2108.141336

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

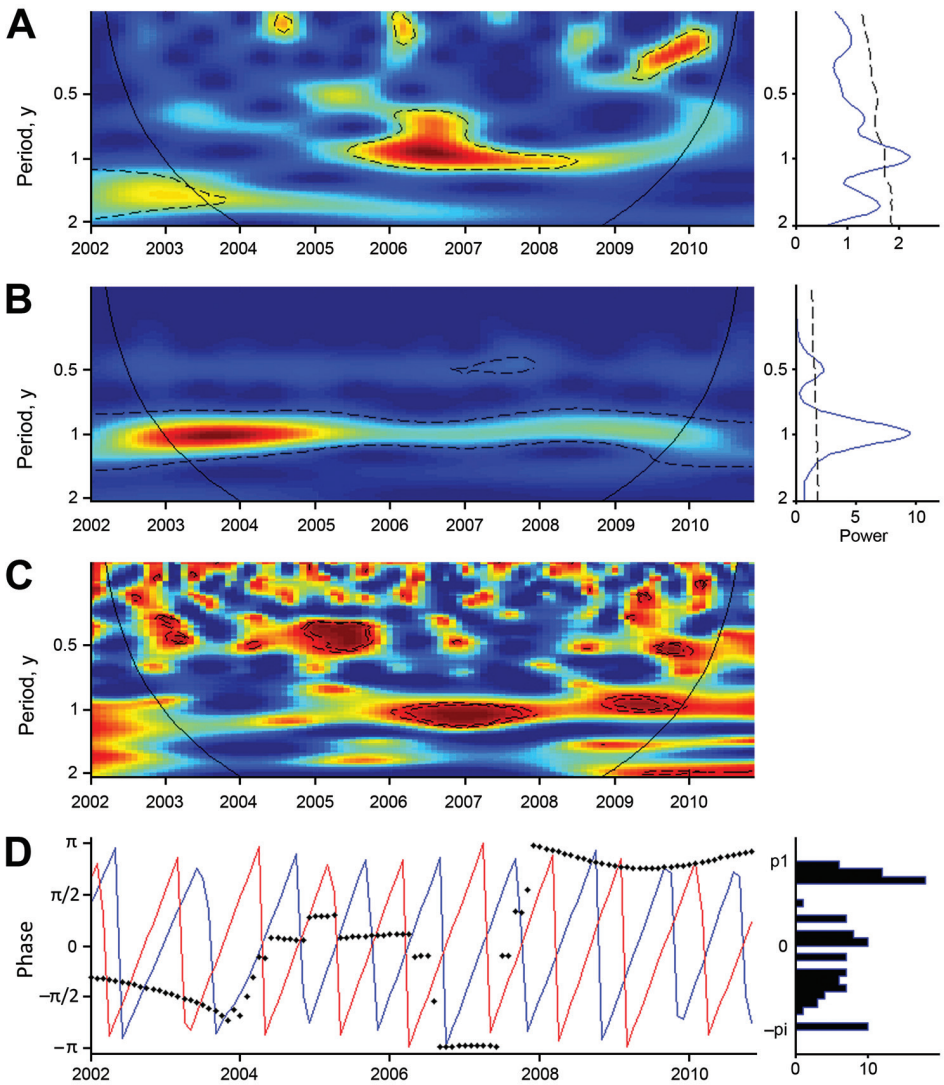


Figure 1. Wavelet analysis of Buruli ulcer (BU) case series and Nyong River flow, January 2002–December 2010. A, B) The color gradient indicates how well the wavelet of a given period adjusted with the series (power). The detection of periodic signals was performed within a confidence cone, which excluded the beginning and the end of the series where edge effects would be too likely (black solid line). Statistically significant zones are circled with dashed lines, indicating detection of significant periodic signals during the corresponding years. A) Wavelet power spectrum for the time series of BU cases: a seasonal signal with a 1-year period was detected from 2005 to 2011 (green to black), and this period was statistically significant from mid-2005 to the beginning of 2009 (dashed contour lines). B) Wavelet power spectrum for the Nyong River flow: the Nyong River flow series exhibits a statistically significant 1-year periodic signal during the whole period. C) Wavelet association between BU incidence and the Nyong River flow signal. The color gradient translates the association between the 2 signals. The dashed lines indicate statistically significant association, and the black line the confidence cone. D) Phase analysis for the 1-year period (expressed in multiples of π); BU cases are represented in blue and Nyong River flow variables in red.

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 ≈4.5 months when data from Australia are used (*10*) and ≈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*).

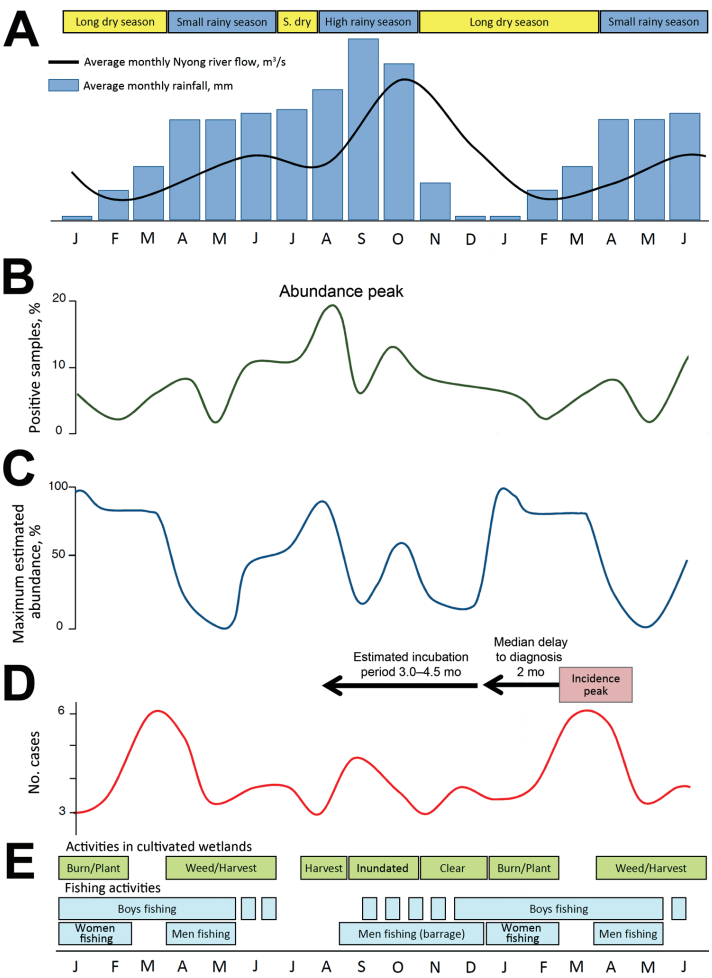


Figure 2. Schematic representation of the seasonal changes and possible links between the environment, *Mycobacterium ulcerans* presence, human exposure, and Buruli ulcer (BU) incidence in the Akonolinga district and the Nyong River valley, Cameroon, 2002–2012. For better visualization of delays, 18 months are shown. A) Average monthly rainfall and mean Nyong River flow (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-1336-Techapp1.pdf). S dry, short dry season. B) *M. ulcerans* prevalence in the aquatic environment (percentage of *M. ulcerans*–positive samples) (*14*). C) Estimated abundance of *M. ulcerans*–positive hemipterans (expressed as % of maximum abundance) (*14*). D) Monthly median number of BU cases detected in the Akonolinga district, 2002–2012 (this study). E) Selected activities involving contacts with environments in which risk for BU is high (T. Giles-Vernick, pers. comm., 2015).

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.

Acknowledgments

We thank the staff of the Akonolinga district hospital, who provided diagnoses and delivered treatment to BU patients, and the staff of the Service de Mycobactériologie at Centre Pasteur du Cameroun, who provided laboratory confirmation of diagnoses.

Dr. Landier specializes in infectious diseases epidemiology. While working on his PhD degree at the Institut Pasteur in Paris, he focused on BU epidemiology and *M. ulcerans* transmission in Central Africa.

References

1. Merritt RW, Walker ED, Small PLC, Wallace JR, Johnson PDR, Benbow ME, et al. Ecology and transmission of Buruli ulcer disease: a systematic review. PLoS Negl Trop Dis. 2010;4:e911. http://dx.doi.org/10.1371/journal.pntd.0000911
2. World Health Organization. Summary data. WHO meeting on Buruli ulcer control and research, 2013 Mar 25–27; Geneva; 2012 [cited 2013 Dec 12]. http://www.who.int/buruli/Summary_data_and_new_target_2013.pdf
3. Landier J, Gaudart J, Carolan K, Lo Seen D, Guégan J-F, Eyangoh S, et al. Spatio-temporal dynamics and landscape-associated risk of Buruli ulcer in Akonolinga, Cameroon. PLoS Negl Trop Dis. 2014;8:e3123. http://dx.doi.org/10.1371/journal.pntd.0003123
4. van Ravensway J, Benbow ME, Tsonis AA, Pierce SJ, Campbell LP, Fyfe JAM, et al. Climate and landscape factors associated with Buruli ulcer incidence in Victoria, Australia. PLoS ONE. 2012;7:e51074. http://dx.doi.org/10.1371/journal.pone.0051074
5. Marston BJ, Diallo MO, Horsburgh CR, Diomande I, Saki MZ, Kanga JM, et al. Emergence of Buruli ulcer disease in the Daloa region of Côte d'Ivoire. Am J Trop Med Hyg. 1995;52:219–24.
6. Revill WD, Barker DJP. Seasonal distribution of mycobacterial skin ulcers. Br J Prev Soc Med. 1972;26:23–7.
7. Uganda Buruli Group. Epidemiology of *Mycobacterium ulcerans* infection (Buruli ulcer) at Kinyara, Uganda. Trans R Soc Trop Med Hyg. 1971;65:763–75. http://dx.doi.org/10.1016/0035-9203(71)90090-3
8. Barker DJP, Carswell JW. *Mycobacterium ulcerans* infection among tse-tse control workers in Uganda. Int J Epidemiol. 1973;2:161–5. http://dx.doi.org/10.1093/ije/2.2.161
9. Cazelles B, Chavez M, De Magny GC, Guégan J-F, Hales S. Time-dependent spectral analysis of epidemiological time-series with wavelets. J R Soc Interface. 2007;4:625–36. http://dx.doi.org/10.1098/rsif.2007.0212
10. Trubiano JA, Lavender CJ, Fyfe JA, Bittmann S, Johnson PD. The incubation period of Buruli ulcer (*Mycobacterium ulcerans* infection). PLoS Negl Trop Dis. 2013;7:e2463. http://dx.doi.org/10.1371/journal.pntd.0002463
11. Ravisse P. Skin ulcer caused by *Mycobacterium ulcerans* in Cameroon. I. Clinical, epidemiological and histological study [in French]. Bull Soc Pathol Exot. 1977;70:109–24.
12. Morris A, Gozlan RE, Hassani H, Andreou D, Couppié P, Guégan JF. Complex temporal climate signals drive the emergence of human waterborne disease. Emerging Microbes & Infections. 2014;3:e56. http://dx.doi.org/10.1038/emi.2014.56
13. Pouillot R, Matias G, Wondje CM, Portaels F, Valin N, Ngos F, et al. Risk factors for Buruli ulcer: a case control study in Cameroon. PLoS Negl Trop Dis. 2007;1:e101. http://dx.doi.org/10.1371/journal.pntd.0000101
14. Garchitorena A, Roche B, Kamgang R, Ossomba J, Babonneau J, Landier J, et al. *Mycobacterium ulcerans* ecological dynamics and its association with freshwater ecosystems and aquatic communities: results from a 12-month environmental survey in Cameroon. PLoS Negl Trop Dis. 2014;8:e2879. PLoS Negl Trop Dis. 2014;8:e2879. 10.1371/journal.pntd.0002879
15. Giles-Vernick T, Owona-Ntsama J, Landier J, Eyangoh S. The puzzle of Buruli ulcer transmission, ethno-ecological history and the end of "love" in the Akonolinga district, Cameroon. Soc Sci Med. 2015;129:20–7.

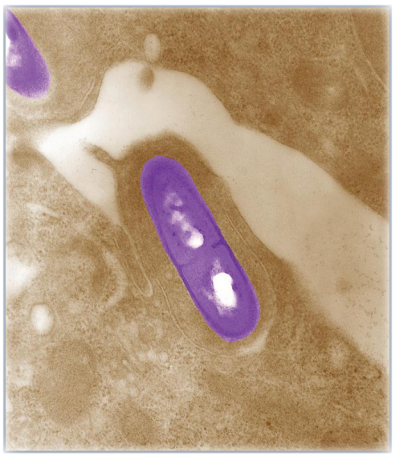
Address for correspondence: Jordi Landier, Unité d'Epidémiologie des Maladies Emergentes, Institut Pasteur, 25–28 Rue du Dr Roux, 75015 Paris, France; email: jordi.landier@gmail.com

EID PODCASTS

http://www2c.cdc.gov/podcasts/player.asp?f=8626870

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.



Article DOI: http://dx.doi.org/10.3201/eid2108.141336

Seasonal Patterns of Buruli Ulcer Incidence, Central Africa, 2002–2012

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.

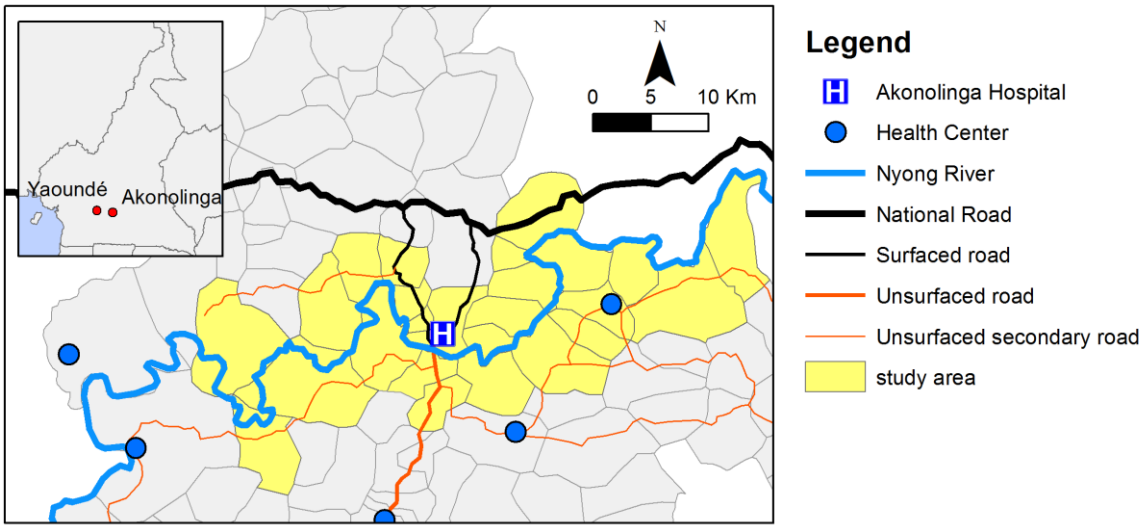
Statistical Methods

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*).

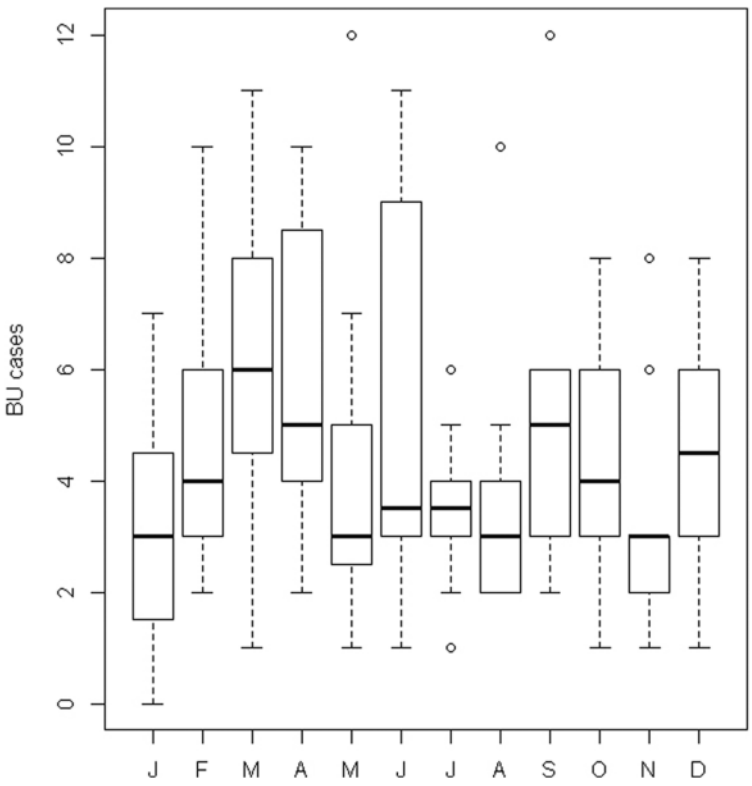
Wavelet coherency was then used to identify and quantify possible statistical associations between two time series, e.g., the BU case time series and one of the environmental variables. Coherency is roughly similar to a classical correlation, but pertains to oscillating components in a given frequency 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

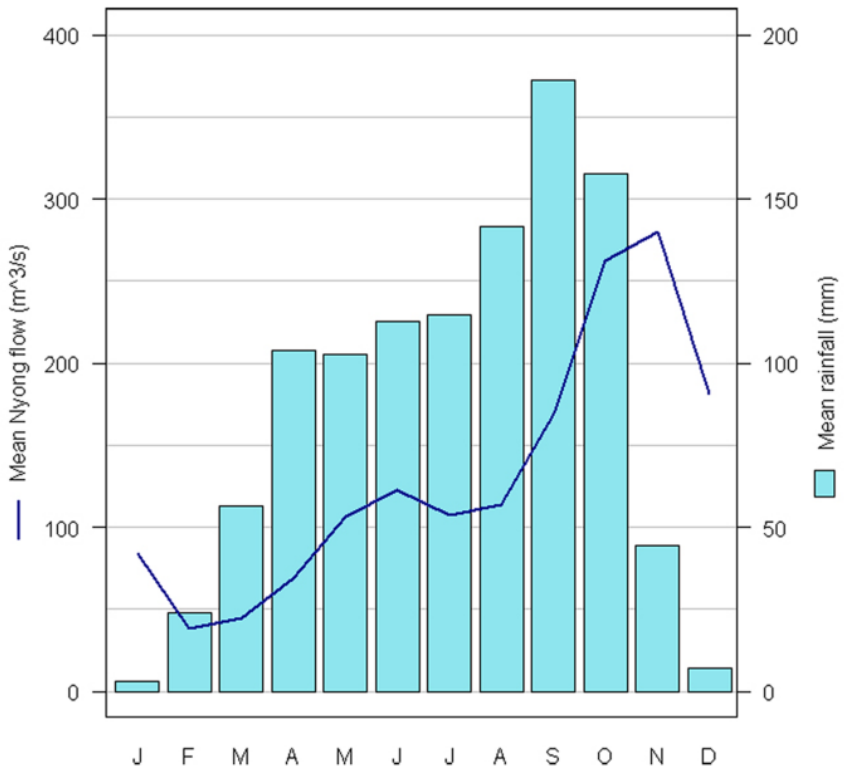
the 1-year mode. The phase difference between the two oscillating components (ΔT) was represented by the black dotted line. The right panel of the phase analysis corresponds to the phase difference histogram over the whole time period (black line). The phase difference can be converted into months (by multiplying by $12/2\pi$): 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

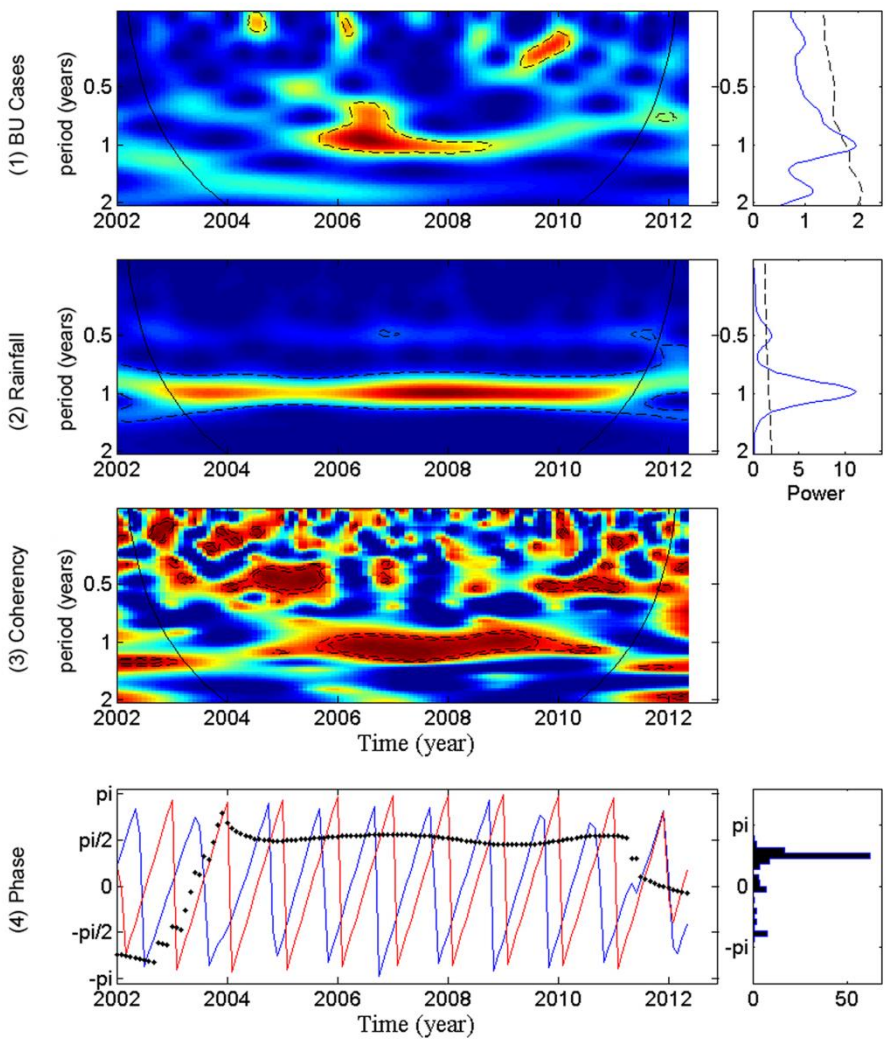


Technical Appendix Figure 1. Presentation of the study area displaying the location of Akonolinga Hospital, of the Health Centers and of the main roads. The boundaries of each village are displayed in dark gray.





Technical Appendix Figure 2. Boxplot of monthly BU incidence data over the study period, and mean monthly rainfall and Nyong River flow.



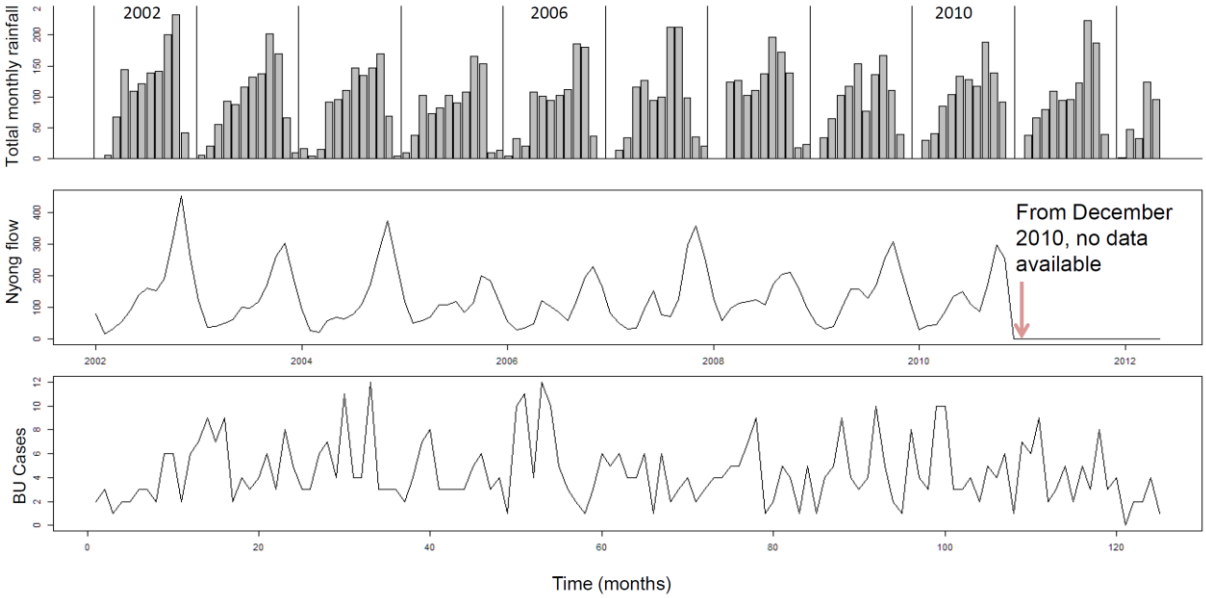
Technical Appendix Figure 3. Wavelet analysis of BU incidence and rainfall.

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

1. Landier J, Gaudart J, Carolan K, Lo Seen D, Guégan J-F, Eyangoh S, et al. Spatio-temporal dynamics and landscape-associated risk of Buruli ulcer in Akonolinga, Cameroon. PLoS Negl Trop Dis. 2014;8:e3123. PubMed http://dx.doi.org/10.1371/journal.pntd.0003123

2. Mensah-Quainoo E, Yeboah-Manu D, Asebi C, Patafuor F, Ofori-Adjei D, Junghanss T, et al. Diagnosis of Mycobacterium ulcerans infection (Buruli ulcer) at a treatment centre in Ghana: a retrospective analysis of laboratory results of clinically diagnosed cases. Trop Med Int Health. 2008;13:191–8. PubMed http://dx.doi.org/10.1111/j.1365-3156.2007.01990.x

3. World Health Organization. Buruli ulcer: diagnosis of Mycobacterium ulcerans disease. Portaels F, Johnson P, Meyers WM, editors. World Health Organization; 2001.

4. Pouillot R, Matias G, Wondje CM, Portaels F, Valin N, Ngos F, et al. Risk factors for buruli ulcer: a case control study in Cameroon. PLoS Negl Trop Dis. 2007;1:e101. PubMed http://dx.doi.org/10.1371/journal.pntd.0000101

5. Monitoring Agriculture with Remote Sensing [Internet]. 2013. Available from: http://www.marsop.info/

6. ORE BVET. BVET, Cameroon, Hydrological data. 2012.

7. Cazelles B, Chavez M, De Magny GC, Guégan J-F, Hales S. Time-dependent spectral analysis of epidemiological time-series with wavelets. J R Soc Interface. 2007;4:625–36. PubMed http://dx.doi.org/10.1098/rsif.2007.0212

8. Constantin de Magny G, Guégan J-F, Petit M, Cazelles B. Regional-scale climate-variability synchrony of cholera epidemics in West Africa. BMC Infect Dis. 2007;7:20. PubMed http://dx.doi.org/10.1186/1471-2334-7-20

9. Agier L, Deroubaix A, Martiny N, Yaka P, Djibo A, Broutin H. Seasonality of meningitis in Africa and climate forcing: aerosols stand out. J R Soc Interface. 2013;10:20120814. PubMed http://dx.doi.org/10.1098/rsif.2012.0814

10. Hashizume M, Chaves LF, Minakawa N. Indian Ocean Dipole drives malaria resurgence in East African highlands. Sci Rep. 2012;2:269. PubMed http://dx.doi.org/10.1038/srep00269